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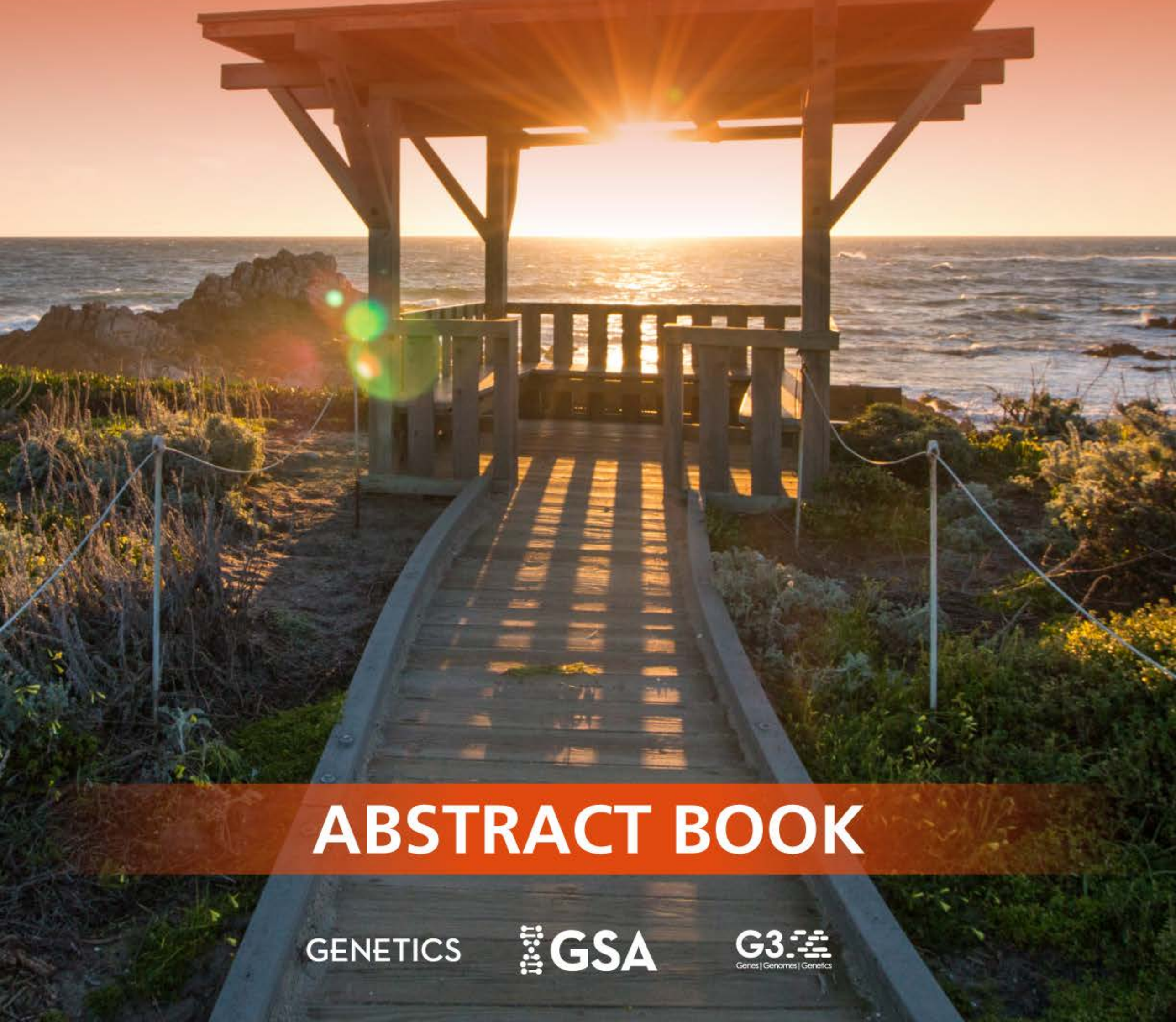

ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

1 **Genome evolution in filamentous plant pathogens** Cristina A. Barragan[1], Sergio M. Latorre[2], Angus Malmgren[1], Adeline Harant[1], Joe Win[1], Yu Sugihara[1], Hernán A. Burbano[2], Sophien Kamoun[1], Thorsten Langner[3] [1]The Sainsbury Laboratory, [2]2Centre for Life's Origins and Evolution, Dept of Genetics, Evolution and Environment, University College London, [3]Max-Planck-Insitute for Biology

Genome structure and maintenance determine the evolvability of organisms. The genomes of fungal plant pathogens are often structured heterogeneously, harboring highly variable compartments and compartments of relative stability. Often, rapidly evolving, virulence-related genes are associated with dynamic regions that are rich in repetitive and transposable elements. In addition, recent advances in whole genome sequencing technologies and pangenome scale studies point towards large repertoires of accessory genomic regions. An extreme case of such a genome structure and genomic structural variation are supernumerary chromosomes, that are present in only some individuals of a species. These chromosomes are associated with intra- and inter-chromosomal rearrangements, copy number variation and horizontal transfer of genetic material that can ultimately increase genetic diversity and the adaptive potential of plant pathogens. Here, we will discuss recent insights into the biology of supernumerary mini-chromosomes in the blast fungus *Magnaporthe* (syn. *Pyricularia*) *oryzae*.

2 **Securing crops against rust pathogens: Robigus in the modern times** Melania Figueroa[1], Eva C Henningsen[1], David Lewis[1], Tim C Hewitt[1], Megan Outram[1], Taj Arndell[1], Cheryl Blundell[1], Jian Chen[1,1], Rohit Mago[1], The Duong Nguyen[1], Emmery Hartwig[2], Rebecca Spanner[2], Eric Nazareno[2], Lee Hickey[3], Yung-Feng Huang[4], Botma Visser[5], Zak Pretorius[5], Willem Boshoff[5], Eric Stone[6], Nathalie Nienser[1], Matthew Moscou[7], Diane Saunders[8], Paula Silva[9], Silvia German[9], Pablo Campos[10], Brian Steffenson[2], Shahryar F Kianian[7], Thomas Vanhercke[1], Jana Sperschneider[1], Peter Dodds[1] [1]CSIRO, [2]University of Minnesota, [3]University of Queensland, [4]National Taiwan University, [5]University of the Free State, [6]Australian National University, [7]USDA-ARS, [8]John Innes Centre, [9]National Agricultural Research Institute, [10]Instituto Nacional de Tecnología Agropecuaria

Rust fungi represent the most complex group of plant pathogens. Among these, the cereal rusts are responsible for severe epidemics that threaten food security and our global economy. Their devastating impact is well-illustrated by the Romans, who made sacrifices to Robigus, the god of rust, to ask for protection of their crops. Centuries later, we are still seeking durable approaches to control rust fungi. The obligate biotrophic nature of rust fungi presents significant research challenges to characterize the underlying mechanisms of virulence evolution and host adaptation. However, knowledge of how rust fungi change to infect otherwise resistant crops is essential to design effective control strategies and surveillance programs. While the genomics era brought opportunities to address these important questions, the dikaryotic nature of these organisms during cereal infection continued to hinder progress. We pioneered the use of long read sequencing platforms and chromatin contact data to generate the first chromosome-level genome references for these pathogens. Importantly, these pipelines allowed us to resolve nuclear haplotypes in the rust dikaryotic phase uncovering a wealth of hidden genetic diversity within individual isolates. High resolution of the haplotype composition demonstrated that nuclear exchange among rust isolates is a widespread phenomenon that underpins the emergence of new rust strains, and consequently epidemics. Furthermore, haplotype-aware pangenome analysis has also provided insights into rare but important genetic recombination events that shape rust populations. By combining genetic information from rust haplotypes and a high-throughput effector library screening platform in plant protoplasts we have developed an effective means to rapidly identify *Avr* effectors, providing a path to understand virulence evolution and improve pathogen surveillance.

3 **Chromosomal engineering in the plant pathogenic fungus *Verticillium dahliae*** Yukiyo Sato, Bart PHJ Thomma Institute for Plant Sciences, University of Cologne, Germany

Plants and pathogens are engaged in everlasting molecular arms-races in which genes encoding host immune receptors and pathogen-secreted virulence factors play central roles. Fungal virulence factors are typically encoded in highly variable genomic compartments that drive pathogen adaptation. In the ascomycete fungus *Verticillium dahliae* such dynamic genomic compartments are called "adaptive genomic regions" (AGRs) that are the result of large-scale genomic rearrangements that have generated segmental duplications that underwent reciprocal gene losses. AGRs are enriched in *in planta* induced virulence genes, active transposable elements, histone H3K27me3, and structural variation. Recently, AGRs were found to physically co-localize in nuclei despite their distribution throughout the genome. However, causal relationships among the various traits that are linked to AGRs remain unclear. To address this, we established CRISPR-Cas9-based chromosomal engineering in *V. dahliae* to artificially engineer large-scale chromosomal rearrangements. We first engineered a reciprocal chromosomal translocation between a large AGR cluster and a core genomic compartment between two chromosomes. RNA-Seq analysis on this chromosomal translocation mutant revealed that genome-wide approximately 2% of genes were differentially expressed when compared with the wild-type strain. This result suggests that the chromosomal translocation impacts gene expression. We are now generating additional chromosomal translocation mutants over different AGR and core regions to collectively analyze whether and how these change

the transcriptome, H3K27me3 profile, and nuclear co-localization profiles. These experiments will reveal which of the traits follow after the large-scale chromosomal rearrangements, providing insight into causal relationships.

4 **Understanding the function of DNA adenine methylation in early-diverging fungi** Victoriano Garre, Carlos Lax, Francisco E Nicolás Universidad de Murcia

Epigenetic modifications and the resulting changes in chromatin structure play a crucial role in the regulation of gene expression. Despite early-diverging fungi (EDF) comprising most of the phylogenetic diversity of the fungal kingdom, the functions of these modifications have received limited attention. However, a pioneering study revealed an unexpected abundance of N6-methyldeoxyadenine (6mA) in their genomes, with dense methylated adenine clusters associated with actively expressed genes. This contrasts with most eukaryotes, including dikarya fungi, where the primary epigenetic mark on DNA is 5-methylcytosine (5mC). To gain a deeper understanding of 6mA regulation and its evolutionary trajectory, we characterized the methylation and transcriptional landscapes in three Mucorales (*Mucor lusitanicus*, *Phycomyces blakesleeanus*, and *Rhizopus microsporus*) representing the different 6mA and 5mC patterns found in EDF. 5mC was abundant only in *P. blakesleeanus*, predominantly located in repeat-rich regions, suggesting its involvement in transposon control. *P. blakesleeanus* and *R. microsporus* exhibited high levels of 6mA, mainly in AT dinucleotides, with both strands methylated, resulting in symmetric methylation (s6mA). In contrast, *M. lusitanicus* showed mostly asymmetric methylation (a6mA), akin to most eukaryotes, including dikarya.

A comparison of transcriptional and the 6mA profiles in different environmental conditions and tissues revealed an association between transcriptomic responses and the methylation landscape. The deletion of several genes encoding putative N6 DNA methyltransferases and subsequent 6mA profiling in *M. lusitanicus* allowed the identification of the writers for both s6mA and a6mA in EDF. Loss of a6mA resulted in higher sensitivity to mutagens, lower virulence, and higher levels of lipids, whereas loss of s6mA led to a reduction in growth. The impossibility to generate knockout mutants lacking s6mA in *R. microsporus*, a fungus with high levels of this epigenetic modification, revealed that this modification is essential. Additionally, analysis of other epigenetic modifications in this fungus unveiled a compartmentalized genome with constitutive heterochromatin and open euchromatin regions, whose transcriptional activity depends on H3K9me3, s6mA, H3K4me3, and H2A.Z. Taken together, these results indicate that 6mA plays an essential role in the genome regulation of EDF.

5 **Single-cell detection of copy number changes reveals dynamic mechanisms of adaptation to antifungals *in vitro* and *in vivo*** Anna Selmecki University of Minnesota

Genomic copy number changes are associated with antifungal drug resistance and virulence across diverse fungal pathogens. Despite the high prevalence of both gain and loss events, the rate and dynamics of these genomic changes in the presence of antifungal drugs is not known. We optimized a dual-fluorescent reporter system in the diploid pathogen *Candida albicans* to quantify copy number variation (CNV) and loss of heterozygosity (LOH) at the single cell level with flow cytometry. We followed the rate and dynamics of CNV and LOH at multiple distinct genomic locations during parallel evolution experiments in the presence and absence of antifungal drugs *in vitro* and in a murine model of infection. Copy number changes were rapid and dynamic during adaptation to three different concentrations of azole drugs. The fluorescent reporters revealed competing sub-populations with distinct genotypes in the drug-evolved lineages. Extensive whole genome sequencing identified recurrent genotypes that cause increased competitive fitness in the presence of antifungal drug, both in vitro and in vivo. In the murine model, copy number changes were only detected in isolates recovered from mice treated with azole drug, and included whole-genome duplication events resulting in polyploidy. This study provides quantitative evidence for the incredible speed at which diverse genotypes arise and undergo dynamic population-level fluctuations during adaptation to antifungal drugs *in vitro* and *in vivo*.

6 **Separation of life stages within anaerobic fungi highlights differences in global transcription and metabolism** Lazarina V Butkovich[1], Patrick A Leggieri[1], Stephen P Lillington[1], Tejas A Navaratna[1], Thea R Zalunardo[1], Anna Lipzen[2], Vivian Ng[2], Mei Wang[2], Juying Yan[2], Igor V Grigoriev[2], Michelle A O'Malley[1] [1]Chemical Engineering, University of California, Santa Barbara, [2]Lawrence Berkeley National Laboratory

Anaerobic gut fungi of the early-diverging fungal phylum Neocallimastigomycota are microbes proficient in valorizing low-cost but difficult-to-breakdown lignocellulosic plant biomass. In order to appreciate how different life stages contribute to biomass breakdown and production of enzymes relevant for biotechnological applications, we aim to better understand the life cycle of anaerobic gut fungi. In this study, we extracted RNA from culture samples of the anaerobic gut fungal strain *Neocallimastix californiae* G1 grown on rumen fluid-based media with glucose as the substrate. We used RNAseq to generate global gene expression profiles to compare two sample types: (1) cell pellets enriched in the young life stage of free-swimming, flagellated zoospores and (2) fungal mats with relatively more vegetative, encysted, mature sporangia. We find evidence that zoospores

transcriptionally prime to encounter plant matter substrate, despite being grown on simple sugar substrate. For example, key catabolic carbohydrate-active enzymes are upregulated in zoospores when compared to fungal mats. Furthermore, we report significant differential gene expression for gene annotation groups, including transporters, transcription factors, and putative secondary metabolites. The described RNAseq dataset and analysis will inform future hypotheses and experiments regarding the different life stages of anaerobic gut fungi.

7 **The Dark Side of Anaerobic Digestion: Carbohydrate-Active Enzymes from Uncultured Rumen Fungi** Katharine L Dickson[1], Itai Brand-Thomas[1], Claire Shaw[1], Charles G Brooke[1], Markus DeRaad[2], Robert Evans[2], Michael Endres[3], Gyorgy Babnigg[3], Vivian Chu[1], Tri Do[1], Abigail Pfefferlen[1], Vincent Lombard[4], Asaf Salamov[2], Alex Copeland[2], Kerrie Barry[2], Hailan Piao[4], Roderick I Mackie[5,6], Scott Baker[4], Samuel Deutsch[2], Susannah G Tringe[2], Bernard P Henrissat[7], Igor V Grigoriev[2], Jan-Fang Cheng[2], Soichi Wakatsuki[8], Yasuo Yoshikuni[2], Trent Northen[9], Andrzej Joachimiak[3], Matthias Hess[1] [1]Dept of Animal Science, University of California, Davis, [2]DoE Joint Genome Institute, Lawrence Berkeley National Laboratory, [3]Argonne National Laboratory, [4]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, [5]Dept of Animal Sciences, University of Illinois, Urbana-Champaign, [6]Dept of Nutritional Sciences, University of Illinois, Urbana-Champaign, [7]Dept of Biotechnology and Biomedicine, Technical University of Denmark, [8]SLAC National Accelerator Laboratory, Stanford University, [9]Environmental Genomics and Systems Biology Division

Anaerobic fungi of the Neocallimastigomycota are important fibrolytic microbes in the gut of large herbivores, degrading plant biomass via mechanical and enzymatic means. Even though anaerobic fungi are known to be vital to biomass digestion, mechanisms underlying anaerobic fungal biomass degradation are poorly understood at the level of individual enzymes. To probe the extent of anaerobic fungal contributions to biomass degradation in the rumen and identify novel carbohydrate-active enzymes (CAZymes) participating in this process, as well as their potential role in in situ biomass conversion, we isolated anaerobic fungal enzyme sequences from rumen microbiome samples, then transformed them into expression vectors and evaluated their capacity to degrade a set of polysaccharide substrates. We introduced samples of switchgrass and corn stover into the rumen of cannulated cows for 48 hours, then isolated polyadenylated RNA from each substrate sample and sequenced it to obtain a polyadenylated metatranscriptome. After annotating metatranscriptomes for their phylogenetic composition and identifying putative CAZyme sequences, a subset of 21 of the 73 glycosyl hydrolase (GH) families probed was selected for further analysis based on increased abundance on either switchgrass or corn stover, relative to rumen fluid, or previously determined functions of importance to biomass degradation. Phylogenetic trees of these sequences were constructed, and a subset of these sequences representing the greatest sequence diversity, novelty, and potential CAZyme activity was selected for transformation into E. coli BL21(DE3) for functional characterization on carboxylmethylcellulose (CMC), as well as the insoluble chromogenic substrates AZCL-Amylose, AZCL-HE-Cellulose, AZCL-Pullulan, and AZCL-Xylan (Oat) (Sigma-Aldrich, St. Louis, MO) (Neogen, Lansing, MI). (something about Western blot analysis). 15 of 196 clones of these selected sequences exhibited activity on at least one substrate, representing members of GH6, GH8, GH9, GH11, and GH48; of these, seven came from the metatranscriptomes and were thus designated novel CAZymes. Two of these clones, IBT63 and IBT64, represent the first GH8s identified from anaerobic fungi. Two additional clones, IBT52 and IBT54, represent the first fungal CAZymes, of family GH6, to incorporate the carbohydrate-binding module CBM10; IBT54 additionally is the first identified fungal CAZyme to incorporate an Fn3-like domain, which form multi-domain complexes with CBM3. In addition, IBT84 and IBT85 contained enzymes combining GH11 and GH10 domains, suggesting a possible synergistic relationship between these two domains. These results further suggest possibilities for acquisition of some of these domains from bacteria via horizontal gene transfer, as well as synergy between some of these CAZymes as deployed in the rumen.

8 **Potential of anaerobic fungi to degrade natural and synthetic biopolymers** Magdalena Calusinska[1,2], Shirley Jin[1], Colleen Ahern[1], Wikram Mubayi[1], Michelle O'Malley[1] [1]Chemical Engineering, University of California Santa Barbara, [2]Environmental Research and Innovation, Luxembourg Institute of Science and Technology

The similarity of bioplastics (BPs) to natural fibers implies similar routes for their degradation through microbial activities. Indeed, studies undertaken in natural environments have uncovered some common mechanisms. Energy recovery through the process of anaerobic digestion (AD) is considered an added-value end-of-life option for BPs waste management. Nevertheless, many BPs cannot biodegrade well under standard AD conditions, and the biodegradability is closely linked to the chemical structure imposed during the production process.

The metabolic versatility of anaerobic fungi (AF) and their ability to degrade complex lignocellulosic polymers indicate that their enzymatic machinery could also enable BPs biodegradation. Indeed, Neocallimastigomycota can produce a large array of hydrolytic enzymes, including different glycoside hydrolases, esterases, pectinases, etc., that synergistically degrade crude biomass. Additionally, the mycelia growth of AF could also cause the mechanical disruption of BPs, thus reducing their durability and

resistance to enzymatic lysis. Despite our incomplete understanding of AF and their involvement in biopolymer degradation, preliminary results suggest that AF represent a great potential for improving BP biodegradation under mesophilic anaerobic conditions. Specifically, cellulose acetate, polyhydroxyalkanoates and different polylactic acid blends have shown promise under these conditions. Still, AF cannot develop well in AD reactors, where they are quickly outcompeted by cellulolytic bacteria. Therefore, improving the characterization of AF and elucidating their nutritional and environmental requirements are crucial to develop future AF bioaugmentation strategies in AD systems.

9 **Unleashing the hidden potential of anaerobic fungi: insights and innovations** Sabine Marie Podmirseg[1], Julia Vinzelj[1], Akshay Joshi[1,2], Sophia FA Strobl[1], Marco Wehner[3], Markus Neurauter[1], Leonie R Sondergger[1], Manuel Karrer[1], Christian Ebner[3], Heribert Insam[1] [1]Universität Innsbruck, Institute of Microbiology, [2]University of Applied Sciences, Biocatalysis, Environment and Process Technology Unit, [3]Unit of Environmental Engineering, Dept of Infrastructure, Universität Innsbruck

In recent years, there has been a growing interest in unlocking the potential of anaerobic gut fungi (AGF) classified under the phylum Neocallimastigomycota. Here, we outline some key findings derived from a comprehensive, multi-year international research endeavor aimed at elucidating the relatively unexplored aspects of this peculiar, basal group of fungi. The investigations encompassed challenges associated with the routine cultivation and preservation of AGF, along with the exploration of their growth requirements and the presumed existence of resting stages.

Furthermore, we present our latest methodologies for detecting and categorizing AGF, utilizing both molecular (FISH) and non-molecular techniques. A critical analysis of the merits and drawbacks associated with these approaches will be discussed. Ultimately, we provide a succinct overview of the environmental distribution of AGF and offers a glimpse into our future strategies for harnessing their biotechnological potential within the specific context of the biogas sector in Central Europe.

10 **Physiological adaptation to changing environments by the polyextremotolerant yeast Aureobasidium pullulans** Audrey Williams[1], Claudia Petrucco[2], Julian Liber[2], Alex Crocker[2,3], Amy Gladfelter[2] [1]Cell Biology, Duke University, [2]Duke University, [3]University of North Carolina Chapel Hill

Fungal life is found across a vast range of environments with extremes of pH, temperature, salinity, water availability, and other abiotic factors. Some fungi (termed *polyextremotolerant*), can grow in multiple extreme (as well as not-so-extreme) environments, and must adapt their physiology dramatically to maintain cell function in the face of these changes. A number of cellular adaptation mechanisms have been described, including changes in solute production, cell wall thickness, ion transport, membrane composition, and cell shape. However, we do not understand how these responses work together to sustain cell organization and biochemistry, the timescales on which different cellular adaptations occur, or how cells adapt to simultaneous changes in multiple environment features. To address these questions, we are developing the widespread, polyextremotolerant yeast *Aureobasidium pullulans* as a cell-biological model of adaptation to extremes. We are building a toolkit of genetically-encoded fluorescent probes with which to measure physiological and morphological traits including intracellular pH, ATP concentration, macromolecular crowding, and cell and vacuole size and shape. With these probes, we will compare the physiology of cells adapted to a range of temperatures, pH, and salinity, and map the dynamic responses of these cells to changes in these factors. We have also found that different *A. pullulans* isolates vary widely in their tolerance for high salinity and other culture conditions. We are sequencing the genomes of 200 isolates to identify genomic loci that correlate with the isolates' ability to grow in different environments. We will draw on these data to identify new candidate cellular processes that contribute to adaptation to extremes.

11 **Structural Adaptation of Fungal Cell Wall in Hypersaline Environment** Liyanage D. Fernando Dr.[1], Yordanis Pérez Llano[2], Malitha C. Dickwella Widanage[1], Anand Jacob[1], Liliana Martínez-Ávila[2], Andrew S. Lipton[3], Nina Gunde-Cimerman[4], Jean-Paul Latgé[5], Tuo Wang[6], Ramon Alberto Batista Garcia[2] [1]Michigan State University, [2]Universidad Autonoma del Estado de Morelos, [3]Pacific Northwest National Laboratory, [4]University of Ljubljana, [5]University of Crete, [6]University of Michigan

Halophilic fungi thrive in hypersaline habitats and face a range of extreme conditions. These fungal species have gained considerable attention due to their potential applications in harsh industrial processes, such as bioremediation and fermentation under unfavorable conditions of hypersalinity, low water activity, and extreme pH. However, the role of the cell wall in surviving these environmental conditions remains unclear. Here we employ solid-state NMR spectroscopy to compare the cell wall architecture of *Aspergillus sydowii* across salinity gradients. Analyses of intact cells reveal that *A. sydowii* cell walls contain a rigid core comprising chitin, β-glucan, and chitosan, shielded by a surface shell composed of galactomannan and galactosaminogalactan. When exposed to hypersaline conditions, *A. sydowii* enhances chitin biosynthesis and incorporates α-glucan to create thick, stiff, and hydrophobic cell walls. Such structural rearrangements enable the fungus to adapt to both hypersaline and salt-deprived

conditions, providing a robust mechanism for withstanding external stress. These molecular principles can aid in the optimization of halophilic strains for biotechnology applications.

12 **How does light affect rock-inhabiting fungi?** Julia Schumacher[1,2], Anna A. Gorbushina[1,2] [1]Bundesanstalt für Materialforschung und -prüfung (BAM), [2]Freie Universität Berlin

Sunlight is an almost unavoidable environmental cue and plays a fundamental role in the biology of pro- and eukaryotic organisms. To cope with sunlight-associated stresses e.g., high temperatures, UV radiation with associated DNA damage, accumulation of reactive oxygen species (ROS), desiccation and osmotic stresses, it is important for organisms to accurately sense and respond to changes in light. The benefits of light are obvious for green organisms such as cyanobacteria, algae and plants which use light as an energy source (photosynthesis). Less apparent are other light-dependent processes such as light-driven DNA repair by photolyases (photoreactivation) or ion pumping by microbial opsins. Fungi that can share light-flooded habitats with phototrophs may profit from their excess photosynthetic products. Rock-inhabiting Dothideomycetes and Eurotiomycetes including *Knufia petricola* possess many proteins for absorbing UV/blue, green, red and far-red light, produce the black 1,8 dihydroxynaphthalene (DHN) melanin and orange-red carotenoids, and may live in multispecies biofilms. Here, we are addressing the question to which extent constitutive pigment formation (melanin and carotenoids) and responses mediated by the stress-activated mitogen-activated protein (MAP) kinase contribute to the observed light (UV-B) tolerance of *K. petricola*.

13 **Long-read sequencing reveals cryptic genome biology of insect gut-associated fungus – *Zancudomyces culisetae* (Harpellales, Zoopagomycota)** Yan Wang University of Toronto

The study of modern genomics has significantly advanced our understanding of evolutionary relationships within the Kingdom Fungi. However, our knowledge of early-diverging fungal lineages remains limited due to the scarcity of reference-quality genomic resources. *Zancudomyces culisetae*, a microbial fungus predominantly found in the digestive tracts of mosquitoes, serves as a valuable model system in investigations of insect gut-dwelling fungi. The initial draft-quality genome of *Z. culisetae* helped unveil hidden interactions between this microbial fungus and its mosquito hosts, including the horizontal gene transfer of a polyubiquitin-coding gene. However, the fragmented genome assembly limited further exploration of this interkingdom transfer event. Here I will share recent genomic progress from our research group, focusing on the PacBio High-Fidelity long-read data obtained for *Z. culisetae.* This data enabled the assembly of a reference-quality genome, shedding light on the cryptic genome biology and evolutionary trajectories of *Z. culisetae*. This work underscores the high resolution of genomic investigation made possible by long-read sequencing techniques.

14 **Functional characterization of sugar transporters in *Saccharomyces cerevisiae* for the improvement of second generation (2G) ethanol production** Roberto N. Silva Biochemistry, University of Sao Paulo

The global interest in reducing the environmental and economic impact associated with the use of fossil fuels argues for new, renewable and cost-effective energy sources. In this scenario, second generation (2G) ethanol proves to be a promising alternative when using lignocellulosic biomass (LCB) as feedstock. However, the degradation of lignocellulose and the fermentation of the resulting sugars are some of the challenges that need to be overcome for an economically viable production of second-generation ethanol. Lignocellulolytic fungi such as *T. reesei* are used industrially due to their potential to produce and secrete holocellulases (cellulases and hemicellulases). On the other hand, *T. reesei* has a complex transport system that is responsible for the effective transport of sugars from LCBs, which act as inducers or repressors of holocellulase biosynthesis. Despite the great interest in this fungus for the bioenergy industry, the sugar transport system of T. reesei is still largely unexplored. Therefore, a better understanding of the transporters of T. reesei can be a valuable strategy to develop improved strains of interest to the 2G industry and overcome the challenges in bioethanol production. In this study, we aim to shed light on this topic through the functional characterization of novel T. reesei transporters. Initially, our phylogenetic analyzes and molecular docking indicated the potential of the new T. reesei transporters to transport the essential sugars of holocellulose (xylose, cellobiose, mannose and glucose). The functional validation was carried out by heterologous expression of the transporters in microbial platforms (EBY.VW4000 and SC9721), which have no functional transport system for these sugars. In this way, it was possible to characterize three xylose transporters (Tr54632, Tr68122 and Tr79202) and one cellobio-oligosaccharide transporter (Tr441745). In addition, expression of the transporters Tr54632 and Tr79202 promoted the low growth of the yeast EBY.VW4000 in other holocellulosic sugars such as glucose and mannose, respectively. In contrast, the yeast with the transporter Tr44175 was able to transport sophorose, a carbon source that induces the expression of hocellulases. In the present work, transporters were characterized for which no transport activity had previously been described. This opens up new avenues for the development of strains that can be used in the bioenergy industry.

15 **Genetic engineering, pilot plant bioprocess development and sustainability assessment of a Trichoderma platform for cellulase production** Mario T Murakami LNBR/CNPEM, Brazilian Center for Research in Energy and Materials (CNPEM)

Enzymatic breakdown of lignocellulose represents one of the major costs in the biotransformation of lignocellulosic agro-industrial residues into sustainable fuels, chemicals and polymers. *Trichoderma reesei* strains have been extensively studied, engineered and employed by industry to produce enzyme cocktails for this purpose in biorefineries. However, the knowledge for the rational development of competitive and robust strains along with an industrially relevant bioprocess to produce these enzyme cocktails remains elusive to public domain. Herein, we have rationally developed a RUT-C30 strain, harboring 7 genetic modifications that (i) mitigate catabolic repression, (ii) increase protein production and secretion, (iii) enhance β-glucosidase activity, (iv) enable the utilization of molasses as carbon source, (v) decrease protease activity levels in the secretion, and (vi) ultimately, amplify the redox power toward crystalline cellulose. Collectively, these modifications resulted in secretion titers higher than 80 g/L of mostly Carbohydrate-Active EnZymes (CAZymes) with high stability and efficiency in the lignocellulose saccharification. Notably, a single modification that augments the cellulolytic redox power of the secretome enhanced the saccharification capacity up to 30% under industrially relevant conditions. The engineered strain is genetically stable during fermentation and degenerated populations are not accumulating. In addition, the bioprocess was optimized in the 300L pilot-plant bioreactors, resulting in integral reproduction of the titer/yield/rate obtained in the bench scale bioreactors in the pilot-plant environment. Sustainability analysis showed that the CO_2 emissions are half to current commercial solutions and cost are highly competitive considering pilot plant data. Taken together, the rational genetic engineering, bioprocess development in pilot plant, strain degeneration studies and sustainability assessments provide a highly competitive and robust platform for lignocellulose valorization in biorefineries.

16 **A Biofoundry for Synthetic Biology and Genetic Tool Development of Anaerobic Gut Fungi** Elaine Kirschke[1], Michelle O'Malley[2] [1]Institute for Collaborative Biotechnologies, University of California Santa Barbara, [2]Chemical Engineering, University of California Santa Barbara

Anaerobic microbial communities possess many characteristics that make them attractive system for industrial application especially for the degradation of recalcitrant polymers and the conversion of renewable resources such as agricultural plant biproducts to biofuels and other value add chemicals. In particular, the anaerobic gut fungi (AGF) found in the digestive tracks of large herbivores, such as *Neocallimastix sp.*, poses impressive ability to break down lignocellulose plant fibers. Correlating with this, their genomes possess a significant expansion of genes associated with lignocellulose degradation. Moreover, recent findings from the O'Malley lab suggest that AGF harbor molecular machinery with the ability to facilitate bond cleavage in one of the most recalcitrant plant polymers, lignin, utilizing a novel uncharacterized anaerobic mechanism. However, our ability to characterize, understand, and exploit these fungal systems and their molecular machinery is severely limited. Challenges include technical consideration in handling obligate anaerobes along with a lack of genetic tools and a limited biological understanding of these understudied organisms. To further scale and accelerate our efforts, and those of others working with anaerobic microbial systems, we are establishing a user facility at UCSB that will house an automated robotic system equipped for high throughput workflows. This fully automatable system consisting of 13 different integrated instruments will enable synthetic biology and genetic tool development of anaerobic microbes and microbial communities. Leveraging the high throughput workflows enabled by this system we aim to develop efficient transformation procedures for AGF and the necessary tools for genome editing and synthetic biology applications. Together, this work should further our understanding of these fungal system and allow their industrial potential to be more fully explored.

17 **Exploring the role of alpha-1,3-glucan synthases on fungal cell wall integrity in Aspergillus niger** Katharina J. Ost[1], Mark Arentshorst[2], Arthur F.J. Ram[2], Bruno M. Moerschbacher[3], Mareike E. Dirks-Hofmeister[1] [1]Food Biotechnology, Osnabrück University of Applied Sciences, [2]Biology, Leiden University, [3]Plant Biology and Biotechnology (IBBP), University of Münster

Alpha-glucan synthases (ags) play a key role in the synthesis of alpha-1,3-glucan, a crucial component of the fungal cell wall that is (i) contributing to its structural integrity and is (ii) involved in cross-linking of different polymers, so that it influences the composition of both: the outer shell and inner membrane of fungal cells. In the filamentous fungi Aspergillus niger five ags genes are annotated, of which agsA and agsE were shown to be the most highly expressed genes during different stages of development. In addition, an agsE deletion mutant caused smaller micro-colonies as well as a shift in the secretome composition of A. niger, without affecting biomass production. Concurrently, intracellular cross-linking between chitin and beta-glucan is primarily mediated by the seven-membered cell wall-related transglycosylase gene family (crh). Although an impact on cell wall integrity has been expected when deleting the entire crh gene cluster in A. niger, significant alterations in cell wall integrity became only evident when the crh gene cluster deletion was combined with the reduction of alpha-glucan and galactomannan by deleting respective agsE or ugmA. With this background, we aimed to further explore the impact of ags gene deletions – both individually

and as an entire family – on fungal cell wall integrity of A. niger. Using targeted CRISPR/Cas9 technology, we engineered various ags-deficient strains, including the deletion of the entire gene family (ΔagsA-E) in a mutant strain lacking all chitin-glucan cross-linking enzymes (Δcrh, TLF39). Subsequent morphological and biochemical characterization of these mutants pinpoint the importance of agsE for maintaining cell wall stability and suggesting its potential influence on protein production and/or secretion. These findings therefor provide not only new insights into fungal biology but also potential targets for biotechnological applications.

18 **Understanding the dynamics of carbon catabolite repression in filamentous fungi** J. Philipp Benz[1], Marcel K. Rüllke[1], Franziska A. Meyer[1], Nils Thieme[1,2], Alexander Karollus[3], Julien Gagneur[3], Elisabeth Tamayo[1] [1]Fungal Biotechnology in Wood Science, TUM School of Life Sciences, Technical University of Munich, [2]Planet A Foods, [3]Computational Molecular Medicine, TUM School of Computation, Information and Technology, Technical University of Munich

Many varieties of waste products accumulate during food processing that contain valuable polysaccharides. The utilization of these compounds could generate added value and the environment would profit from a more holistic usage of these resources. Filamentous fungi like *Neurospora crassa* and *Aspergillus niger* possess various enzymes for depolymerization of complex carbohydrates. However, the synthesis of biomass-degrading hydrolases is tightly regulated. A major challenge particularly at industrial scale is carbon catabolite repression (CCR), which leads to a preferred utilization of easily metabolizable carbon sources like monosaccharides over complex sugar polymers. The transcription factor CRE-1/CreA is the main inhibitor of genes coding for hydrolytic enzymes, sugar transporters and regulators. In presence of high amounts of e.g. glucose, CRE-1/CreA binds to specific motifs in the promoter regions of its target genes and thereby inhibits transcription. While CCR is a highly conserved process and the components involved in the signaling pathway are well studied in many organisms, it is notoriously hard to quantify, and the dynamics of this repression differ between sugars and fungi. To overcome these limitations, new approaches and tools are needed.

Aiming to better understand and track CCR dynamics, we took two approaches: first, we analyzed transcriptomes from a nutritional array in *N. crassa* to identify CCR-signatures across conditions. Second, we developed a luciferase-based reporter system for real-time quantification of CRE-1/CreA-dependent CCR effects. The reporter is controlled by an artificial and tunable promoter system, leading to down-regulation of the luciferase in CCR-inducing conditions. Variants of CRE-1/CreA-binding motifs were tested for their responsiveness to a glucose gradient. The best performing construct was then used to compare the CCR-triggering of several monosaccharides in *A. niger* and *N. crassa* over time. It was observed that many monosaccharides elicit CCR effectively, while the timing, strength and duration of the response differed considerably.

Our results demonstrate that the luciferase reporter can be applied equally well across diverse fungi to study the role of CRE-1/CreA as a central regulator of carbohydrate utilization and to optimize fermentation conditions for biotechnologically relevant platform species growing on lignocellulosic biomass.

19 **Understanding the inner workings of the basidiomycete Fomes fomentarius for materials applications** Carsten Pohl[1], Bertram Schmidt[1], Ulla Simon[2], Tamara Nunez-Guitar[1], Carsten Lühr[3], Justus Zillessen[1], Fangxing Zhang[4], Heiko Briesen[4], Hans-Jörg Gusovius[3], Vera Meyer[1] [1]Applied and Molecular Microbiology, Technische Universität Berlin, [2]Chair of Advanced Ceramic Materials, Technische Universität Berlin, [3]Dept Systems Process Engineering, Leibniz-Institute for Agricultural Engineering and Bioeconomy (ATB), [4]Chair of Process Systems Engineering, Technical University of Munich

To achieve climate neutrality, fundamentally new concepts of circularity need to be implemented by the building sector, a significant contributor of anthropogenic CO2 emissions. Recently, we have shown that the polypore Fomes fomentarius feeds well on renewable lignocellulosic biomass and produces composite materials that could potentially replace fossil fuel-based expanded polystyrene as insulation material.

In follow up work, we explored the mechanical and physical properties of F. fomentarius-based composite materials in more detail and determined key performance parameters that are important to evaluate the usability of F. fomentarius-based composite materials in the construction sector. These parameters were determined according to European standards and included compressive strength, modulus of elasticity, thermal conductivity, water vapour permeability, and flammability of uncompressed composites as well as flexural strength, transverse tensile strength, and water absorption capacity of heat-pressed composites, among others.

We furthermore show that heat-pressing can be used to reliably generate stiff and firm particleboards that have the potential to replace current wood-based particleboards that contain synthetic additives. X-ray microcomputed tomography finally visualized

for the first time the growth of hyphae of F. fomentarius on and into the hemp shive substrates and generated high-resolution images of the microstructure of F. fomentarius-based composites.

Lastly, we used transcriptomics to characterize the expression of cell wall genes under various growth conditions and attempt to link the impact of the cell wall composition to the observed material properties.

20 **Characterizing the effects of simulated space environmental conditions on the biological and mechanical properties of fungal composite biomaterials** Rolando Perez[1,2], Katheryn Kornegay[2], Hannah Krivic[3], Victoria Porto[2], Sujith Pakala[4], Monika Brandic Lipinska[5], James Head[4], Christopher Maurer[6], Martyn Dade-Robertson[5], Maikel Rheinstadter[3], Debbie Senesky[2], Lynn Rothschild[7] [1]NASA Ames Research Center, Blue Marble Space Institute of Science, [2]Stanford University, [3]McMaster University, [4]Brown University, [5]Newcastle University, [6]Redhouse Studio, [7]NASA Ames Research Center

With increasing interest in long-term space exploration there is a need for technologies that enable *in situ* resource utilization (ISRU) to reduce the dependance of future missions on resupply services from Earth. For example, current proposals for lunar civil infrastructure construction, such as habitats for humans, use steel structures delivered from Earth. While it is acceptable to use steel infrastructure for lunar missions, and initial missions beyond the Moon, a sustained presence beyond the Moon would incur significant upmass costs in the long term. With resupply services to the Moon feasible, the lunar surface is increasingly being viewed as a testbed for technologies that enable longer-term and -distance space exploration. ISRU using lunar regolith for material production has been proposed but it relies on resource-intensive processes, such as lunar concrete production via laser melting. In contrast, biologically-based processes exhibit potential benefits, *e.g.*, regeneration. An alternative approach, working with fungi to bind biomass supplied from Earth or produced *in situ*, or lunar regolith mined *in situ*, could enable significant benefits in terms of cost, performance, function, and aesthetics. To this end, we have characterized the effects of simulated space environmental conditions, such as reduced pressure and UV exposure, on the biological and mechanical properties of fungal composite biomaterials, such as growth performance and compressive strength. Our initial results suggest that fungal composite biomaterials may be a viable alternative to current methods for producing valuable materials, *e.g.*, construction materials for habitats. If humanity is to explore beyond low-Earth orbit we will need advanced ISRU, material production capacities and bioregenerative life support systems to sustain life. Fungal composite biomaterials may be a viable solution to this need.

21 **Biomineralization-Enabled Self-Growing Building Blocks for Habitat Outfitting on Mars** Nisha Rokaya[1], Richard Wilson[1], Congrui Jin[2] [1]Plant Pathology, University of Nebraska-Lincoln, [2]Texas A&M University

The outfitting of inflatable habitat on Mars currently relies on launching necessary equipment from a second spacecraft. Rather than shipping prefabricated materials from earth, a biological toolkit that would allow to self-growing of the habitat outfitting on Mars by utilizing the initial in-situ resources on Mars, including sunlight, water, CO_2, N_2, and trace minerals enable long-term human space exploration and colonization. The focus of this research is the development of a synthetic lichen system using a phototroph-heterotroph symbiosis capable of thriving in the Martian atmosphere. The system would produce abundant biominerals and biopolymers, effectively binding Martian regolith into consolidated building blocks. To construct this synthetic community, two key species are employed: diazotrophic cyanobacteria, responsible for extracting CO_2 and N_2 from the air, and filamentous fungi, which excrete Ca^{2+} and stimulate the formation of substantial $CaCO_3$ precipitates. Experimental results confirm that these co-culture systems can grow very well on air and light in an inorganic liquid with a Martian regolith simulant (MRS) medium, without the need for additional carbon or nitrogen sources. Among ten tested filamentous fungi in co-culture with the diazotrophic cyanobacterium *Anabaena* sp., three fungi (*Trichoderma reesei, Aspergillus niger*, and *T. viride*) exhibited significantly enhanced growth of both cyanobacteria and fungi compared to the other seven fungi, highlighting the crucial role of mutual interactions with specific fungi. Since Mars' atmosphere consists of only traces of oxygen, we further tested axenic and co-culture growth of diazotrophic cyanobacteria and fungi under anaerobic conditions. Results reveal the oxygen produced by a diazotrophic cyanobacterium in 20% CO_2 and 80% N_2 supports the growth of fungi. In addition, the Mars atmosphere has a pressure of 7.5 mbar, 2.8% N_2, which is incompatible with the metabolism of most microbes. Therefore, to implement this technology, a photobioreactor will be utilized to provide the microbes with tightly regulated atmospheric conditions, as well as other necessary parameters inside the habitat, such as illumination, heating to optimal temperatures, and protection against harmful radiation.

22 **Structural genomics insights onto the evolution of generalist parasitism in Ascomycetes** Darcy AB Jones[1], Mark C Derbyshire[2], Sylvain Raffaele[1] [1]LIPME, INRAE, [2]Centre for Crop and Disease Management, Curtin University

The range of hosts that parasites can infect is a key determinant of the emergence and spread of disease. In fungal pathogens, host range varies from a single host genotype (specialists) to hundreds of unrelated species (generalists). In these interactions, pathogen small-secreted protein effectors play a major role in manipulating plants to facilitate disease. While molecular processes

contributing to host specialization have been relatively well studied, the molecular and genetic bases of host range expansion and the evolution of generalism remain elusive. To search for candidate effectors associated with a generalist lifestyle, we analyzed the predicted structure of small-secreted proteins lacking functional annotation (orphans) across Ascomycete genomes. We show that a majority belong to families broadly conserved at the structure level, that diversified through changes to their surface energetic landscape. The underlying mutations tend to increase the robustness of the overall effector structures while also contributing to evolvability. To test for functional diversification within families of structurally-related effectors, we focused on the white mold pathogen *Sclerotinia sclerotiorum*. We performed gene co-expression analyses to determine the extend to which structurally-related effectors are co-regulated and associate with genes of similar function. We will report on the evolution of frustration patterns at effector surfaces in species from the *Sclerotiniaceae* and their predicted protein-protein interactions. These predictions further our understanding of the long term evolution of biological activity in conserved effector families, and open new avenues for the experimental validation of evolutionary scenarios for fungal effectors.

23 **Machine learning-enabled prediction of genes associated with drug resistance and thermotolerance in Saccharomycotina yeasts** Marie-Claire Harrison[1], Abigail LaBella[2], Stella Lo[2], William White[1], Marizeth Groenewald[3], Chris T Hittinger[4], Antonis Rokas[1] [1]Vanderbilt University, [2]Bioinformatics and Genomics, University of North Carolina at Charlotte, [3]CBS Yeast Microbial Genetic Resources, Westerdijk Fungal Biodiversity Institute, [4]University of Wisconsin-Madison

The recently characterized genomes, isolation environments, and qualitative patterns of growth on 122 substrates and conditions from 1,154 (nearly all known) yeast species in the subphylum Saccharomycotina (Y1000+ Project) provide a powerful but complex dataset for studying the evolution of the genotype-phenotype map. We recently generated high accuracy predictions of growth on several carbon substrates from genetic data and identified a novel utilization pathway using a random forest classification algorithm, demonstrating the effectiveness of using machine learning to analyze this dataset. In this study, we trained a random forest classification algorithm to predict the clinically-relevant but complex traits of drug resistance and salt/thermotolerance at 37˚C for each species from Y1000+ Project genomic data. This analysis revealed the ability of machine learning to pick up on relevant genes and predict these complex traits when trained on genomic datasets. In this presentation, I will be talking about the genes (and gene attributes) most strongly associated with the ability to predict drug resistance and temperature/salt tolerance across species in the subphylum; these include genes previously implicated in cell wall composition, drug resistance and thermotolerance, as well as novel genes not previously known to be associated with these traits. We conclude that machine learning is a powerful tool for investigating the macroevolution of the genotype-phenotype map in fungi.

24 **Applied machine learning models for elucidating complex relationships between epigenomic regulatory design rules and gene expression between fungal species across phylogenetic distances.** Laura Weinstock, Cameron Kunstadt, Anna Fisher, Jenna Schambach, Elizabeth Koning, Wittney Mays, Raga Krishnakumar Sandia National Laboratories

Engineered fungi are promising chassis for future sustainable biomanufacturing and bioproduction. Reliable regulation of the functionality in diverse fungi at scale remains a significant challenge to commercialization. Determining ground rules of gene regulation and their applicability across fungal species is critical for minimizing the number of conditions that need to be tested to achieve optimally engineered fungi across species. Epigenetic modifications play a crucial role in regulating gene expression and having a handle on regulation of epigenetics across fungal species will significantly improve engineering prospects, both in existing and emerging synthetic biology chassis. Recently, there have been efforts to better understand the relationship between gene sequence, epigenetic modifications, and gene expression within fungal species through the use of machine learning and deep learning (ML/DL) methods that are able to ingest the high-dimensional, complex sequencing data. However, there remains limited understanding of how similar epigenetic modifications control gene expression across fungal species. The discovery of conserved epigenetic modification design rules that control gene expression would greatly improve the efficiency of engineering across diverse fungal species. In this study, we aimed to predict gene expression levels based on combinatorial epigenetic modification expression within and across fungal species. By predicting the effect of epigenetic modifications on gene expression, we can a) predict more accurately how a given engineered strain might optimally leverage epigenetics and b) determine how to engineer strains to take full advantage of these epigenetic pathways. We trained and tested ML/DL models within and across fungal species to identify the relationship between epigenetic modification features and gene expression. We tested a battery of models, ranging from regression to deep neural networks, on increasingly complex engineered data features to not only predict gene expression based epigenetic modifications, but also to understand the degree of complexity required to make accurate predictions. Overall, there is some conservation of epigenetic mechanisms across related species, though the predictive capacity of our cross-species epigenetic models was limited, which may be due to underlying biological constraints or limited data availability.

25 **Global analysis of circuitry governing *Candida albicans* morphogenesis within host immune cells and identification of inhibitors of morphogenesis** Nicola T Case[1], Johannes Westman[2], Michael T Hallett[3], Jonathan Plumb[2], Aiman Farheen[1], Michelle E Maxson[2], Mami Yoshimura[4], Takeshi Sonoda[4], Toshie Kaizuka[4], Makiko Itou[4], Hiroyuki Hirano[4], Jessie MacAlpine[1], Sean D Liston[1], Bernard Hube[5,6], Yoko Yashiroda[4], Nicole Robbins[1], Luke Whitesell[1], Hiroyuki Osada[4], Minoru Yoshida[4], Charles M Boone[1,4], Sergio Grinstein[1,2,7], Leah E Cowen[1] [1]University of Toronto, [2]The Hospital for Sick Children, [3]Western University, [4]RIKEN Center for Sustainable Resource Science, [5]Hans Knoell Institute, [6]Schiller University, [7]St. Michael's Hospital

The evasion of killing by immune cells is crucial for fungal survival in the host. For the human fungal pathogen *Candida albicans*, the morphogenetic transition from yeast to filament upon internalization by macrophages is a key intracellular survival strategy that occurs through mechanisms that remain unclear. Here, we employed functional genomic screening of conditional expression mutants covering >50% of the *C. albicans* genome to identify genes selectively required for filamentation inside macrophages. Through manual and machine learning-based image analyses, we uncovered a role for the mitochondrial ribosome, respiration, and the SNF1 AMP-activated kinase complex in governing filamentous growth within the phagosome, suggesting that *C. albicans* relies on respiration to evade the antifungal activities of macrophages. We demonstrated that downregulating the expression of these genes reduces ATP levels and impedes filamentation as well as growth under monoculture conditions in medium lacking glucose. In co-culture with physiological glucose concentration, downregulation of genes involved in mitochondrial function and respiration prevented *C. albicans* from expanding within the phagosome, escaping, and inducing immune cell death. Additionally, we screened ~50,000 compounds for their ability to inhibit *C. albicans* filamentation using a dual-strain screening strategy where a nourseothricin (NAT)-resistance marker was placed downstream of the filament-specific promoter *HWP1p* or downstream of the constitutive promoter *TEF1p*. This enabled us to identify compounds that specifically inhibit filamentation using optical density as a readout. Through this approach, we identified 259 putative inhibitors of *C. albicans* filamentation and prioritized 16 based on potent activity only against the *HWP1p*-NAT strain. We subsequently focused on three compounds with available chemical-genomic profiles and confirmed their ability to inhibit filamentation without substantially affecting growth. Moreover, we determined that these compounds inhibit filamentation across diverse filament-inducing cues and in some cases, inhibit filamentation induced by overexpression of transcription factors that positively regulate filamentation. Together, our work highlights respiration and the SNF1 AMP-activated kinase as key effectors of *C. albicans* metabolic flexibility and filamentation within phagocytes and identifies novel inhibitors of the yeast-to-filament transition.

26 **A deep learning strategy for biosynthetic gene cluster prediction in fungal genomes** Stephen F Harding, Robert Proctor, Hye-Seon Kim ARS, U.S. Dept of Agriculture

Fungi produce numerous secondary metabolites (SMs) that can function as plant hormones, pigments, or toxins, including mycotoxins (e.g., fumonisins and trichothecenes) that are of concern to food and feed safety. Genes directly involved in synthesis of the same SM are typically adjacent to one another in a biosynthetic gene cluster (BGC). Multiple programs have been developed to predict fungal BGCs from genome sequence data. However, some are constrained to detect specific BGC types (e.g., Ribosomal synthesized and post-translationally modified peptides) while others yield inaccurate predictions by over or underestimating the numbers of genes in the predicted BGC or fail to detect or identify known clusters. The application of machine learning (ML) based programs to fungal BGC discovery addressed these limitations and demonstrated increased BGC detection accuracy relative to fungiSMASH and DeepBGC; however, so far, published analyses with those platforms have been limited to the *Aspergillus* species *A. niger and A. nidulans*. Thus, ML based model performance with other fungi is unreported. Therefore, the application of ML methods for fungal BGC discovery is an emerging area of study. We developed a ML model for BGC discovery and classification by combining deep learning and other ML methods. Our model adapted the PFAM2Vec embedding method, bidirectional long-short term memory network, and ML classification approach implemented by DeepBGC. In addition, because the original DeepBGC's dictionary was constructed using bacterial genomes, we constructed a fungal PFAM dictionary using >4,000 fungal genomes. We also incorporated sequence similarity networks (SSNs) in our BGC classification methods to help infer the chemical structure and biological activity of metabolic products of the novel BGCs. Through our research, we aim to provide a ML-based fungal BGC discovery model that is readily applied to all fungal genera while also addressing limitations of other BGC mining programs.

27 **EffectorGeneP: accurate effector gene annotation in pathogen genomes from infection transcriptomes** Jana Sperschneider[1], Eva Henningsen[1], Taj Arndell[2], Megan Outram[1], Cheryl Blundell[1], Jian Chen[1], Thomas Vanhercke[1], Melania Figueroa[1], Peter Dodds[1] [1]CSIRO, [2]Queensland University of Technology

Gene annotation is crucial for accurate inference of biological knowledge from genomes. Automated gene annotation methods rely on decades-old methods biased towards model species and conserved genes, whilst manual curation is inefficient and can be error-prone. Incorporation of transcription evidence such as RNA-seq data has vastly improved accurate gene annotation.

However, non-canonical genes such as those lacking homologs, those residing in rapidly evolving genomic regions or single-exon genes are still routinely dismissed in annotation pipelines as transcriptional noise. In fungal pathogen genomes, this disproportionately affects the accurate annotation of genes encoding disease-causing effector proteins. We introduce EffectorGeneP, a machine learning tool that self-trains on infection RNAseq data to distinguish coding sequences of non-secreted proteins, secreted proteins and candidate effector proteins from other genomic regions. EffectorGeneP predicts the most likely coding sequence from transcripts and effectively addresses transcript fusions which frequently occur in compact fungal genomes, whilst separating *bona fide* genes from non-coding RNAs, incorrectly spliced transcripts and transcriptional noise. We show that EffectorGeneP annotates over 90% of known effectors correctly, while other RNAseq-based methods only annotate 17%-75%. We demonstrate the utility of EffectorGeneP as a tool for effector library design from infection transcriptomes and show that pooled effector library screening in plant protoplasts uncovers previously poorly annotated *Avr* genes in wheat stem rust. Combined with high-throughput effector validation, EffectorGeneP will be invaluable for uncovering effectors in other high-priority pathogens for which not a single effector has been found.

28 **Modernizing high-throughput mycology with robotics and artificial intelligence** Johan V Christiansen, Søren D Petersen, Vilhelm K Møller, David Llorente, Parvathy Krishnan, Steen S Brewer, Katharina Steinert, Alexander R Brems, Sabrina M Pittroff, Mathilde Nordgaard, Vincent Wiebach, Niels Bjerg, Lars Jelsbak, Ling Ding, Jakob B Hoof, Jens C Frisvad, Rasmus J N Frandsen Bioengineering, Technical University of Denmark

Filamentous fungi and their secondary metabolites have been proposed as solutions for many global crises, including as biocontrol agents of agricultural pests. However, high throughput functional screening of filamentous fungi is currently challenging due to the large morphological and physiological diversity, and as most mycological experimental methods have not been developed with a high throughput in mind. Therefore, our project has taken on the task of modernizing mycological methods to create a high throughput screening platform through robotic workflows, automatic data processing and machine learning. The goal is to be able to screen the extensive IBT fungal collection with 38,000+ isolates. To achieve this, we are transferring single-vial strain spore stocks to 96-well plates compatible with robotic workflows, such as cultivation on various defined and complex growth media, metabolite extraction, bioactivity assays and taxonomic profiling using genetic barcodes. We are systematically gathering high resolution mass spectrometry metabolomics data for all isolates to allow for artificial intelligence-based data mining focused on identifying industrial relevant metabolites and predicting which metabolites and species carry bioactivity. For unbiased, fast, and automatic metabolomics data processing and analysis, we have developed a pipeline using state of the art tools wrapped in a Python framework. Additionally, we automated image analysis is used to score the outcome of fungal-fungal interactions and capture basic growth characteristics of the strains. The automated analysis workflow has proven extremely important as our current robotics workflow supports the analysis of 800 strains every two-to-three weeks.

We are currently using this platform to screen IBT isolates for their ability to in vitro inhibit growth of the plant pathogens *Fusarium graminearum* and *Zymoseptoria tritici*. The metabolomics database and taxonomic genotyping data allows for deselection of fungi that produce mycotoxins or are classified as pathogens. The best in vitro performing isolates are continuously tested in planta (green house and field trials) by our commercial partner, FMC. The combined dataset aims to identify fungal isolates that can be used as control agents in agriculture.

29 **MycoAI: Artificial Intelligence for fungal identification** Duong Vu Westerdijk Fungal Biodiversity Institute

Studying microbial biodiversity is crucial to understanding the causes and consequences of environmental changes. The rapid development of sequencing technologies enables us to explore microbes in their natural environments using the metabarcoding approach without culturing them. Metabarcoding targets specific genetic markers, known as DNA barcodes, to provide taxonomic profiles of the microbes. Species identification plays an essential role in generating these taxonomic profiles. The traditional method for species identification is BLAST (Basic Local Alignment Search Tool). While BLAST has proven to be efficient, it encounters scalability issues when dealing with millions of sequences from environmental samples. As alternatives, machine learning-based classifiers such as the Ribosomal Database Project (RDP) Bayesian classifier have been proposed in the literature for rapid taxonomic identification.

Deep learning has emerged as a successful paradigm for big data classification. We aim to build an open-source application called MycoAI that employs deep learning techniques efficient with regard to input data, allowing for a quick determination of taxonomic profiles of microbes while maintaining high accuracy. In this talk, I will discuss the advantages and disadvantages of deep learning models for fungal identification, including convolutional neural networks (CNNs) and deep belief networks (DBNs), in comparison

with traditional methods such as BLAST and RDP. Additionally, I will present new results on employing the state-of-the-art BERT model for fungal identification.

30 **Transposon mobility in serial isolates of *Cryptococcus* from patients with recurrent cryptococcal meningitis** Anna Mackey, Vesper Fraunfelter, Callan Schroeder, John Perfect, Sue Jinks-Robertson, Asiya Gusa Duke University

Cryptococcus are environmental fungi that cause life-threatening diseases primarily in populations with weakened immune systems. Species of *Cryptococcus* can cause a fatal inflammation and swelling of the brain known as cryptococcal meningitis (CM), which is responsible for up to 20% of AIDS-related deaths. Inhaled into the lungs, *Cryptococcus* can persist for months to years in patients with recurrent CM, particularly when drug treatment fails. Understanding the types of genetic and genomic adaptations that occur during infection to cause drug resistance and persistent disease is critical in developing effective treatment strategies. Transposable elements (TEs) are endogenous mobile elements that can insert within or between genes to disrupt function or alter expression. We previously reported TE mobilization as a significant driver of mutations in *Cryptococcus deneoformans* during murine infection and in response to heat stress in vitro, contributing to an increased rate of antifungal drug resistance at 37°C (human body temperature). Subsequently, we demonstrated that TE mutations are the predominant source of genome-wide sequence variation under conditions of heat stress in *C. deneoformans*, compared to base substitutions or small insertions and deletions (Gusa et al, *PNAS* 2020 & 2023).

To investigate TE mobilization in *Cryptococcus neoformans*, the major disease-causing species, and its potential contribution to genetic changes during human infection, we obtained serial isolates of *C. neoformans* from patients with recurrent CM. Importantly, several virulence-related phenotypes observed in the relapse compared to incident isolates (e.g., increased virulence and drug resistance) could not be explained by the genotypic changes identified (Chen et al, *mBio*, 2017). In this study, we identified several characterized and uncharacterized TEs (DNA transposons and retrotransposons) as mobile in *C. neoformans* under conditions of heat stress and compared the rate of drug resistance at 30° vs 37° in clinical isolates with demonstrated TE mobility. We then prepared telomere-to-telomere genome assemblies of several paired incident and relapse isolates in efforts to identify the sum of genetic and genomic changes likely to have occurred during infection. Analyses are underway to 1) characterize the genetic differences between isolates, 2) link genotype to observed phenotypic changes, and 3) determine whether there is evidence of TE mobilization or TE-mediated rearrangements.

31 **Transposable elements as hidden players in fungal evolution** Ursula Oggenfuss[1], Anna M Selmecki[2] [1]Microbiology and Immunology, University of Minnesota, [2]University of Minnesota, Microbiology and Immunology

Transposable elements (TEs) play an important role in genome evolution. TEs drive genomic instability, gene expression and gene evolution, frequently leading to phenotypic changes. The impact of novel TE insertions is likely deleterious; thus, ongoing TE activity is limited by purifying selection and diverse defense mechanisms, including the fungal specific repeat-induced point mutations. However, in rare cases novel TE insertions are adaptive. We recently argued that some fungal species make a “Devil’s bargain”, where a moderate activity of TEs is tolerated to create novelty. In these cases, most TEs are located in TE-rich regions containing effectors but lacking any essential genes. Other fungal species, including the Saccharomycotina, contain only few TE families with each just a handful of copies. Consequently, Saccharomycotina species have very gene-dense genomes, and the impact of a novel TE insertion is very likely deleterious, indicating that strong purifying selection against TE activity. The loss of a TE copy can likely mean the loss of the whole family, and it would be expected that over time, all TE copies would be lost. However, no Saccharomycotina species with a complete lack of TEs has been described so far. We recently found that TE-derived domains are more likely to be domesticated in Saccharomycotina, compared to other fungal species. Despite this, full-length TE copies with the potential to be active are still detected. We observed small bursts of TEs in clinical isolates of *Candida albicans*, preferred location of TEs in the subtelomeric region in *C. auris*, as well as individual specific location of the 1-3 copies of the only TE family in *C. glabrata*. Using both short- and long read sequencing, we described pangenome-wide structural variants and the activity of TEs over all known clades of *C. albicans* and *C. auris*. The ongoing activity of these TEs and the effect of TEs on virulence and drug resistance will be discussed.

32 **Chromosome-level genome assemblies from *Fusarium graminearum* populations highlight the distribution of structural variants** Upasana Dhakal[1], Hye-Seon Kim[2], Christopher Toomajian[3] [1]Kansas State University, [2]Mycotoxin Prevention and Applied Microbiology Research Unit, USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, [3]Plant Pathology, Kansas State Univ

Structural rearrangements, such as inversions, translocations, duplications, and large insertions and deletions, are large-scale genomic variants that can change gene content and the copy number, product, and expression regulation of individual genes. Little

is known about the role of these structural rearrangements in shaping phenotypic variation and the evolution and adaptation of plant pathogenic fungi due to the limited number of high-contiguity assemblies. We used chromosomal-level assemblies from eight *Fusarium graminearum* isolates to determine the number, frequency, and predicted effect of structural variants and infer their role in fungal evolution. We generated the assemblies of four of these genomes after Oxford Nanopore sequencing of isolates representing the major *F. graminearum* populations from the U.S. These assemblies and their gene annotations can serve as additional reference genome resources for this species. For instance, when characterizing variants unique to a single isolate by mapping sequence reads to only a single reference genome, a poorly-matching reference can mean a substantial portion of unmapped reads, missing important variation. A total of 87 inversions, 159 translocations, 245 duplications, and tens of thousands of insertions and deletions were detected. Regions of high recombination rate are associated with structural rearrangements, and a significant proportion of inversions, translocations, and duplications overlap with the repeat content of the genome, suggesting recombination and repeat elements are major factors in the origin of structural rearrangements in *F. graminearum*. Large insertions and deletions introduce presence-absence polymorphisms for many genes, including secondary metabolite biosynthesis cluster genes and predicted effectors genes. Translocation events were found to be shuffling predicted effector-rich regions of the genomes and are likely contributing to the gain and loss of effectors facilitated by recombination. Breakpoints of some structural rearrangements fall within coding sequences and are likely altering the protein products. Structural rearrangements in *F. graminearum* thus have an important role to play in shaping pathogen-host interactions and pathogen evolution through genome reorganization, the introduction of presence-absence polymorphisms, and by altering the regulation and protein products of individual genes.

33 **Evolutionary playgrounds and how to find them** Jake Elton[1], Ester Gaya[2], Alexandra Dallaire[2,3] [1]Queen Mary University of London, [2]Royal Botanic Gardens Kew, [3]Dept of Biochemistry, University of Cambridge

Fast-evolving genomic regions of filamentous phytopathogens are often enriched in transposable elements (TEs) and *in planta*-induced genes that mediate infection (Dong *et al*., 2015). In at least five lineages of mutualistic fungi, TEs repeatedly associate with symbiosis-related gene innovations, to which they may contribute new *cis*-regulatory elements or epigenetic regulation (Hess *et al*., 2014; Dallaire *et al*., 2021; Looney *et al*., 2022; Wu *et al*., 2022; Plett *et al*., 2023). Evolutionary and functional compartmentalisation of genes appears not limited to species with pathogenic lifestyles, and the roles of TEs in generating variation in genome architecture and regulation are now under investigation across the Fungal Tree of Life. Association between TEs and gene families may point to ecologically relevant loci that encode the basis for lineage-specific adaptations. Using the Fungal Tree of Life as a model system, I investigate how associations between TEs and specific gene families may support fungal evolution. Here, I will present a statistical method to identify TE-associated gene families in fungal genomes, and will discuss how this information can be used to discover fast-evolving molecular functions and lifestyle-associated genes in fungi.

34 **Entanglement of transposable elements and virulence in rapid crop pathogen adaptation** Daniel Croll University of Neuchatel

Adaptation in plant pathogens proceeds at speeds that easily overwhelm the rate of resistant cultivar deployment and fungicide development. Hence, understanding the molecular basis of adaptation is critical to define more sustainable containment strategies. Key features of genomic variation in crop pathogens are transposable elements (TEs). Many pathogen species carry compartmentalized genomes with gene-rich and gene-poor regions where genes involved in virulence (i.e., effectors) are often located near TEs. TEs govern the regulation of effector genes in TE-rich compartments through epigenetic effects. This enables tight regulatory timing with the plant infection stages from early contact to the establishment of large lesion areas. De-repression of TE control during infection imposes though a challenge to maintain genome integrity as TEs may jump and insert into new locations in the genome. How pathogens may benefit or suffer from active TEs remains largely unexplored. I will address this question using large-scale genomic and transcriptomic datasets of *Zymoseptoria tritici,* a major fungal pathogen of wheat having spread to all continents over the past centuries. Using a reference-quality pangenome and large resequenced panels of strains across the world, I will recapitulate first the spread of the pathogenicity-associated *Styx* TE. The element likely originated in the *Zymoseptoria* genus and underwent multiple independent reactivation events. Importantly, we find that new copies of the element are not affected by genomic defenses revealing a recent loss of control against the element. Beyond the *Styx* element, the species experiences a broad pattern of TE reactivation concurrent with weakened genomic defences. The newly inserted TEs make a vast contribution to regulatory variation within populations and are overpresented at loci associated with variation in virulence. In conjunction, the pool of active TEs in the species underpins both a vast potential to adapt to the host and carries long-term risks to the integrity of the genome.

35 **Horizontal transfers between fungal *Fusarium* species contributed to successive outbreaks of coffee wilt disease** Lily D Peck[1], Timothy Barraclough[2] [1]Ecology and Evolutionary Biology, University of California Los Angeles, [2]University of Oxford

Outbreaks of fungal disease have devastated plants and animals throughout history. Over the past century, the repeated emergence of coffee wilt disease caused by the fungal pathogen *Fusarium xylarioides* severely impacted coffee production across sub-Saharan Africa. To improve disease management of such pathogens, it is crucial to understand their genetic structure and evolutionary potential.

We compared the genomes of 13 historic strains spanning six decades and multiple disease outbreaks to investigate population structure and host specialisation. We found *F. xylarioides* comprises at least four distinct lineages: one host-specific to *Coffea arabica*, one to *C. canephora* var. *robusta*, and two historic lineages isolated from various *Coffea* species. Mapping variation onto a new long-read reference genome showed that host-specificity appears to be acquired through horizontal transfer of effector genes from members of the *F. oxysporum* species complex. This species complex is known to cause wilt disease in over 100 plant species. Multiple transfers into the *F. xylarioides* populations matched to different parts of the *F. oxysporum* mobile pathogenicity chromosome and were enriched in effector genes and transposons. Effector genes in this region and other horizontally transferred carbohydrate-active enzymes important in the breakdown of plant cell walls were shown by transcriptomics to be highly expressed during infection of *C. arabica* by the fungal arabica strains. Widespread sharing of specific transposons between *F. xylarioides* and *F. oxysporum*, and the presence of large *Starship* elements, indicate that transposons were involved in horizontal transfers.

Our results support the hypothesis that horizontal gene transfers contributed to the repeated emergence of this fungal disease.

36 **Sanctuary I: A Starship transposon mediating the horizontal transfer of the necrotrophic effector ToxA** Hannah Wilson[1], Angus Bucknell[2], Ryan Gourlie[3], Peter Solomon[1], Reem Aboukhaddour[3], Megan McDonald[2] [1]Division of Plant Sciences, The Australian National University, [2]Division of Plant Sciences, University of Birmingham, [3]Agriculture and Agri-food Canada

Horizontal gene transfer (HGT) is a tool that many organisms use to rapidly adapt to novel hosts or environments. One well-known example of HGT is the movement of the necrotrophic effector *ToxA* between three fungal wheat pathogens, Parastagonospora nodorum, *Pyrenophora tritici-repentis* and *Bipolaris sorokiniana*. Defining the extent of horizontally transferred DNA is important because it can define the mechanisms that facilitate HGT. Our previous analysis of *ToxA* and its surrounding 14 kb showed that this region was a class II DNA transposon we named ToxhAT due to the hAT-like transposase gene near to *ToxA*. Importantly, there was some evidence that this transposon may remain active and mobile in *B. sorokiniana*. Long-read genome sequencing of eight ToxhAT carrying *B. sorokiniana* isolates confirmed that ToxhAT is an active transposon with a two base-pair "TA" target site duplication. This feature suggests that it should be re-classified as a member of the Tc1/Mariner transposon superfamily. In addition to confirming ToxhAT is an active transposon, these assemblies revealed that ToxhAT was a passenger within a giant transposon (~200kb). This transposon, *Sanctuary I*, has been classified as a giant Starship transposon a new transposon family found in fungi. In parallel, the region carrying ToxhAT in *Pyrenophora tritici-repentis* has also been shown to be a mobile Starship, named "*Horizon*". This indicates two independent captures of the smaller ToxhAT by these large transposons.

37 **Gene acquisition by giant transposons primes fungi for rapid evolution via horizontal gene transfer** Andrew S Urquhart[1], Aaron A Vogan[1], Emile Gluck-Thaler[2] [1]Systematic Biology, Uppsala University, [2]Dept of Plant Pathology, University of Wisconsin-Madison

The significance of Horizontal Gene Transfer (HGT) in fungi is not well established. However a growing number of examples of HGT are being reported in the literature. The critical missing information is the mechanism by which these HGT events occur. We now have found evidence that mobile elements are active agents of HGT in fungi. This evidence comes from a novel class of giant transposons called Starships, which we recently demonstrated to be mobile within a single genome. These elements are massive (up to 700kb) and thus are capable of carrying large amounts of host-beneficial DNA including entire gene clusters. This breaks the paradigm that transposons are typically small "selfish" DNA regions which do not carry host-beneficial gene cargo. To demonstrate that Starships are mobile not only within genomes but also between genomes, we examined a gene cluster, which contributes to formaldehyde resistance and is found in some Starships. Remarkably, we found four instances where this gene cluster has been independently acquired by distantly related Starships, and show that each acquisition event coincided with the element's horizontal transfer (at least 9 HGT events in total). Phenotyping of strains confirmed the effect of carrying the gene cluster in Paecilomyces variotii and Aspergillus fumigatus. Our results demonstrate that acquisition of host-beneficial cargo by Starships prime fungi for rapid and repeated adaptation via HGT, elevating the role of HGT in fungi.

38 **Small molecules restore azole activity against drug-tolerant and drug-resistant Candida isolates** Iuliana V Ene Mycology, Institut Pasteur

Each year, fungi cause more than 1.5 billion infections worldwide and have a devastating impact on human health, particularly in immunocompromised individuals or patients in intensive care units. The limited antifungal arsenal and emerging multidrug resistant species necessitate the development of new therapies. One strategy for combating drug resistant pathogens is the administration of molecules that restore fungal susceptibility to approved drugs. Accordingly, we carried out a screen to identify small molecules that could restore the susceptibility of pathogenic *Candida* species to azole antifungals. This screening effort led to the discovery of novel 1,4-benzodiazepines that restore fluconazole susceptibility in resistant isolates of *Candida albicans*, as evidenced by 100-1000-fold potentiation of fluconazole activity. This potentiation effect was also observed in azole-tolerant strains of *C. albicans*and in other pathogenic *Candida* species. The 1,4-benzodiazepines selectively potentiated different azoles, but not other approved antifungals. A remarkable feature of the potentiation was that the combination of the compounds with fluconazole was fungicidal, whereas fluconazole alone is fungistatic. Interestingly, the potentiators were not toxic to *C. albicans* in the absence of fluconazole, but inhibited virulence-associated filamentation of the fungus. We found that the combination of the potentiators and fluconazole significantly enhanced host survival in a *Galleriamellonella* model of systemic fungal infection. Taken together, these observations validate a strategy wherein small molecules can restore the activity of highly used anti-infectives that have lost potency.

39 **Most azole antifungal resistance mutations in the drug target provide cross-resistance and carry no fitness cost** Camille Bédard[1,2,3,4,5], Isabelle Gagnon-Arsenault[1,2,3,4,5], Jonathan Boisvert[2,3,4,5], Samuel Plante[1,2,3,4,5], Alexandre K Dubé[1,2,3,4,5], Alicia Pageau[1,2,3,4,5], Jehoshua Sharma[6], Laetitia Maroc[6], Rebecca S Shapiro[6], Christian R Landry[1,2,3,4,5] [1]Dept of Biochemistry, Microbiology and Bioinformatics, Université Laval, [2]Dept of biology, Université Laval, [3]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, [4]Le regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines (PROTEO), Université Laval, [5]Centre de Recherche sur les Données Massives (CRDM), Université Laval, [6]Dept of Molecular and Cellular Biology, University of Guelph

C. albicans is the most frequent cause of fungal infections in humans, and these infections are routinely treated with azole drugs, which inhibit the enzyme cytochrome P450 lanosterol 14-alpha-demethylase (Erg11/Cyp51). Amino acid substitutions in and around the drug binding site of this protein are a common resistance mechanism in pathogenic yeasts. However, despite the key importance of this species and this protein in clinical microbiology, our picture of the drug resistance mechanisms conferred by mutations in *ERG11* is incomplete. In fact, the number of resistance mutations inventoried in the literature is relatively small and, most importantly, only rarely has the link between a mutation and its resistance profile been experimentally validated. Therefore, our knowledge of resistance mutations is insufficient to confidently determine if an amino acid change in Erg11 will lead to resistance or not, and what would be the cost associated with these mutations.

Here, using a deep mutational scanning approach, we created a library of nearly 4,000 variants of *C. albicans* Erg11 ligand binding pocket (CaErg11). This library was used to characterize resistance mutation to six medical azoles. For each azole, an average of 1,000 variants were found to confer resistance, which led to nearly 6,000 experimentally validated drug-resistance associations. We found that a large fraction of amino acid substitutions lead to resistance (33%), most resistance mutations confer cross-resistance to two or more azoles (88%), and only a handful of resistance mutations show a significant fitness cost in the absence of drug (9%). These results reveal that selection for azole resistance can arise through a large set of mutations, and this will likely lead to azole pan-resistance, with minimal evolutionary compromise.

The creation of an extensive catalog of CaErg11 mutations leading to azole resistance will improve genotype interpretation and help treatment choice in clinical settings. In addition, the systematic characterization of CaErg11 variants allowed a better understanding of cross-resistance and trade-off, which we hope will impact regulations linked to azole usage and help develop new drugs in the future. Using this approach on orthologs and other fungal pathogen genes could help in our fight against fungal infections, and even give us a step ahead by identifying resistance mutations before their apparition in clinics and agriculture.

40 **Dynamics of copy-number variation in response to fluconazole are dependent on drug concentration and temperature** Saaz Sakrikar, David Gresham New York University

Treatment of fungal infections relies on a limited series of drugs of which triazoles (like fluconazole) are the most commonly used class. However, heritable resistance to these drugs has been documented in common fungal pathogens. A commonly observed mechanism of resistance is the amplification of the ERG11 gene, whose product is the target of fluconazole.

Here, we investigate the dynamics of ERG11 copy-number variants (CNVs) in *Saccharomyces cerevisiae* in the adaptation to fluconazole. We use a fluorescent reporter system developed in the lab to track the CNVs at a single-cell level during experimental evolution in different fluconazole concentrations and growth temperatures.

We found that ERG11 CNVs arise repeatedly in the lower tested concentrations (16 and 32ng/µL), but not at higher concentrations or in the absence of fluconazole. Further, temperature plays a key role in the dynamics of ERG11 CNVs, with higher temperatures favouring quicker emergence and high prevalence of these CNVs. Sequencing revealed that the evolved strains were found to be whole chromosome aneuploidies, rather than local amplifications. This study clarifies the role of aneuploidies in rapid adaptation to a widely used drug, and will be used as a basis for further work in pathogenic fungal species, as well as longer-term evolution to understand the stability of CNVs as an adaptive mechanism.

41 **Exposure to agricultural DHODH inhibitors result in cross-resistance to the novel antifungal olorofim in *A. fumigatus*** Norman van Rhijn[1], Michael Bottery[2], Isabelle Storer[2], Johanna Rhodes[3], Mike Bromley[2] [1]Manchester Fungal Infection Group, University of Manchester, [2]Manchester Fungal Infection Group, [3]Radboud UMC

Pesticides, including fungicides, are extensively used in agricultural practice to protect plants from unwanted growth of weeds, plant pathogens and other pests. Dual use of antifungals in the environment and in the clinic, with similar mode of actions, has been shown to drive the development of resistance. Although not a plant pathogen, *A. fumigatus*is ubiquitous in the environment and therefore exposed to agricultural fungicides. Extensive use of triazoles in the environment has led to high rates of resistance found in clinical *A. fumigatus* isolates. The development of novel antifungals is paramount to be able to treat azole-resistant aspergillosis. Olorofim is a novel antifungal for clinical use, targeting the essential protein DHODH, for which resistance is rare. Recently, several agricultural DHODH inhibitors, including ipflufenoquin, quinofumelin and tetflupyrolimet, have gone through the approval process. We show that these DHODH inhibitors are active against *A. fumigatus*, and have the same mode of action as olorofim. Spontaneous mutation analysis revealed we can select for ipflufenoquin resistant *A. fumigatus* isolates. These ipflufenoquin resistant mutants show cross-resistance to olorofim. Furthermore, other agricultural DHODH inhibitors recently approved as herbicide have the potential to result in cross-resistance to olorofim. Lastly, we show that *A. fumigatus* isolates which are multi-drug resistant to a range of agricultural fungicides and clinically used antifungals are more fit under exposure to sub-inhibitory concentrations of ipflufenoquin and olorofim. Our results highlight the potential dangers of using DHODH inhibitors in agriculture and the future threat of resistance development to novel antifungals by selection in the environment.

42 **Induction of *Aspergillus fumigatus* zinc cluster transcription factor OdrA/Mdu2 provides combined cellular responses for oxidative stress protection and multiple antifungal drug resistance** Christoph Sasse[1], Emmanouil Bastakis[2], Fruzsina Bakti[2], Annalena M. Höfer[2], Isabella Zangl[3], Christoph Schüller[3], Anna M. Köhler[2], Jennifer Gerke[2], Sven Krappmann[4], Florian Finkernagel[5], Rebekka Harting[2], Joseph Strauss[3], Kai Heimel[2], Gerhard H. Braus[2] [1]Institute of Microbiology and Genetics, Dept of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), University of Göttingen, [2]University of Göttingen, [3]University of Natural Resources and Life Sciences, Vienna (BOKU), [4]University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nürnberg, [5]Philipps University, Marburg

The filamentous fungus *Aspergillus fumigatus* represents a prevalent opportunistic human pathogen, which can develop resistance mechanisms against antifungal drugs. A genomic wide overexpression screen of 228 zinc cluster transcription factor encoding *zcf* genes of *A. fumigatus* revealed 11 genes conferring increased resistance to to the broadly applied azole voriconazole, to the polyene amphotericin B or to both. These include four *odrA-D* genes encoding oxidative stress and drug resistance factors, which provide even broader cellular stress protection. Thereby, the corresponding fungal OdrA/Mdu2 and AtrR/OdrD-dependent genetic networks are interconnected. OdrA/Mdu2 activates *atrR/odrD* transcription by direct binding to the promoter, whereas AtrR/OdrD functions as repressor of *odrA/mdu2* expression. *odrA/mdu2* overexpression provides combined resistance to amphotericin B, voriconazole, itraconazole and to reactive oxygen species generated by menadione. OdrA/Mdu2 mediated itraconazole resistance is evoked by direct regulation of the transporter encoding gene *mdr1*. Oxidative stress-inducing substances like amphotericin B and menadione promote OdrA/Mdu2 accumulation in the nucleus to regulate stress response genes like *mdr1* and the putative glutathione-S-transferase encoding gene *gstD*. The expression levels and external stress conditions fostering nuclear accumulation of OdrA/Mdu2 determine the regulation of the target genes. Hence, OdrA/Mdu2 provides a combined adaptation strategy for survival in nature or within a potential host, where this fungus represents the most common agent for human mold pneumonia worldwide. The OdrA/Mdu2 controlled genetic network highlights the tight connection between oxidative stress response and antifungal drug adaption to secure *A. fumigatus* survival in various hostile environments.

43 **Fungicides alternation and mixture lead to *in vitro* selection of generalist resistance mechanisms (MDR) in *Zymoseptoria tritici*** Elza Neau[1], Anaïs Lalève[2], Sabine Fillinger[2] [1]Université Paris Saclay, [2]Université Paris-Saclay, INRAE, UR BIOGER

The agricultural sector faces the increasing emergence and development of fungicide resistance in *Zymoseptoria tritici*, the agent of septoria leaf blotch in wheat. Since the early 2010s, strains with 'Multi-Drug Resistance' (MDR) phenotype have been detected in natural populations. This phenotype has been associated with the overexpression of the membrane efflux pump gene *MFS1*, resulting in increased efflux of fungicides. In this context, understanding the evolution and spread of resistances in this fungus is crucial to protect the effectiveness of treatments.

Recent experimental evolution studies, aiming to investigate the selection of resistance mechanisms under various fungicide application conditions, have led to the selection of resistant strains. Surprisingly, some of them display MDR phenotype, which does not seem to be linked to known *MFS1* expression regulation. This suggests that previously undescribed MDR mechanisms may have been selected through these experimental evolutions.

To elucidate whether increased efflux is involved in these phenotypes, two distinct efflux tests were carried out on a selection of 53 MDR isolates. The first test assessed the efflux of a fluorescent molecule (Nile Red), while the second test investigated the interaction between terbinafine (fungicide) and transport modulators (inhibitors of ABC and MFS transporters) on fungal growth. The majority of the studied isolates exhibited an increase in Nile Red efflux in comparison to the ancestor sensitive strain and/or clear synergy between at least one transport modulator and terbinafine on growth inhibition. This suggests that their MDR phenotype may be attributed to increased fungicide efflux. Other isolates showed different phenotypes, indicating the involvement of alternative MDR mechanisms, not necessarily through increased efflux.

Additionally, whole-genome sequencing data are currently being analysed and may provide further insights into the molecular mechanisms underlying MDR.

44 **Constraint on boric acid resistance and tolerance evolvability in Candida albicans** Aleeza Gerstein[1], Yana Syvolos[2], Ola Salama[2] [1]Microbiology, University of Manitoba, [2]University of Manitoba

Boric acid is a broad-spectrum antimicrobial used to treat vulvovaginal candidiasis when patients relapse on the primary azole drug fluconazole. *Candida albicans* is the most common cause of vulvovaginal candidiasis, colloquially referred to as a "vaginal yeast infection". Little is known about the propensity of *C. albicans* to develop boric acid resistance or tolerance (the ability of a subpopulation to grow slowly in high levels of drug). We evolved 96 replicates from eight genotypically diverse *C. albicans* strains to increasing boric acid concentrations to examine whether they would evolve boric acid resistance and/or tolerance. We found that many replicates went extinct quickly. Although some replicates were able to grow in much higher levels of boric acid than the ancestral strains, evolved populations isolated from the highest terminal boric acid levels surprisingly showed only modest growth improvements and only at low levels of boric acid. No large increases in resistance or tolerance were observed in the evolved replicates. These results will be directly contrasted to experimental evolution experiments in fluconazole, where we and others observe the propensity to evolve resistance and tolerance depends on both the drug concentration and the dosing regimen. Overall, our findings illustrate the evolutionary constraints limiting the emergence of boric acid resistance and tolerance, which could explain why it remains an extremely effective treatment for recurrent yeast infections.

45 **Identification of protein kinases that govern the susceptibility of *C. albicans* to antifungal drugs** Laura J Ristow, Juraj J Kramara, Tomye J Ollinger, Damian J Krysan Pediatrics, University of Iowa

The ability of a fungal pathogen to develop clinically significant resistance to an antifungal drug is dependent on two factors: 1) the acquisition of a specific mutation that dramatically decreases the susceptibility of the pathogen to the drug and 2) the sufficiently robust function of stress responses or other physiologic processes triggered by inhibition of the drug target. Inhibition of the drug-induced stress response pathways represents a potential approach to overcoming drug resistant mutations through combination therapy of the legacy antifungal drug and a drug that inhibits the specific stress response pathways. Protein kinases are one of the most common new drug targets in modern medicine. Here, we describe a new library of protein kinase deletion mutants generated in the widely used SN *C. albicans* genetic background. We have used this library in a systematic screen of protein kinases for those that affect the susceptibility of *C. albicans* to clinically used antifungal drugs including manogepix, a mechanistically novel drug in late-stage clinical development. These data were generated using flow cytometry-based competition experiments that allow high throughput quantitative characterization of large numbers of mutants. The mechanistic and drug discovery implications of this work will be discussed as well.

46 **The Spitzenkorper: engine and guide of hyphal growth** Salomon Bartnicki-Garcia Microbiology, CICESE

In 1959, M. Girbardt demonstrated that the Spitzenkorper (SPK) discovered by H. Brunswik, exactly 100 years ago, was a real structure with an essential function in hyphal growth. Later, electron microscopy revealed that the SPK consisted of accumulations of vesicles of two different sizes, small ones in the center and large ones at the periphery. By confocal microscopy and genetic engineering, their biochemical functions were disclosed: microvesicles carry chitin synthase (i.e. they are the chitosomes discovered in 1974), while macrovesicles transport glucan synthase. It remains to be discovered why this duality is necessary. A cybernetic-mathematical analysis revealed that the SPK functions as a vesicle supply center (VSC) whose advancement produces a continuous discharge of vesicles. This exocytic process generates: 1) a gradient of apical wall construction and 2) gives the hyphal tips their peculiar shape (hyphoid). There is emerging evidence that SPK vesicles are transported from the SPK to the cytoplasmic membrane by an actin skeleton. There is also evidence that formin triggers the assembly of the actin skeleton in the VSC. By manipulation with laser tweezers, C. Bracker and R. Lopez-Franco discovered that SPK position determines the direction of growth of hyphae. One of the most critical points of SPK behavior remains to be demonstrated, namely the mechanism that advances it forward, either by being pushed forward by the cytoskeleton or being dragged by tethering to the advancing cell wall.

47 **Hyphal characteristics among the fungi** Robby Roberson School of Life Sciences, Arizona State University

The highly polarized mechanism of hyphal growth requires a robust secretory system for the delivery of materials (e.g., membrane, proteins, cell wall components) to sites of cell growth. This results in the apical assembly of a Spitzenkörper (Spk) in members of the Basidiomycota, Ascomycota, and Blastocladiomycota, or an apical vesicle crescent (AVC) in most Mucoromycota and Zoopagomycota. The Spk is a complex apical body composed of secretory vesicles, cytoskeletal elements, and signaling proteins. The AVC appears less complex than the Spk, though little is known of its composition other than secretory vesicles. Both bodies influence hyphal growth and morphogenesis. Bioimaging investigations of subcellular structures and cytoplasmic behaviors are presented and provide a better understanding hyphal biology and phylogenetic relationships of fungi.

48 **The striatin-interacting protein phosphatase and kinase complex (STRIPAK complex) in Ustilago maydis** Julia Dennig[1], Lea Morbe[1], Kerstin Schmitt[2], Oliver Valerius[2], Gerhard Braus[2], Joerg Kaemper[1] [1]Genetics, Karlsruhe Institute of Technology, [2]Georg-August-University Göttingen

The striatin-interacting phosphatases and kinases (STRIPAK) complex is evolutionary highly conserved in eukaryotes. It functions as a node, by physical interaction with conserved signaling complexes to establish larger networks. In fungi, STRIPAK affects sexual development, growth and cell fusion. In fungi, investigation of STRIPAK has been focused on Ascomycetes as *S. macrospora*, *N. crassa* or *S. cerevisiae*. Here, we present characterization of STRIPAK components in the dimorphic basidiomycete *Ustilago maydis*, a pathogen of maize plants.

The main components of STRIPAK are kinases, tail-anchored proteins, developmental proteins and the phosphatase consisting of regulatory, catalytic and scaffolding subunits. The regulatory subunit, striatin, mediates interaction of the tail-anchored protein and the developmental protein, resembling the inner framework of the complex. The tail-anchored protein ensures localization at the membrane of mitochondria or nuclei, while the developmental protein is linked to internal membrane systems.

In contrast to Ascomycetes, both striatin (Far8, Umag03784) as well as the developmental protein (Far11, Umag10285) are essential in *U. maydis*, which might indicate supplementary pathways orchestrated by STRIPAK.

Deletion of the anchor protein (Far10, Umag04391) in *U. maydis* mirrors phenotypes observed in Ascomycetes, as cytokinesis defects, defects in hyphal fusion, premature release of pheromone-induced cell cycle arrest, altered cell wall and membrane integrity. *Δfar10*-strains are apathogenic as fungal cells are neither able to attach to the plant surface nor capable to form infection structures. Concomitantly with the observation in other systems, a complex of Far8, Far10 and Far11 localizes to the ER and mitochondria in sporidia (haploid cells that propagate by budding). However, colocalization of Far8 and Far10 or of Far11 with either Far8 or Far10 was lost after the switch from budding growth to filamentous growth or during filamentous growth. Only Far8 remains at mitochondria in filaments. The colocalization of each Far8, Far10 and Far11 with the nuclear envelope disappear during the dimorphic shift, pending on the migration of the nucleus from the initial sporidia to the emerging hypha. Our results indicate a restructuring of the STRIPAK complex during the dimorphic shift of *U. maydis* that precedes plant infection.

49 **Is there localized mRNA translation at the hyphal tip?** Domenico Modaffari, Edward W J Wallace, Kenneth E Sawin University of Edinburgh

Hyphal growth is driven by vesicle fusion at the cell tip. In many fungal species, a vesicle organizing center called the Spitzenkörper (SPK) forms at the cell tip. Electron micrographs show ribosomes at the base of the SPK. However, the molecular components of the SPK remain largely uncharacterized. The possible regulation of local protein translation at the SPK and hyphal cell tip has not been yet investigated.

Using the model mold *Aspergillus nidulans*, we show that the RNA-binding protein SsdA travels towards the hyphal tip on microtubules. SsdA is the ortholog of *S. cerevisiae* translational-repressor protein Ssd1 and *N. crassa* GUL-1. Ssd1 recognizes a conserved RNA motif. The motif is enriched on genes encoding cell wall proteins which localize at the hyphal tip. Overall, SsdA could be part of a greater system that regulates local protein production at the hyphal tip.

We also report a straightforward CRISPR-Cas9 system for scarless genetic engineering of *Aspergillus nidulans* and *Aspergillus* codon-optimized latest-generation fluorescent protein tags.

50 **Probing the *Candida albicans* Spitzenkörper** Charles Puerner, Patricia Silva, Allon Weiner, Miguel Basante-Bedoya, Antonio Serrano, Priya Jaitly, Robert Arkowitz, Martine Bassilana University Cote d'Azur/CNRS/INSERM

Apical growth is critical in a range of fungal pathogens for tissue invasion and host cell evasion and, in fungal hyphae, a vesicle cluster referred to as a Spitzenkörper is observed at the growing apex [**1**], including in the human fungal pathogen *Candida albicans* [**2, 3**]. To study the dynamics and organization of the *C. albicans* secretory pathway, we used live-cell imaging and three-dimensional electron microscopy. Furthermore, we probed the function of the Spitzenkörper using genetics and synthetic physical interactions, as well as investigated the dynamics and composition of this vesicle cluster using optogenetic manipulation of cell polarity to reset growth in filamentous cells. From these studies, we were also able to identify critical components of the Spitzenkörper, and delineate their function using a synthetic physical interaction approach to restrict/stabilize these components at the Spitzenkörper, including the lipid flippase Drs2 [**4**]. Together, our results indicate that the *C. albicans* secretory pathway is organised in distinct domains, with a Spitzenkörper comprised of uniformly sized secretory vesicles [**5**]. Quantitative analyses also revealed a strong correlation between filament diameter and extension rate, a central prediction of the vesicle supply center model [**6-8**], and suggest that the Spitzenkörper is important for growth robustness [**9**]. Intriguingly, optogenetic perturbation of polarized filamentous growth resulted in a striking *de novo* cluster of secretory vesicles, similar to the Spitzenkörper, that was highly dynamic, indicating that secretory vesicle clustering can occur in the absence of directional growth [**10**]. We are currently investigating the interplay between perturbation of cell polarity and the actin cytoskeleton.

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51 **Lipid rafts in *Schizophyllum commune* – insights in localization and composition** Berit Frizzy Porsche, Robert Jesse, Katrin Krause, Erika Kothe Friedrich Schiller University

Schizophyllum commune, a filamentous basidiomycete fungus, is noted for unique growth patterns and ecological significance. This white rot fungus breaks down organic matter, contributing to nutrient cycling in diverse ecosystems, and is known to interact with other organisms of the community. Polarized hyphal growth, a key aspect, requires the supply of proteins and lipids to the hyphal tip by vesicle trafficking. Cellular membranes feature the interplay of lipids and proteins and are visualized as a dynamic fluid lipid structure by the fluid mosaic model of Singer and Nicolson. Cell membranes consist of different membrane microdomains as lipid raft domains, enriched in sterols, sphingolipids, and specific proteins, serving as platforms for signal transduction, membrane trafficking, protein sorting, and polarized growth. Crucial in establishing and maintaining hyphal polarity are the actin cytoskeleton and raft domains. The aim of this work is to investigate the composition of raft domains as well as the localization and role of rafts in *S. commune* using two raft-associated proteins, stomatin and striatin. They were reported to play a role in cell signalling and signal transduction, as well as in numerous protein-protein interactions and the assembly of proteins in distinct signalling complexes in mammals, but not much is known for fungi. Sterol-enriched raft domains were visualized by Filipin staining at hyphal tips and septation sites. Further, stomatin was labelled with the fluorescence protein dTomato and visualization by laser

scanning microscopy showed a shift from young branch tips to the apex over time. In the dikaryotic stage, stomatin co-localizes with eGFP-labeled actin. Stomatin deletion in *S. commune* and RNA sequencing were performed and demonstrate its impact on morphology and genetics, upregulating MAPKs, GPCRs, and cytoskeleton-related genes. This study highlights raft-associated proteins' crucial role in hyphal growth and cell signaling in *S. commune*, offering potential for therapeutic development through raft-mediated strategies.

52 **Investigation of Differing Roles of Ammonium Transporters in the Nematode-trapping Fungus *Arthrobotrys oligospora*** Sheng-Chian Juan[1,2], Yen-Ping Hsueh[1,2] [1]Institute of Molecular Biology, Academia Sinica, [2]Molecular and Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Science, National Defense Medical Center

To adapt to environmental changes, cells require the ability to sense external signals. *Arthrobotrys oligospora*, a fungus belonging to a non-monophyletic group known as nematode-trapping fungus (NTF), senses prey signals and initiates trap morphogenesis under starvation. While a previous study has shown that ammonium suppresses trap formation in *A. oligospora* that the underlying mechanism is still unknown. Ammonium, a major nitrogen source that promotes growth for many microbes, is transported through the Ammonium transporter (Amt)/Methylammonium permease (Mep)/Rhesus protein (Rh) family. Therefore, to investigate how ammonium transport affects trap formation in *A. oligospora*, we first identified three potential ammonium transporters in *A. oligospora*, *MEP1, MEP2*, and *MEP3*. Phylogenetic analysis revealed that *MEP2* and *MEP3* are both high-affinity and low-capacity transporters, whereas *MEP1* is a low-affinity and high-capacity transporter. *MEP* gene expression is elevated in response to nematode exposure and subsequently downregulated upon prey capture, indicating their possible roles in the initiation of trap formation. Under low-nutrient conditions, single deletion mutants of *mep1* and *mep3* show decreased trap numbers, while traps numbers in *mep2* remain similar to wildtype. Double *mep* deletion mutants without enhanced phenotypes and the elevated gene expression of the remaining *MEP*(s) suggest functional redundancy among the three ammonium transporters. In contrast, upon addition of ammonium, *mep1* mutants were the only *mep* mutants to exhibit normal growth and suppression of trap formation, suggesting that Mep1 is the primary transporter involved in trap formation under ammonium treatment. Together, these results suggest that the Mep transporters are involved in trap formation initiation in *A. oligospora*. As nematode-trapping fungi (NTF) like *A. oligospora* have been proposed to be a biocontrol agent, an enhanced understanding of ammonium transport in this fungus is essential as agricultural environments are normally ammonium-rich.

53 **Re-routing of MAP kinase signaling for pentration peg formation in predator yeast** Mareike L Rij[1], Yeseren Kayacan[2], Florian Michling[3], Beatrice Bernardi[3], Juergen W Wendland[4] [1]Dept of Microbiology and Biochemistry, Hochschule Geisenheim University, [2]Vrije Universiteit Brussel, [3]Hochschule Geisenheim University, [4]Microbiology and Biochemistry, Hochschule Geisenheim University

Predator yeasts are either homothallic or heterothallic ascomycetes of the genus *Saccharomycopsis*. These yeasts represent a unique genus of necrotrophic mycoparasites that predate a wide range of yeasts and filamentous fungi. Their mycoparasitism can be divided into several phases including recognition of and adhesion to prey cells, penetration of prey cells, their killing and nutrient uptake. For the penetration of a prey cell a dedicated structure called penetration peg is formed. Penetration pegs grow in a polarized manner into the prey cells and these pegs show strong fluorescence with dyes recognizing carbohydrate moieties suggesting active protein secretion. In contrast to penetration hyphae of plant pathogenic fungi, predator yeast penetration pegs do not grow out of their prey cells, do not contain nuclei and thus do not become daughter cells. Each penetration peg is, therefore, a one-time investment for an attack of a single cell. Homologs of the mating and filamentation MAP kinase cascade of *Saccharomyces cerevisiae* have been shown to regulate appressorium formation e.g. in *Magnaporthe oryzae*. Similarly, deletion of the single *KSS1*/*FUS3* map kinase homolog *KIL1* in *Saccharomycopsis schoenii* generated non-predacious strains. *S. schoenii kil1* mutant cells were unable to form penetration pegs under nutrient-limiting and predation-promoting conditions. A downstream target of the *S. cerevisiae* Kss1/Fus3 MAP kinases is the transcription factor Ste12. We show that *S. schoenii ste12* mutants showed comparable phenotypes as *kil1* mutants indicating that Ste12 is a major target for Kil1-signaling. Comparative RNAseq transcriptomics identified genes involved in either hunger or predation. Particularly, amongst the predation response genes a shared promoter motif was identified with resemblance to the *S. cerevisiae* Ste12 DNA-binding site. Our data suggest a re-routing of MAP-kinase signaling in predator yeasts to regulate penetration peg formation in a similar way to what has been observed for *Magnaporthe,* even though these species are separated by >400 million years of evolution.

54 **In-host profiling of transcription factor activity yields insights into fungal colonization of the gut** Suzanne M. Noble Microbiology & Immunology, UCSF School of Medicine

Candida albicans is a fungal component of human gut microbiota, as well as an opportunistic pathogen. In forward screens of *C. albicans* mutants in a mouse model of fungal gut colonization, we identified numerous *C. albicans* transcription factors (TF) with strong effects on commensal fitness. To capture transcription factor activity during host colonization, we performed RNA-seq and Calling Card-seq (a transposon-based technique that marks DNA binding events in living cells) on yeasts propagated in the animal model. The results revealed extensive cross-regulation among key TFs. We next used a "regulation-function coupling" approach to identify highly regulated genes whose expression correlates with relative fitness in the gut. This analysis revealed two fungal effectors of gut colonization, a secreted chitinase (Cht2) and a secreted aspartyl protease (Sap6). In ongoing work, we are evaluating host transcriptional responses to first-time gut colonization with *C. albicans* to elicit clues about effector activity in the host.

55 **On the mechanism of RNAi-mediated silencing of repetitive DNA in *Cryptococcus neoformans*** Sheng Sun, Vikram Ponnusamy, Vikas Yadav, Francis Fang, Anna Floyd Averette, Joseph Heitman Molecular Genetics and Microbiology, Duke University Medical Center

RNA-induced silencing is ubiquitous throughout eukaryotes, and serves central functions including regulation of gene expression, repression of transposable elements, and maintenance of genome stability. In *Neurospora crassa*, genes involved in DNA repair such as *RAD52* are known to be involved in transgene silencing. Additionally, studies have shown that formation of R-loops/RNA:DNA hybrids can also result in gene silencing. In the human fungal pathogen *Cryptococcus neoformans*, transgene tandem repeats are silenced by two RNAi silencing pathways: sex-induced silencing (SIS) and mitotic-induced silencing (MIS). These two pathways share common components but also show distinct characteristics. How transgene repeats are recognized to trigger silencing is unknown. In this study, we analyzed genes that are involved in DNA-repair (*RAD51*, *RAD52*, and *RAD54*), as well as genes required for R-loop resolution (*RNH1* and *RNH2*), for possible roles in silencing of a *URA5* transgene array in *C. neoformans*. Mutation of the *RAD51*, *RAD52*, and *RAD54* genes conferred hyper-sensitivity to DNA damaging agents and abolished sporulation during bi-lateral crosses, consistent with roles in homologous recombination mediated DNA repair. Notably, none of the three *RAD* genes was required for MIS transgene silencing. On the other hand, deletion of the *RNH1* and *RNH2* genes significantly increased MIS during vegetative growth, suggesting formation of RNA:DNA hybrids may promote transgene array silencing. Furthermore, we discovered significantly increased retention of the two introns of the *URA5* gene in strains in which the transgene array was silenced. Consistent with this, deletion of the *GWC1* gene, which is a component of the SCANR complex that recognizes stalled spliceosomes at unspliced introns, led to complete abolishment of MIS. Taken together, our studies provide evidence that transgene silencing does not involve a *RAD* gene mediated DNA homology search but instead involves: 1) intron retention and activation of the SCANR complex, and 2) RNA-DNA interaction, possibly via RNA from one gene repeat involving a neighboring DNA repeat to form an R-loop.

56 **The role of extracellular vesicles in cross-kingdom RNA trafficking** Baoye He[1], Angela Chen[1], Shumei Wang[1], Qiang Cai[2], Hailing Jin[1] [1]University of California, Riverside, [2]Wuhan University

Cross-kingdom RNA trafficking between plants and fungal pathogens is a crucial process that regulates gene expression in both organisms during infection. We demonstrate that extracellular vesicles (EVs) serve as a means for both plants and fungal pathogens to deliver RNA to their respective partners. We performed RNA seq analysis and revealed that both small RNAs (sRNA) and message RNAs (mRNA) were found in plant EVs, which can be transferred to the fungal pathogen Botrytis cinerea. The transferred plant sRNA can silence fungal virulence-related gene expression. The transferred plant mRNAs can be translated into proteins to modulate fungal mitochondrial morphology and functions to suppress fungal infection. B. cinerea EVs can also deliver fungal sRNAs into plant cells that suppress plant immune-related gene expression and enhance infection. B. cinerea EVs can be internalized by plants via clathrin-mediated endocytosis (CME). Both plant and fungal EVs are isolated based on the minimal information guidelines for studies of extracellular vesicles 2018 (MISEV2018).

57 ***Trichoderma atroviride* small RNA1 targets the Arabidopsis *PRIM2* gene to establish a mutulaistic relationship** Sergio Casas-Flores, Eyra Judith Hernández-Hernández, Mitzuko Dautt-Castro Molecular Biology, Institute for Scientific and Technological Research of San Luis Potosi

In their natural settings, plants interact with both, pathogens and beneficial microorganisms. To defend from pathogens, plants have evolved several layers of defense, including basal chemical defenses, structural barriers and innate immunity. Once pathogens

surpass the first defense barriers, plants trigger sophisticated mechanisms to neutralize pathogen attack, which initiates with the detection of pathogen-associated molecular patterns (PAMP) to activate PAMP-triggered immunity (PTI), that limits the pathogen spreading from the original site of infection. However, some pathogens have developed effector molecules to suppress PTI. It has been shown that small RNAs (sRNAs) produced by pathogens can act as effectors molecules to suppress plant immunity. *Trichoderma* spp. are plant beneficial fungi that colonize plant roots, conferring beneficial effects to plants by promoting their growth and inducing the systemic disease resistance. Here, we show that the *Arabidopsis* DNA primase large subunit encoding gene, *PRIM2* is targeted by the *T. atroviride* small sRNA1 (*Ta*_sRNA1). *Ta*_sRNA1 is accumulated in the presence of Arabidopsis, which anticorrelates with the downregulation of *PRIM2*. Arabidopsis overexpressing lines of *Ta*_sRNA1 (*At*_OE_sRNA1) showed differential accumulation levels of *Ta*_sRNA1, which agree with low levels of *PRIM2*. Furthermore, co-expression assays of *Ta*_sRNA1 and *PRIM2* wild-type (wt) gene in tobacco, showed decreased accumulation of *PRIM2* transcript, whereas a *PRIM2* version bearing synonymous mutations on the *Ta*_sRNA1 target site was resistant to mRNA slicing. Arabidopsis transgenic lines bearing a short tandem target mimic (STTM) to interfere with the activity of *Ta*_sRNA1 showed increased accumulation of *PRIM2* during its interaction with *T. atroviride*. In addition, both *prim2* mutants and *At*_OE_sRNA1 plants presented enhanced resistance to the fungal pathogen *Botrytis cinerea*. Intriguingly, *At*_OE_sRNA1 and Col-0 (wt) plants inoculated with a *T. atroviride* strain overexpressing the *Ta*_sRNA1, manifested enhanced susceptibility to *B. cinerea*. Together, our results indicate a role of *Ta*_sRNA1 in stablishing of mutualistic relationship of *T. atroviride* with plants

58 **Investigating the role of chromatin dynamics in *Histoplasma* morphogenesis** Nebat Ali, Mark Voorhies, Anita Sil UCSF
The ability to sense and adapt to the environment is a hallmark of clinically relevant microbial pathogens. This phenomenon is exemplified in thermally dimorphic fungi such as *Histoplasma,* where temperature is a critical signal that triggers a dramatic shift between cell states. At ambient temperatures, *Histoplasma* grows as filamentous hyphae that can be aerosolized and inhaled into the lungs of mammals, where elevated temperature (37°C) is sufficient to induce the switch to growth as a pathogenic budding yeast. Prior studies aimed at understanding the regulation of this switch identified a key network of transcription factors (TFs) Required for yeast phase growth (Ryp 1,2,3,4) that globally reprogram the transcriptome to establish yeast cells at 37°C. Given that chromatin state can influence TF activity and DNA-binding, we sought to investigate if chromatin dynamics underlie the global gene expression changes required to establish cell state. Studies in related fungi that undergo morphology changes have identified histone modifying enzymes that are critical for proper initiation and maintenance of morphogenesis. In *C.albicans,* histone deacetylases (HDACs) work in conjunction with TFs to direct the regulatory landscape of the cell throughout transitions. To broadly query a putative role for HDACs in *Histoplasma,* we sought to test the morphological effect of chemical compounds that act as HDAC inhibitors (HDACi). We selected a panel of HDACi to comprehensively target all classes of HDAC orthologs conserved in *Histoplasma.* Interestingly, treatment of *Histoplasma* yeast cells with a Class-I HDACi disrupts yeast-phase growth and induces improper hyphal growth at 37°C. Transcriptome analyses of Class-I HDACi-treated cells reveals large global changes in gene expression and disruption of the canonical Ryp regulon at 37°C. Furthermore, HDACi treatment triggers inappropriate accumulation of hypha-specific transcripts at 37°C, suggesting a potential role for Class I HDACs in temperature-dependent transcription. Phylogenetic analysis reveals Histoplasma encodes two highly conserved Class I HDACs: Rpd3 and Hos2. Genetic studies leveraging CRISPR/Cas9 gene editing are currently underway to investigate the function of Rpd3 and Hos2. Preliminarily, strains undergoing disruption of Rpd3 display aberrant morphology. These studies will uncover how chromatin remodeling factors contribute to regulating this critical developmental switch in thermally dimorphic fungi.

59 ***Cryptococcus neoformans* Adaptation to the Host is Regulated by the RAM Pathway** Emma E Blackburn[1], Benjamin Chadwick[2], Xiaorong Lin[2] [1]Microbiology, University of Georgia, [2]University of Georgia
Cryptococcus neoformans is an opportunistic fungal pathogen, responsible for cryptococcal meningitis. Despite existing antifungal therapies, this disease kills over 180,000 people annually. There are no vaccines available, making this fungal pathogen deadly to immunocompromised individuals. Adaptation to host physiological conditions for this environmental fungus is a prerequisite for its pathogenesis. *C. neoformans* can adapt to host high temperatures (≥37°C) and CO_2 levels (≥5%), and the latter differs drastically from its normal niche of ~0.04% CO_2 in the atmosphere; however, the molecular basis of such adaptation to the host conditions are poorly characterized. Our previous research into thermotolerance and CO_2 tolerance placed the *Regulator of Ace2 Morphogenesis* (RAM) pathway at the center of the signaling network allowing this fungus to adapt to host conditions. Consequently, disruption of Cbk1, the terminal kinase of the RAM pathway, prevents growth at ≥37°C or ≥5% CO_2. Through a *cbk1*Δ natural suppressor screen, we found that loss of the novel ribonuclease domain-containing protein Psc1, an RNA binding protein Ssd1, and an uncharacterized protein Psc2 partially restored *cbk1*Δ's growth defects in CO_2 and high temperature. Ssd1 is characterized in *Saccharomyces cerevisiae* and is phosphorylated by Cbk1; the phosphorylation state of Ssd1 dictates its subcellular localization and its ability to suppress translation. We hypothesize that in *C. neoformans* Cbk1 interacts with RNA-binding factors Psc1, Psc2, and Ssd1 to regulate subcellular localization and translation of mRNAs required for CO_2 adaptation. We expect that investigation will reveal the underlying mechanism of post-transcriptional control that enables this fungus to adapt to the host environment.

60 **Continual propagation of [D1,2] stwintrons in divergent *Xylariales*** Erzs?bet Fekete, Norbert Ág, Viktória Ág-Rácz, Alexandra Márton, Vivien Bíró, Michel Flipphi, Levente Karaffa University of Debrecen

Spliceosomal twin introns consist of two nested U2 introns excised consecutively. In a [D1,2] stwintron, an internal intron interrupts the 5'-donor of an external intron between the 1st and 2nd nucleotide (nt) (5'-$G_1|U_2$). For *Hypoxylon* sp. CO27-5, one can classify [D1,2]'s in two groups. Of these, sequence-similar "sister" stwintrons cross-identify by blastn, and occur at new intron positions in narrow taxa (species, variants). When reciprocal blastn screens were performed in genomes of other *Xylariales* species–using proven CO27-5 sister stwintrons as primary queries–258 new sequence-similar stwintrons were revealed in 12 species. Some species contain > 50 sister stwintrons, others < 10. All of them are integrated seamlessly in seemingly random exonic sequences, excluding transposon-driven mechanisms or splice-site co-option for their propagation. One observes essentially species-specific clades of sister stwintrons in maximum likelihood trees, implying vertical transmission of sequence-diverging duplication-competent [D1,2]'s. *Xylaria* sp. MSU SB201401 and *X. striata* RK1-1 are intimately related–like strains of the same species–albeit isolated from very different plant species, growing on different continents. 4 stwintrons unique to MSU SB201401 are phylogenetically clustered and the 3 most similar ones are >99 % identical, while the genes harboring them are completely unrelated in sequence, intron-exon structure and function. This lineage involves consecutive strain-specific stwintron duplication events, arguably the most recent duplications in our set. Although the continuous 11-nt near-terminal inverted repeat in these 4 MSU SB201401 stwintrons is not as long as those in prototypical CO27-5 sisters, the near-terminal stem structure is the stand-out common feature. We propose that this stem structure can bring the donor G_1 of the internal- and the acceptor G_3 of the external intron in close proximity, necessary for the rare duplication of [D1,2] stwintrons into double-stranded DNA breaks, including those with smaller or less perfect terminal inverted repeats. We also identified one potential lateral transfer: one MSU SB201401 stwintron is >93 % sequence-identical with two different [D1,2]'s in *Xylariaceae* sp. FL1651. The 3 genes harboring them are completely unrelated. This may imply that duplication-competent stwintrons can be (re)acquired by lateral transfer. Such rare events could contibute to the periodicity of overall (stw)intron gain and loss.

61 **tRNA Modification and The Rice Blast Fungus** Rongrong He[1], Ziwei Lv[2], Yinan Li[2], Xiao-Lin Chen[2], Zhipeng Zhou[2] [1]Huazhong Agricultural University, [2]Huazhong Agricultral University

The rice blast fungus, *Magnaporthe oryzae* (*M. oryzae*), causes one of the most destructive diseases of cultivated rice crops worldwide. Infections caused by this devastating pathogen lead to substantial economic losses globally. Transfer RNA (tRNA) is an essential component of the translation machinery, serving as an adaptor molecule between mRNA and amino acids. A distinctive feature of tRNA is its extensive post-transcriptional modifications. However, the biological roles and the underlying regulation mechanisms in *M. oryzae* remain unclear. In this study, we utilize tRNA-seq to analyze tRNA expression and modification profiles during the development of *M. oryzae*. Through genetic and biochemical experimental methods, we demonstrate that tRNA methylation and thiolation are required for the growth and full virulence of *M. oryzae*. Our study reveals the significance of translational regulation in the pathogenicity of plant fungal pathogens and sheds light on the development of effective and sustainable fungicides.

62 **From ascomycete reference strain to quirky anaerobe; how fungi degrade plant biomass** Jolanda M van Munster Dept of Plant and Soil Sciences, SRUC

Understanding effective biodegradation of plant cell wall lignocellulose is essential to advancing renewables-based biotechnology. Similarly, in farming of ruminants like cows and sheep, lignocellulose degradation by the rumen microbiome is essential for feed digestion, and therefore critically impacts animal health, farming efficiency and greenhouse gas production. Fungi are important as degraders in both arenas, and we therefore study their degradative mechanisms, integrating approaches in mycology, glycobiology and biochemistry.

We investigated how industrial workhorse *Aspergillus niger* regulates gene expression and enzyme secretion in response to lignocellulose. We linked this to assessments of how lignocellulose composition and structure changed after exposure to this fungus, to create a full picture of its degradative mechanism. Building on insights gained from working with this reference strain, we assess how less well explored fungi behave. We found that species of anaerobic fungi (Neocallimastigomycota), key degraders of raw lignocellulose in the rumen, leverage degradative mechanisms with strongly distinct effects on lignocellulose. We work towards elucidating both these effects on plant cell wall architecture, as well as the underlying fungal biology and regulatory mechanisms in more detail. Via understanding these distinct degradative mechanisms, we aim to help elucidate the roles of different anaerobic fungi in the rumen microbiome. Integrating this with our work on understanding fungal interactions with bacterial and archaeal microbiome partners, we hope to open up new avenues to manipulation of the rumen microbiome activity for more sustainable ruminant farming.

63 **Codon usage variation, selection, and evolution in a fungal subphylum** Bryan Zavala Martinez[1,2], Colin Speer[1], Stevie Clemens[1], Dana Opulente[3], Chris Todd Hittinger[4], Antonis Rokas[5], Abigail LaBella[6] [1]UNC Charlotte, [2]FDA, [3]Villanova, [4]University of Wisconsin-Madison, [5]Vanderbilt University, [6]Bioinformatics and Genomics, University of North Carolina at Charlotte

The genomes of the Saccharomycotina, commonly referred to as yeasts, are highly diverse; levels of gene sequence divergence across yeasts are comparable to levels observed across plants and animals. This includes vast diversity in the usage of synonymous codons. While changes in synonymous codon usage have been traditionally considered silent, emerging work suggests that synonymous codon usage plays an active regulatory role in gene expression. We have used the Saccharomycotina as a model system to explore the evolution of codon usage biases and the associated changes in tRNAs. We are leveraging machine learning, evolutionary, and experimental studies to capture the critical role of codon usage in metabolism, pathogenicity, horizontal gene transfer, and gene regulation. Codon usage bias is a treasure trove of genetic information that has been broadly overlooked and may have broad applications to fungal genetics studies.

64 **Epigenetic control of *Neurospora* development** Zachary Lewis Microbiology, University of Georgia

Chromatin modifications and their binding proteins impact DNA accessibility. Proper spatiotemporal regulation of chromatin structure is central to DNA-based processes including replication, repair, and execution of gene expression programs. Polycomb repressive complex 2 (PRC2) is a conserved chromatin modifying complex that methylates H3 lysine-27 in animals, plants, and many fungi. In *Neurospora crassa*, di- and tri-methylated H3K27 is highly enriched across large, gene-rich chromosome domains. PRC2 target domains have low chromatin accessibility and contain a high proportion of hypothetical and/or fungal-specific proteins. These genes are strongly repressed in mycelia grown under diverse conditions, but they are coordinately induced during perithecial development. These data suggest that PRC2 represses the perithecial gene expression program. Indeed, deletion of genes encoding PRC2 subunits leads to aberrant production of perithecia-like structures in the absence of a mating partner. In mammals, assembly of Polycomb repressed chromatin depends on positive feedback between PRC2, its enzymatic product, and PRC1, another chromatin modifying complex that was apparently lost in an early fungal ancestor. To identify gene products that control assembly or maintenance of PRC2-repressed chromatin in *N. crassa*, we performed a mutant screen of *N. crassa* gene knockout strains. Together, our results indicate that PRC2 works in concert with a network of chromatin factors to maintain mycelial cell fate in *N. crassa*.

65 **Echinocandin heteroresistance causes prophylaxis failure and facilitates breakthrough *Candida parapsilosis* infection** Bing Zhai[1,2], Chen Liao[2], Siddharth Jaggavarapu[3], Yuanyuan Tang[1], Thierry Rolling[2], Yating Ning[4], Tianshu Sun[4], Sean A Bergin[5], Mergim Gjonbalaj[2], Edwin Miranda[2], N. Esther Babady[2], Oliver Bader[6], Ying Taur[2], Geraldine Butler[5], Li Zhang[4], Joao B Xavier[2], David S Weiss[3], Tobias M Hohl[2] [1]Shenzhen Institute of Advanced Technology, [2]Memorial Sloan Kettering Cancer Center, [3]Emory University, [4]Peking Union Medical College Hospital, [5]University College Dublin, [6]University Medical Center Göttingen

Breakthrough infections of patients on antimicrobial prophylaxis represent a significant and often unexplained cause of morbidity. Here, we reveal that in high-risk patients on micafungin prophylaxis heteroresistance – the presence of a phenotypically unstable, low frequency subpopulation of resistant cells (~1 in 10,000) – underlies breakthrough bloodstream infections by *Candida parapsilosis* misclassified as susceptible by standard antimicrobial susceptibility testing. By analyzing 219 clinical *C. parapsilosis* isolates from North America, Europe, and Asia, we demonstrate widespread micafungin heteroresistance. To facilitate detection of micafungin heteroresistance, we constructed a predictive machine learning framework that classifies isolates as heteroresistant or susceptible by a maximum of ten genomic features. Our results connect heteroresistance to unexplained prophylaxis failure and demonstrate a proof-of-principle diagnostic approach with the potential to inform clinical decisions.

66 **Insights into metabolism from transcriptional regulators** Chris Koon Ho Wong Faculty of Health Sciences, University of Macau

Metabolism and gene expression are tightly interconnected processes. The orchestration of global gene expression involves complex networks of transcriptional regulators, including kinases, chromatin modifiers, and transcription factors. The functions of these regulators are influenced by the metabolic state of the cell, and in turn, their effects on global gene expression shape cellular metabolism. Thus, studying the functions and regulation of transcriptional regulators can provide valuable insights into metabolism. In our laboratory, we employ a Functional Genomics approach to investigate the genome-wide targets and transcriptional effects of various transcriptional regulators involved in metabolism. By deciphering the regulatory networks and transcriptional responses associated with metabolic processes, we aim to gain a broader understanding of cellular metabolism and its regulation and develop new strategies for manipulating metabolic processes in fungi.

67 **The role of cell wall remodeling in innate immunity of early divergent Mucoromycotina fungi** Hana Barrett[1], Maria Laura Gaspar[1], Carlos Lax[2], Victoriano Garre[2], Teresa E Pawlowska[1] [1]Plant Pathology and Plant Microbe Biology, Cornell University, [2]University of Murcia

The fungal cell wall plays a crucial role in the maintenance of cell integrity and homeostasis, including immune defense. Since the mechanisms of innate immunity in early divergent fungi are not well understood, our research focuses on elucidating the role of the cell wall in immune defense. To examine this process, we use the antagonistic interaction between the fungus Rhizopus microsporus (Mucoromycotina) and the bacterium Mycetohabitans spp, since certain fungal strains harbor his bacteria as an endosymbiont while other strains exhibit an ROS response to the same bacteria. Previous work from our lab has found significant differential gene expression in non-host fungi in coculture with bacteria vs fungi alone. Notably, genes associated with cell wall components chitin, fucose, glucan, mannose, and galactose were differentially expressed in the antagonistic interaction. We are currently quantifying and visualizing cell wall remodeling in the antagonistic interaction between nonhost fungal germlings and Mycetohabitans bacteria by fluorescently probing cell wall components and using microscopy and flow cytometry for visualization of the stains. We have found an increase in the amount of exposed chitin on the surface of the wildtype fungal cell wall in the antagonistic interaction vs the fungus grown alone. No change in mannose content between the two conditions has been seen. We also generated CRISPR/Cas9 disruption mutants of two adenylyl cyclase (cyr1 and cyr2) genes that encode PGN receptors in other fungi to examine their role in our study system. Disruption of the adenylyl cyclase 1 (Δcyr1) led to increased exposed chitin in the fungus alone vs antagonistic interaction and disruption of the adenylyl cyclase 2 (Δcyr2) had decrease chitin in fungus alone vs antagonistic interaction. The increase in chitin in wildtype may indicate that the fungus is reinforcing the cell wall as a defense response. The decreased chitin in the coculture in Δcyr1 may be due to the activity of bacterial chitinases. In Δcyr2 the cell wall remodeling response seems to be heightened. These results suggest that the change in chitin content is an immune defense response that is influenced by the activity of adenylyl cyclase proteins.

68 **Unraveling the 6mA-regulated transcriptional regulatory networks in the early diverging fungus *R. microsporus*** Carlos Lax[1], Leo A Baumgart[2], Yu Zhang[2], Ghizlane Tahiri[1], Stephen Mondo[2], Ronan C O'Malley[3], Igor Grigoriev[2], Eusebio Navarro[4], Francisco E Nicolás[4], Victoriano Garre[4] [1]Departamento de Genética y Microbiología, Facultad de Biología, Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia, [2]U.S Dept of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, [3]Dept of Human Genetics, University of Chicago, [4]Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia

The cistrome is defined as the complete set of transcription factor (TF) binding sites (cis-elements) in an organism. Understanding the intricate regulatory mechanisms governing gene expression is pivotal for unraveling the complexities of fungal biology. Unfortunately, the lack of cost-effective and scalable approaches for TF binding has provoked detailed cistrome information to be restricted to a very few model species. Usually neglected and overlooked compared to higher fungi, early diverging fungi (EDF) possess unique biological features that remain mainly unexplored. In this work, we surveyed the transcription factor with a focus on the basal fungus *R. microsporus*. This EDF representative possesses a DNA epigenetic landscape dominated by 6mA, a rare epigenetic mark in eukaryotes that is associated with transcriptional regulation in this organism. Since some of the 6mA marked genes code for transcription factors, we aimed to characterize the indirect role of this epigenetic modification in transcriptional regulatory networks. DNA Affinity Purification sequencing (DAP-seq) offers a high-throughput and precision in identifying TF-DNA interactions. This pioneering approach allowed us to comprehensively examine 57 TF binding profiles belonging to the main fungal TF families. Our results uncover the binding patterns and functional regulatory networks governed by each TF family. Moreover, integrating these results with expression data has provided valuable insights into the dynamic nature of transcriptional regulation in response to environmental and growth conditions, including light exposure and zinc availability. We deepened into the binding dynamics of the 6mA-regulated white collar-2 (*wc2*) and characterized wc2-dependent and independent light-regulated genes in *R. microsporus*. Additionally, considering the pathogenicity of this species, we focus on the study of transcription factors that participate in the virulent response of *R. microsporus* to the host and identify the regulatory networks that govern the pathogenic capacity of this fungus. The identification and functional characterization of the binding patterns of the TF here studied and its conservation in other species could be a useful tool to reveal unknown regulatory networks in other fungal species, which could broaden the resources available to explore transcriptional regulation across the fungal tree of life.

69 **Spores of arbuscular mycorrhizal fungi host surprisingly diverse communities of endobacteria** Olga Lastovetsky[1], Tancredi Caruso[2], Susanna Pylni[2], Fiona Brennan[3], David Wall[3], Evelyn Doyle[2] [1]School of Biology and Environmental Science, University College Dublin, [2]University College Dublin, [3]Teagasc - Crops Environment and Land-Use Programme
Arbuscular mycorrhizal fungi (AMF) are ubiquitous plant root symbionts which can house two endobacteria: Ca. Moeniiplasma glomeromycotorum (CaMg) and Ca. Glomeribacter gigasporarum (CaGg). However, little is known about their distribution and

population structure in natural AMF populations and whether AMF can harbour other endobacteria. We isolated AMF from two environments and surveyed the surface-sterilized spores for endobacteria. Consistent with previous reports, we found that CaMg were extremely abundant (80%) and CaGg were extremely rare (2%) in both environments. Unexpectedly, we discovered an additional and previously unknown level of bacterial diversity within AMF spores which extended beyond the known endosymbionts, with as many as 277 other bacterial taxa detected in individual spores. Detailed analysis of endobacterial communities inside AMF spores revealed that: (i) CaGg were not limited in distribution to the Gigasporacea family of AMF, as previously thought, (ii) CaMg population structure was driven by AMF host genotype, (iii) a significant inverse correlation existed between the diversity of CaMg and diversity of all other endobacteria. The latter suggests the existence of competition dynamics between different bacterial populations inside AMF spores and provides a basis for generation of testable hypotheses regarding the function of CaMg in AMF biology

70 **Anaerobic fungi are an untapped reservoir of biosynthetic potential** Michelle A O'Malley Chemical Engineering, Bioengineering, UCSB

Anaerobic fungi colonize biomass within the digestive tract of large herbivores, where they have evolved unique abilities to break down lignin-rich cellulosic biomass through invasive, filamentous growth and the secretion of powerful lignocellulolytic enzymes. Despite these attractive abilities, considerably less genomic and metabolic data exists for gut fungi compared to well-studied anaerobic bacteria and aerobic fungi that hydrolyze cellulose. We have addressed these knowledge gaps by isolating and characterizing a collection of anaerobic fungi from large herbivores using a combination of 'omics' tools, from genome sequencing to untargeted metabolomics. Along with a wealth of carbohydrate active enzymes, we also uncovered a plethora of gene clusters encoding biosynthetic enzymes for secondary metabolites from diverse chemical classes by mining the genomes and transcriptomes across the *Neocallimastogomycetes*. Key secondary metabolite clusters include those that build polyketides and non-ribosomal peptides, and several of these clusters appear to be horizontally transferred from anaerobic bacteria. Unlike many aerobic fungi that transcribe a low percentage of clusters under standard laboratory conditions, several anaerobic fungal isolates transcribe and produce greater than 30% of these biosynthetic gene clusters during lab-scale growth on various cellulosic substrates. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) characterization of fungal supernatants detected 72 likely natural products from one strain, and a compound produced by all analyzed strains of fungi was putatively identified as the polyketide baumin. Molecular networking quantified similarities between tandem mass spectrometry (MS/ MS) spectra among anaerobic fungi, enabling three groups of natural products to be identified that are unique to anaerobic fungi. Moreover, synthetic co-culture experiments suggest that the products of anaerobic fungi are antagonistic to rumen bacteria. Overall, the metabolism of anaerobic fungi represents a completely untapped reservoir of biosynthetic potential, which could be drawn on for therapeutics, new chemical building blocks, and enzymes for bioengineering natural products.

71 **Fucose - an overlooked sugar in fungal metabolism** Malgorzata Orlowska[1], Drishtee Barua[2], Sebastian Pilsyk[2], Anna Muszewska[2] [1]Institute of Evolutionary Biology, Faculty of Biology, University of Warsaw, [2]Institute of Biochemistry and Biophysics, PAS

Fucose is a deoxyhexose sugar present and studied in mammals. The process of fucosylation has been the primary focus in studies relating to fucose in animals due to the presence of fucose in Lewis antigens. Very few studies have reported its presence in Fungi, mostly in Mucoromycotina. The constitution of 25% and 12% of this sugar in the carbohydrates of the cell wall in the respective Umbelopsis and Mucorales strains boosts the need to bridge the knowledge gap on fucose metabolism across the FToL. In the absence of a network map involving fucose proteins, we carried out an in-silico approach to construct the fucose metabolic map in Fungi. We analyzed the taxonomic distribution of 85 protein families in Fungi including diverse early diverging fungal lineages. The expression of fucose-related protein-coding genes proteins was validated with the help of transcriptomic data originating from representatives of early diverging fungi (EDF). We found proteins involved in several metabolic activities apart from fucosylation such as synthesis, transport and binding. Most identified protein families are shared with Metazoa suggesting an ancestral origin in Opisthokonta. However, the overall complexity of fucose metabolism is greater in Metazoa than in Fungi. Massive gene loss has shaped the evolutionary history of these metabolic pathways leading to a repeated complete deletion in most yeast-forming lineages. Our results point to a distinctive mode of utilisation of fucose among fungi belonging to Dikarya and EDF. While Dikarya used fucose as a source of nutrients for metabolism, the EDF depended on fucose as a building block and signalling compound.

72 **A novel link between calcineurin, amino acid permease, and protein kinase A in virulence in pathogenic fungi** Madeline P Giner[1], Courtney P Smith-Cornitius[1], Alexis P Garcia[1], Sandeep P Vallenki[1,2], Soo Chan Lee[3] [1]The University of Texas at San Antonio, [2]Dartmouth College, [3]University of Texas

In recent decades, life-threatening fungal infections have become more prevalent due to the growing number of susceptible immunocompromised cohorts. Antifungal treatments often fail due to the intrinsic resistance of some species and the increasing incidence of acquired drug resistance. The clear medical importance and the dearth of effective treatments underscore the need for a better understanding of pathogenesis in fungi, which will subsequently contribute to the development of therapeutic options to combat fungal infections.

One very well-known virulence factor in fungi is **calcineurin**, a serine/threonine phosphatase required for the growth at the human body temperature, 37°C in *Cryptococcus neoformans* and the invasive hyphal growth in *Mucor*. This attribute of the phosphatase makes a highly attractive target of antifungal drugs.

We revealed that a **novel downstream** of calcineurin, a **fungus specific amino acid permease**, that links to pathogenesis in *Mucor* and *C. neoformans.* During preliminary studies, we discovered that, in *Mucor*, (1) calcineurin plays key roles in invasive hyphal growth, where inhibitions of calcineurin results in loss of hyphal growth and virulence, (2) *bycA* encoding an amino acid permease is negatively regulated by calcineurin in a transcription level and functions to inhibit hyphal growth by regulating the activity of protein kinase A (PKA). In *C. neoformans*, we revealed that 3) the amino acid permease Byc1 is also negatively regulated by calcineurin and 4) overexpression of Byc1 results in thermos-sensitivity and lowered virulence. 5) *A link between calcineurin, Byc, and protein kinase A for virulence is conserved in two fungal systems.* These observations have never previously been reported in any fungal system and will therefore lead research in a completely different direction in the study of calcineurin as a virulence factor and a target for antifungal drugs.

73 **Dispersal and biotic filtering structure Mucoromycota fungal communities and their associated bacteria across two different biomes** Nicole Reynolds[1], Kevin Amses[2], Jessie Uehling[3], Rasheed Adeleke[4], Margaret Branine[5], Teresa E. Pawlowska[6] [1]Integrative Plant Science, Cornell University, [2]Perelman School of Medicine, University of Pennsylvania, [3]Dept of Botany and Plant Pathology, Oregon State University, [4]Unit for Environmental Sciences and Management, North-West University, [5]Graduate Field of Microbiology, Cornell University, [6]School of Integrative Plant Science, Cornell University
Despite the ecological importance of Mucoromycota fungi as mycorrhizal symbionts, opportunistic human and plant pathogens, and post-harvest spoilage agents, they remain understudied compared to Dikarya. Fundamental aspects such as geographical distribution, dispersal patterns, and community structure remain unclear. Furthermore, recent and ongoing discoveries about the endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions regarding their effects on fungal host evolution. EB have different effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. Our investigations of Mucoromycota and their associated bacteria communities are focused on testing three main community filtering hypotheses: dispersal (based on geographic distance), biotic (influenced by plant communities), and environmental (incorporating abiotic variables). We collected rhizosphere soils from four total locations in California representing two biomes (Desert and Mediterranean scrub) with three transects and two different plant species sampled from each site. These samples are being analyzed using both culture dependent and culture independent (metabarcoding) methods. Metabarcoding data were generated using bacterial (16S rDNA) and fungal (28S) primers and show desert communities had higher proportions of Zoopagomycota taxa, whereas the coastal samples had more mycorrhizal taxa (Glomerales and Endogonales). Both biotic filtering and dispersal filtering significantly affected fungal and bacterial communities; however, dispersal filtering was only significant over larger distances (km rather than m scale). In addition, network analyses show differential structural characteristics as well as unidentified OTUs as potentially influential hub organisms. Ongoing culture-based screening results have yielded lower diversity than amplicon data, but potentially new fungal/EB combinations. Phylogenetic analyses indicate several distantly related species from each biome with varying EB associations.

74 **Lichtheimia corymbifera as model system for mucormycosis** Kerstin Voigt[1,2] [1]Jena Microbial Resource Collection, University of Jena, [2]Jena Microbial Resource Collection, Leibniz Institute for Natural Product Research and Infection Biology

The basal fungal lineage order Mucorales comprise more than 30 species which are human pathogenic causing life-threatening infections known as invasive mucormycosis (IM). Mortality rates range from 40-80% in immunocompromised background. Rapid progression and difficult diagnosis are major hallmarks of the disease. Comorbidities such as diabetes mellitus, hematological malignancies, organ transplantations, and most recently COVID-19 predispose individuals to Mucormycosis. The increased incidences of these underlying diseases over the last years are leading to a worldwide spread of incidences for mucormycosis.

Besides *Rhizopus*, *Mucor* and *Rhizomucor* spp., *Lichtheimia corymbifera* ranges among the most prominent causative agents of IM. In 1885, Arnold Paltauf published the first case of disseminated mucormycosis, which he named "Mycosis mucorina" and was most probably caused by *L. corymbifera*.The current abstract provides an overview about (i) the genome architecture, (ii) the interaction with immune cells of the innate immune system, (iii) expression signatures based on dual-transcriptomics and (iv) potenmtial virulence markers derived from spore surface proteomics, secretomics and iron uptake profiling. All these features are discussed in the light of potential targets for diagnostics and therapy.

75 **Role of potassium transport for *Candida auris* biofilm formation and skin colonization** Emily Eix, Chad J Johnson, Maisy Andes, Angela Gibson, Dhanansayan Shanmuganayagam, Jeniel Nett University of Wisconsin-Madison

Candida auris poses an urgent public health threat due to its propensity to colonize human skin, rapidly spread in healthcare settings, and cause invasive disease with high mortality. It is critical to understand how *C. auris* grows on skin to prevent skin colonization. We used synthetic sweat medium and ex vivo skin models to identify environmental conditions necessary for *C. auris* skin colonization. After systematically removing individual components of the sweat medium and assessing biofilm growth, we found that removing K+ inhibited *C. auris* growth and biofilm formation. To begin to understand K+ utilization for *C. auris*, we used a homologous recombination system to disrupt putative potassium transport genes. We found that a *trk1*Δ mutant exhibited defects in biofilm formation and skin colonization and these phenotypes were reversed with the addition of high K+ concentrations. These studies reveal the importance of potassium uptake in *C. auris* skin colonization and biofilm growth. As potassium transport in *C. auris* is not well-understood, further exploration of the mechanisms of potassium transport and its influence on biofilm growth provides new insight into skin colonization for the emerging pathogen.

76 **Genome of endophytic *Fusarium oxysporum* from the strawberry root microbiome lacks common virulence factors** Samantha Gebben-Hernandez, Fiona C Harrigian, Nicholas LeBlanc USDA-ARS

Fungi in the *Fusarium oxysporum* species-complex have primarily been studied as plant pathogens. However, many of these fungi commonly associate with plants as nonpathogenic endophytes and can have beneficial effects on host growth. Understanding the underlying genetic bases of endophytic interactions requires a high-quality genome assembly. The goal of this study was to generate a genome assembly for a novel endophytic strain of *F. oxysporum* isolated from roots of healthy strawberry plants. Genomic DNA was sequenced from a single spore isolate for 24 hours using Nanopore technology. An assembly was generated and polished using Flye, genes were predicted using Funannotate, and completeness was measured using a set of conserved orthologs and BUSCO. Homologs of proteins required for fungal production of the plant hormones indole-acetic acid (IAA) and gibberellic acid (GA) as well as Secreted In Xylem (SIX) effector proteins were identified using blast. Additional putative effector proteins were identified using SignalP and EffectorP and annotated with PHI-base. Approximately 3 million Nanopore reads assembled into a genome of ca. 50 Mb, composed of 86 contigs, with an N_{50} of 4,529,961 bp, and 99.4% completeness. The genome lacked SIX effectors and proteins required for production of plant hormones IAA and GA. Of the total 15,716 predicted proteins, 487 were identified as putative effectors. Only one putative effector showed homology with a known virulence factor of pathogenic *F. oxysporum*, a secreted metalloprotease (FoMep1). Outcomes from this research demonstrate the potential for using Nanopore data to generate fungal genome assemblies and will provide a platform for identifying genes required for host infection by endophytic forms of *F. oxysporum*.

77 **Study of the interaction of the pathogen *Zymoseptoria tritici* with wheat endophytic fungi** Agustina de Francesco[1], Pilar Vesga[1], Rocio Tirado-Conejo[1], Federico Lopez-Moya[2], Andrea Sánchez-Vallet[1] [1]Centro de Biotecnologia y Genomica de Plantas, Universidad Politécnica de Madrid, [2]Dept of Marine Sciences and Applied Biology, Universidad de Alicante

Zymoseptoria tritici is a very damaging pathogen of wheat that interacts with endophytic microorganisms during host colonization and formation of the reproductive structures. In this work, we isolated 206 fungal endophytes from different durum wheat cultivars grown in different regions of Spain. We demonstrated that *Z. tritici* can inhibit the growth of 3 fungal endophytes, two of them belonging to the *Tricharina* genus. Additionally, we demonstrated that 31 of the fungal endophytes hampered the growth of the pathogen in axenic conditions, and that two of them also inhibited the infection process *in planta*. We further demonstrated that the endophyte-pathogen interactions is genotype-specific since different *Z. tritici* strains interact differently with the fungal endophytes. Our work provides further insights on the interaction of *Z. tritici* with endophytic fungi from durum wheat.

78 ***Pochonia chlamydosporia* chitosan metabolism: A way to modulate its pathogenicity during plant parasitic nematodes-plant interaction** Federico Lopez-Moya Marine Science and Applied Biology, University of Alicante

Nematophagous fungi have been employed in biological control to protect crops of interest because of their ability to manage plant parasitic nematodes sustainably. *Pochonia chlamydosporia,* a worldwide fungal parasite root knot nematodes (RKN), is responsible for natural suppression of soils to plant parasitic nematodes. This fungus is also a true endophyte in both mono and dicot crop plants. *P. chlamydosporia* can modulate plant local and systemic defenses. Extracellular depolymerases of nematophagous fungi reflect their parasitic, endophytic, and saprophytic traits. Cell wall degrading enzymes play a key role during fungus-nematode interaction. RKN eggshell is enriched in chitin and glycoproteins. *P. chlamydosporia* a highly expanded family of hydrolases related with chitin modification. Chitosan is a highly deacetylated form of chitin. Most biocontrol fungi are resistant to chitosan. Genomes of most isolates of *P. chlamydosporia* from worldwide origin show genes encoding chitin deacetylases and chitosanases. These enzymes are overexpressed during Pc infection on RKN eggs. Most of these isolates display high parasitism to nematode eggs and degrade chitosan. Chitin perception is a key component of the Plant Immune System (PTI). Chitin shielding/deacetylation in fungi is a way to circumvent plant defenses. Plant chitinases show less affinity for chitosan than chitin. Besides, chitosan is a less efficient plant defense elicitor than chitin. We propose that chitosan metabolism allows endophytic biocontrol fungi such as *P. chlamydosporia* for evading plant defenses in the rhizosphere and allows them to parasitize efficiently endoparasitic nematodes embedded in root tissues.

79 **Atpenin A5 - Elucidating the function of a succinate dehydrogenase inhibitor produced by the poplar pathogen *Sphaerulina musiva*** Cole Sawyer[1,2], Kelsey Sondreli[3], Tomas Rush[1,2], Sameer Mudbhari[1,2], William Alexander[2], Carrie Eckert[1,2], Jared LeBoldus[3], Paul Abraham[1,2], Joanna Tannous[1,2] [1]University of Tennessee Knoxville, [2]Oak Ridge National Lab, [3]Oregon State University

Production of poplar trees for biofuel production is a vital mission of the Dept of Energy which has largely been hampered by fungal infection. *Sphaerulina musiva*, the causal agent of leaf spot and stem canker disease, is the most economically impactful poplar pathogen. The traditional geographic range of this fungus is limited to the eastern coast of the United States, but breeding efforts of poplar strains has led to the anthropogenic movement of this pathogen westward. Recently, the succinate dehydrogenase inhibitor and potent fungicide Atpenin A5 was found to be encoded in the genome of *S. musiva*. The exact role of this metabolite in the biology of *S. musiva* and its downstream effects on a naïve ecosystem is currently unknown.

Bioinformatic prediction of atpenin A5 production was performed using antiSMASH across a 122-member population. Eight hypovirulent strains from a British Columbia clade were found to be missing the backbone PKS-NRPS from the atpenin a5 biosynthetic gene cluster. This finding led us to hypothesize that the metabolite might exacerbate infection on poplar trees. To study this possibility, atpenin A5 production was disrupted by targeting a pathway-specific transcription factor predicted to control regulation of the corresponding gene cluster. Five mutants were generated using a CRISPR-Cas9 ribonucleoprotein knockout protocol and subjected to Oxford nanopore sequencing. No off target effects of Cas9 were detected in three selected mutants. Absence of atpenin A5 production and significant down-regulation of its corresponding gene cluster was confirmed via metabolomics and transcriptomics. Comparing mutant and wild-type strains revealed a minor, strain-specific effect on 1 of 7 poplar genotypes. As Atpenin A5 is a known antifungal compound, we then tested the metabolite using co-culture assays with two beneficial, poplar-associated fungi. The ectomycorrhizal fungus *Laccaria bicolor* showed reduced hyphal growth in the presence of atpenin A5 positive strains of *S. musiva*. The dark septate endophyte *Hyaloscypha finlandica* showed no reduction in growth, indicating resistance to the compound. Further analysis of atpenin-like clusters using cBlaster located an intact atpenin A5 cluster in the genome of *H. finlandica.* Herein, we successfully disrupted atpenin A5 production in a non-model plant pathogen. Atpenin A5 likely does not play a substantial role during infection but instead mediates fungal-fungal antagonism between beneficial and detrimental poplar-associated fungi.

80 **Surprising strain-specific molecular determinants of *Aspergillus fumigatus* pathogenicity revealed by new cancer small molecule therapies** Katherine E Doss[1], Matthew R James[1], Andrew Wishart[2], Tobias M Hohl[3], Michail S Lionakis[2], Robert A Cramer[1] [1]Dartmouth College, [2]National Institutes of Health, [3]Memorial Sloan Kettering Cancer Center

Clinical risk factors for diseases caused by *Aspergillus fumigatus* have expanded beyond neutropenia and high-dose corticosteroid therapy. As treatments move toward small molecule drugs that target specific host pathways, novel immune states that facilitate infection have emerged. An example of this precision therapy is the drug ibrutinib (IBT) that inhibits Bruton's tyrosine kinase (BTK) and is used for the treatment of B-cell malignancies like chronic lymphocytic leukemia. Patients receiving IBT are at a higher risk of *A. fumigatus* infection. Surprisingly, reference *A. fumigatus* strains AF293 and CEA10 are not pathogenic in the setting of IBT treatment or genetic BTK-/- deficiency in mice. Rather, only certain strains of *A. fumigatus* are pathogenic in IBT-treated and BTK-

deficient mouse models. These data challenge the long-standing paradigm that any *A. fumigatus* strain can cause disease in an immune compromised host.

Mechanistic studies of IBT-mediated susceptibility to *A. fumigatus* revealed an unexpected role of p40phox, a component of the neutrophil NADPH oxidase, and RAC2, which regulates NADPH oxidase. IBT treatment thus results in defective production of reactive oxygen species (ROS) that are a crucial aspect of host defense against *A. fumigatus*. We have tested the hypothesis that *A. fumigatus* strain-specific pathogenicity is ROS-mediated. To test this hypothesis, we are defining the genetic network that mediates *A. fumigatus* responses to NADPH oxidase-dependent host defense mechanisms. By utilizing the recently available protein kinase, phosphatase, and transcription factor null mutant collections, we are identifying key regulators of the fungal ROS response. In addition, to define the genetic and phenotypic variants associated with the strain-specific pathogenicity in the setting of BTK inhibition, we are utilizing both whole genome sequencing and ROS-related phenotyping of a unique collection of *A. fumigatus* isolates from patients on IBT. Preliminary results in both a biofilm and germling model suggest that five of seven isolates show reduced susceptibility to hydrogen peroxide. Using these approaches will allow us to define the cause-and-effect relationship between allelic variants in the *A. fumigatus* population and murine model disease outcomes. Defining these mechanisms is expected to promote new insights into both fungal pathogenicity and host response in specific patient populations.

81 **Genome engineering of filamentous fungi for the production of bioactive compounds** Arnold Driessen Molecular Microbiology, University of Groningen

Filamentous fungi are a rich reservoir of bioactive compounds that are applied in a myriad of fields ranging from crop protection to medicine. The surge of genomic data available shows that fungi remain an excellent source for new pharmaceuticals. However, most of the responsible biosynthetic gene clusters are transcriptionally silent under laboratory growth conditions. Hence, there is a high demand for novel tools for fungal genome engineering.

We have developed several generic strategies for activation of these clusters including CRISPR/cas9-based gene inactivation [1] used for targeting of regulatory genes including histone deacetylases, as well as a genome-editing-free, transcriptional regulation tool for filamentous fungi, based on the CRISPR activation (CRISPRa) methodology [2]. A collection of genetic parts compatible and interchangeable with the Modular Cloning system was generated for filamentous fungi and made available through AddGene, and that includes natural and synthetic promoters (constitutive and inducible), terminators, fluorescent reporters, and selection makers [3].

Further, a *Penicillium rubens* strain with an industrial background was developed in which the four highly expressed biosynthetic gene clusters (BGC) required to produce penicillin, roquefortine, chrysogine and fungisporin were removed [4]. This resulted in a minimal secondary metabolite background. This platform strain was repurposed for expression of the polyketide calbistrin gene cluster as well as for the production of plant metabolites such as naringenin. Pathways were introduced through the *in vivo* assembly of DNA segments with short overlaps and integrated into a highly expressed genomic region corresponding to the former penicillin-BCG. Our studies pave the way for fast combinatorial assembly and expression of biosynthetic pathways in a fungal strain with low endogenous secondary metabolite burden.

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82 **Investigating interactions between *Zymoseptoria tritici* and *Pseudomonas* bacteria through multi-omic approaches** George Lund[1], Susan Mosquito[1], David Hughes[2], David Withall[3], Ian Clark[1], Morten Lindqvist Lindqvist Hansen[4], Lars Jelsbak[4], Jason Rudd[3], Tim Mauchline[1] [1]Sustainable Soils and Crops, Rothamsted Research, [2]Intelligent Data Ecosystems, Rothamsted Research, [3]Protecting Crops and the Environment, Rothamsted Research, [4]DTU Bioengineering, Technical University of Denmark

Zymoseptoria tritici, the causal agent of Septoria tritici blotch disease (STB), is the most economically important wheat pathogen in Western Europe, resulting in up to 50% yield losses. Resistance to conventional fungicides is becoming an increasing problem, with very few effective control strategies currently available to farmers. For this reason, new chemistry and fungicide targets are urgently required. *Pseudomonas* are ubiquitous, gram-negative bacteria that can act as biocontrol agents of a range of plant pathogens and produce a range of antimicrobial compounds. *Pseudomonas* bacteria possess a largely untapped diverse secondary metabolite repertoire, which has potential for the discovery of new high value fungicides or *Z. tritici* antagonistic compounds.

We present the use of an *in vitro Z. tritici* antagonism assay, that can be used both qualitatively and quantitatively, to identify *Pseudomonas* isolates with the potential to antagonise *Z. tritici* through the production of secreted secondary metabolites. Used in combination with genome-mining approaches and gene disruption, we are working to identify and characterise bacterial secondary metabolite biosynthetic gene clusters (BGCs) involved in the interaction between antagonistic *Pseudomonas* isolates and *Z. tritici*. Using this approach, we have characterised *Pseudomonas* bacteria producing the known antifungal compound 2,4-diacetylphloroglucinol using site-directed mutagenesis, to link the BGC to antagonism phenotype. We also identified *Pseudomonas* isolates where the mechanisms of i*n vitro* fungal antagonism are currently unknown. We found statistically significant differences in the responses of genetically distinct *Z. tritici* isolates to antagonism by pseudomonads *in vitro*.

These results indicate that a combination of *in silico* prediction of secondary metabolite BGCs, and *in vitro* assessment of antagonism of *Z. tritici* blastospores has the potential to help decipher mechanisms of antagonism by generating leads for further characterisation of novel BGCs with analytical chemistry approaches. In a wider context, these results highlight the potential need for multiple genotypes of fungal pathogen to be assessed in biocontrol studies, particularly for highly genetically diverse species such as *Z. tritici*, as resistance to bacterial antagonism may exist as a quantitative trait within natural populations.

83 **Understanding the mechanisms that regulate H3K27me3 in the model fungi *Neurospora crassa*** Felicia Ebot Ojong, Aileen R Ferraro The University of Georgia
Neurospora crassa is a filamentous fungus with a rich history in epigenetics research. Several conserved epigenetic pathways operate in *N. crassa* to silence gene expression, including RNAi, DNA methylation, H3K9 methylation, and H3K27me3, which is absent in yeast models. Like higher eukaryotes, H3K27me3 is catalyzed by a conserved Polycomb Repressive Complex 2 (PRC2). Neurospora also contains the HCHC complex, which contains Histone Deacetylase 1 (HDA-1) and is targeted to H3K9me3 by Heterochromatin protein 1 (HP1). The regulation of constitutive heterochromatin is also accomplished by the HCHC deacetylase silencing complex, composed of HP1, CDP-2, HDA-1 and CHAP. The HCHC complex removes acetyl groups from histones in constitutive heterochromatin. We carried out a genetic screen to identify genes required for Polycomb repression in N. crassa. We found that HDA-1 is required for repression of PRC2-targeted genes. In the absence of HDA-1, H3K27me2/3 is lost from typical PRC2-targeted domains and accumulates aberrantly at constitutive heterochromatin domains marked by H3K9me3. We also showed that CDP-2, another HCHC mutant, is important for proper localization of the H3K27me2/3 methylation mark. Together, our results show that H3K9me3 recruits' components of the HCHC deacetylation complex to prevent aberrant recruitment of PRC2 to constitutive heterochromatin domains.

84 **Impacts of the Epigenome Beyond Transcriptional Regulation** David Rowe[1], Jun Huang[1,2], Wei Zhang[1,3], Divya Mishra[1,4], Katherine Jordan[5], Barbara Valent[1], Sanzhen Liu[1], David Cook[1] [1]Plant Pathology, Kansas State University, [2]Dept of Molecular Genetics and Microbiology, Duke University Medical Center, [3]Dept of Botany and Plant Sciences, University of California-Riverside, [4]National Institute of Plant Genome Research, [5]USDA-ARS, Hard Winter Wheat Genetics Research Unit

Genomes exhibit non-random organization of processes such as transcription, replication, nuclear arrangement, and repair. The epigenome plays an important regulatory role in these genome functions, many of which are documented in filamentous fungi. The direct causative role that the epigenome plays in genome evolution remains less clear. Experimental evidence in fungal and plant systems highlights an association between chemical histone modifications and the accumulation of DNA variation. Here we took a comparative genomic and epigenomic approach to establish if such an association exists in *Magnaporthe oryzae* (synonoym of *Pyricularia oryzae*) the causative agent of numerous blast diseases of monocots. We analyzed sequence data from 86 *Magnaporthe oryzae* isolates infecting rice, along with new complete genome assemblies of four rice infecting strains. Histone modification profiles for these four strains were obtained through genome-wide chromatin immunoprecipitation-sequencing that

revealed an association between SNP and INDEL frequency and repressive histone marks (H3K27me3, H3K9me3). Within the analyzed SNPs, we identified numerous clusters of dense SNPs that reside in heterochromatin but were often outside of transposable elements, highlighting the link between heterochromatin and DNA variation. When controlling for selection, silent SNP frequency/kb were higher in H3K27me3-associated genes, providing evidence for an unexplained association between variation and the epigenome. Larger classes of DNA variation were identified through comparison of the reference strains, and we found that euchromatic regions were often syntenic, while heterochromatic regions trended towards non-synteny. Heterochromatin emerged as a major factor associated with diverse DNA variations in *M. oryzae*, despite efforts to account for selection. Numerous questions remain regarding epigenetic networks and mechanisms creating DNA variation, but our results underscore heterochromatin's role in shaping genetic diversity in these mainly asexually reproducing fungi.

85 **Histone binding of ASF1 is required for fruiting body development, but not for genome stability in the filamentous ascomycete *Sordaria macrospora*** Jan Breuer, Minou Nowrousian Ruhr-University Bochum

Histone chaperones are proteins that are involved in nucleosome assembly and disassembly and can therefore influence all DNA-dependent processes including transcription, DNA replication and repair. The highly conserved eukaryotic histone chaperone ASF1 is involved in the assembly and disassembly of nucleosomes during transcription, DNA replication and repair. It was the first chaperone discovered to be involved in all three of these processes. The filamentous fungus *Sordaria macrospora* is one of only two multicellular organisms where *asf1* deletions are viable, which makes it useful for *in vivo* analysis of this central regulator of eukaryotic chromatin structure. Deletion of *asf1* in *S. macrospora* leads to sterility, a reduction of DNA methylation, and upregulation of genes that are usually weakly expressed in the wild type. Here, we focused on the functions of the highly conserved core and the divergent C-terminal tail of ASF1, studied the effects of ASF1 on histone modifications and tested its relevance for genomic stability. By Co-IP and complementation analysis we showed that substitutions of amino acid V94 or truncations of the C-terminal tail abolish histone binding and do not complement the sterile mutant phenotype. Δasf1 is sensitive to the DNA damaging agent MMS, while complementation strains, even those with non-histone-binding variants, regain wild type-like resistance. Western blot analysis revealed a global increase of H3K27me3 in Δasf1, accompanied by a global decrease of H3K56ac; these effects can be rescued by transformation with *asf1* variants that allow histone binding. A deletion mutant in the predicted histone acetyltransferase gene *rtt109* showed a similar decrease in H3K56ac, sensitivity to MMS, and a sterile phenotype, suggesting a close functional association between ASF1 and RTT109 in the context of development, histone modification and DNA damage response. By using Hi-C we detected a tandem duplication of around 600 kb on chromosome 2 in the Δasf1 mutant (the *asf1* gene locus is on chromosome 6). Crossing experiments indicated linkage between the viability of Δasf1 strains and the presence of the duplication. Other phenotypes like fruiting body formation or the tested histone modifications do not change in the presence or absence of the duplication. In summary, our data help to unravel roles for the histone chaperone ASF1 in multiple cellular pathways including histone modifications, genome stability, and multicellular development.

86 **Argonaute proteins are important for RIPping in *Fusarium graminearum*** Zeyi Wang, Zhuyun Bian, Jin-Rong Xu Purdue University

The RID DNA demethylase is essential for repeat-induced point (RIP) mutation during sexual reproduction in Sordariomycetes. In this study, we used affinity purification and mass spectrometry analysis to identify putative FgRid1-interacting proteins in the wheat scab fungus *Fusarium graminearum*. One of them is FgAgo2 that is specifically expressed during sexual reproduction. The interaction of FgRid1 with FgAgo2 was confirmed by co-immunoprecipitation assays. To determine the role of AGO proteins in RIPping, we generated the *Fgago1 Fgago2* deletion mutant in a reporter strain containing direct repeats of an 802-bp sequence. The resulting double mutant had no detectable defects in vegetative growth, perithecium formation, and ascosporogenesis but was defective in ascospore discharge. However, in comparison with the wild type, the ripping efficiency was reduced over 80% in the *Fgago1 Fgago2* mutant. To determine the effects of *AGO* deletion on RIPing of unlinked repeats, we also generated the *Fgago1 Fgago*2 double mutant with two dispersed hygromycin phosphotransferase (*hph*) cassettes (one full-length and one truncated non-functional). Preliminary data showed that none of the 1,500 ascospore progenies isolated from this mutant with dispersed *hph* repeats mutant were sensitive to hygromycin. Sequencing analysis with 30 randomly selected ascospore progenies did not identify C-to-T mutations in both *hph* copies, indicating the importance of AGO proteins in RIPpping in *F. graminearum*. Further characterization of RIPping defects in ascospore progeny of both double mutants with linked and dispersed repeats is in progress.

87 **Exploring the role of Spoks (Spore Killers) in chromosome dynamics of *Fusarium oxysporum*** Gema Puebla Planas[1], Dilay H. Ayhan[2], Pilar Gutiérrez Escribano[1], Lucía Gómez Gil[1], Cristina López Díaz[1], Li-Jun Ma[3], Antonio Di Pietro[1], Manuel Sánchez López-Berges[1] [1]Genetics, Universidad de Córdoba, [2]Biochemistry and Molecular Biology, University of Massachusetts Amherst, [3]University of Massachusetts Amherst

The ascomycete *Fusarium oxysporum* causes vascular wilt disease in more than a hundred crop species and opportunistic infections in humans. Its genome is compartmentalized into conserved core chromosomes and lineage-specific accessory regions, which are involved in adaptation to different environments such as the plant host. Some of these regions are highly dynamic and undergo spontaneous loss or duplication, but the mechanisms controlling chromosome dynamics remain largely unknown. It was previously observed that the accessory genome of *F. oxysporum* contains multiple copies of Spoks (Spore Killers), a class of genetic elements that act as meiotic drivers by actively killing neighboring cells that lack the element. Interestingly, we found that a single copy of Spok was either present or absent on a conserved chromosome exhibiting differential stability in two different *F. oxysporum* isolates. To experimentally test the role of Spok in chromosome stability, we transferred either a wild-type or a loss-of-function copy of the Spok element from the strain with the stable chromosome into the strain with the unstable chromosome, together with a fluorescence and an antibiotic resistance marker. To test the effect of chromosome stability, passaging experiments are performed under conditions favoring loss of the unstable chromosome followed by quantitative monitoring of chromosome loss by flow cytometry. The results of this work will shed new light on the role of Spoks in the dynamics of accessory genomic regions.

88 **Recent co-evolution of two pandemic plant diseases in a multi-hybrid swarm** Mostafa Rahnama[1], Bradford Condon[2], João P Ascari[3], Julian R Dupuis[4], Emerson M. Del Ponte[3], Kerry F. Pedley[5], Sebastián Martinez[6], Barbara Valent[7], Mark L Farman[8] [1]Tennessee Tech University, [2]University of Kentucky, [3]Universidade Federal de Viçosa, [4]Entomology, University of Kentucky, [5]USDA, [6]Instituto Nacional de Investigación Agropecuaria, [7]Plant Pathology, Kansas State University, [8]Plant Pathology, University of Kentucky

Pyricularia oryzae (synonymous with *Magnaporthe oryzae*) is primarily recognized as the "rice blast fungus" due to its significant and global impact on rice crops. More recently, it has become a concern for potentially affecting global wheat production. Wheat blast, a new disease, was first detected in 1985 in Paraná, Brazil, and by 1990, it was widespread throughout all wheat-growing regions and neighboring countries. In recent years, wheat blast outbreaks have been reported in Asia and Africa, making it an emerging concern for global agriculture. Another disease caused by *P. oryzae*, gray leaf spot (GLS), was initially identified in 1971 in annual ryegrasses in Louisiana and Mississippi, USA. By the mid-1990s, GLS had caused widespread outbreaks of perennial ryegrass and the related species, tall fescue, in the central United States. In this study, we reconstruct the evolutionary history of two recent populations of *P. oryzae* that are responsible for these two novel diseases (wheat blast and GLS). We provide evidence that wheat blast/GLS evolved through two distinct mating episodes: the first occurred around 60 years ago when an *Eleusine*-adapted fungal individual mated with a *Urochloa*-adapted individual. In the subsequent decade, a single progeny of this cross was mated with a small number of individuals from three additional host-specialized populations. As a result of these matings, non-functional alleles of two key host-specificity factors, whose recombination in a multi-hybrid swarm probably facilitated the host jump, were introduced into the population. Additionally, we demonstrate that a very small number of mutations have occurred since the founding event and that the majority are private to individual isolates. Finally, our results showed adaptation to the wheat or *Lolium* hosts appears to have been instantaneous and driven solely by selection on repartitioned standing variation, with no apparent role being played by newly formed mutations.

89 **Arbuscular mycorrhizal fungi heterokaryons have two nuclear populations with distinct roles in host–plant interactions** Gokalp Yildirir[1], Jana Sperschneider[2], Yanina S Rizzi[3], Mathu Malar C[4], Ariane Mayrand Nicol[4], Essam Sorwar[4], Matthew Villeneuve-Laroche[4], Eric CH Chen[5], Wataru Iwasaki[5], Elizabeth K Brauer[6], Whynn Bosnich[6], Caroline Gutjahr[3], Nicolas Corradi[1] [1]Biology, University of Ottawa, [2]CSIRO, [3]Technical University of Munich, [4]University of Ottawa, [5]University of Tokyo, [6]Agriculture and Agri-Food Canada

Arbuscular mycorrhizal fungi (AMF) are prominent root symbionts that can carry thousands of nuclei deriving from two parental strains in a large syncytium. These co-existing genomes can also vary in abundance with changing environmental conditions. Here we assemble the nuclear genomes of all four publicly available AMF heterokaryons using PacBio high-fidelity and Hi-C sequencing. We find that the two co-existing genomes of these strains are phylogenetically related but differ in structure, content and epigenetics. We confirm that AMF heterokaryon genomes vary in relative abundance across conditions and show this can lead to nucleus-specific differences in expression during interactions with plants. Population analyses also reveal signatures of genetic

exchange indicative of past events of sexual reproduction in these strains. This work uncovers the origin and contribution of two nuclear genomes in AMF heterokaryons and opens avenues for the improvement and environmental application of these strains.

90 **Assessing the plasticity of the *Neurospora crassa* genome organization** Sara Rodriguez, Clayton Hull-Crew, Andrew T Reckard, Yulia Shtanko, Tiffany J Lundberg, Farh Kaddar, Andrew D Klocko Chemistry & Biochemistry, University of Colorado Colorado Springs

Eukaryotic genomes must be properly organized for their correct function. The chromosomes of most filamentous fungal genomes are organized into a Rabl conformation, in which centromeres cluster independent of telomere bundles. The chromatin within each chromosome is also compartmentalized, with the open and active euchromatin found in the center of the nucleus, while the more condensed, silent heterochromatic regions, including the centromere cluster and telomere bundles, localize to the nuclear periphery. Despite these features of fungal genome organization, it is still unclear whether this organization is maintained when structural variants in the underlying genomic DNA occur, including deletions or large genome rearrangements, such as translocations or inversions. Many structural variants result from improperly repaired chromosomal breaks and can result in aberrant transcriptional regulation due to incorrect long-range interactions between promoters and enhancers. Work in humans has shown structural variants are not tolerated well, with translocations/inversions often observed in oncogenic cell lines. However, little is known about how structural variants impact fungal genome organization and function, the latter assessed by changes in transcriptional regulation or deposition of histone post-translational modifications (PTMs), which are often considered "epigenetic". To understand how genome translocations/inversions and deletions impact the conformation and activity of fungal genomes, we used strains of *Neurospora crassa* that either harbor a single translocation or inversion, or strains deleted of individual heterochromatic regions; these single structural variant strains are better models than often-heterogeneous human cancer cell lines. We assess genome organization by chromosome conformation capture coupled with high-throughput sequencing (Hi-C) and genome function using Chromatin Immunoprecipitation-sequencing (ChIP-seq) of histone PTMs or RNA-sequencing of total messenger RNAs (RNA-seq). Here, we will provide our latest findings on how single genomic translocations/inversions or deletions impact fungal genome organization and function.

91 **Molecular mechanisms of peroxisome movement** Bellana Driscoll, Madison Fountain, John Salogiannis Molecular Physiology and Biophysics, University of Vermont Larner College of Medicine

In Aspergillus nidulans, peroxisomes move on microtubules by hitchhiking on motile early endosomes (EEs) at membrane contact sites. We previously identified the protein PxdA as an EE-marker critical for peroxisome hitchhiking. However, the molecular mechanisms that dictate how peroxisomes attach to PxdA-marked EEs is not known. We undertook a mutagenesis screen, as well as a candidate and interactor-based approach, to identify new factors involved in peroxisome hitchhiking. Our findings suggest that peroxisome hitchhiking is a multi-step process requiring the motility of early endosomes, the recruitment of endosome-specific factors, and the recognition of proteins at the EE-peroxisome contact site.

92 **Microtubule-dependent endosomal mRNA transport** Senthil Devan, Sainath Shanmugasundaram, Kira Muentjes, Florian Altegoer, Sander HJ smits, Michael Feldbrugge Heinrich-Heine University

Efficient long-distance transport is essential for polar growth, ensuring the precise distribution of cellular cargo such as proteins and mRNAs. In fungal pathogens, this growth mode depends on microtubule-associated endosomal transport. The precise mechanism by which mRNAs are linked to the endosomal surface is poorly understood. Our structural analysis revealed that the key RNA-binding protein Rrm4 of *Ustilago maydis* contains a new type of MademoisLLE domain that harbours a seven-helical bundle providing a distinct binding interface. A comparison with the canonical MLLE domain of the poly(A)-binding protein Pab1 revealed distinct features of the two MLLE domains. Deciphering the MLLE-binding code allowed us to predict and verify previously unknown Rrm4 interactors. Importantly, we demonstrate that also the human MLLE domains of PABPC1 and UBR5, use a similar principle to differentiate among interaction partners. Thus, we provide unprecedented mechanistic insights into how structural variation of the widely distributed MLLE domain determines mRNA attachment during endosomal transport.

93 **The role of the GTPase Cdc42 in *Cryptococcus neoformans* stress response** Hannah Segbefiah Akahoho[1], Congyue Peng[2], Nabanita Saikia[3], Feng Ding[3], Lukasz Kozubowski[1] [1]Genetics and Biochemistry, Clemson University, [2]Clemson University, [3]Physics and Astronomy, Clemson University

In ascomycete yeast, *Saccharomyces cerevisiae*, the conserved Rho-family GTPase Cdc42 is essential for establishment of cell polarity and cytoskeletal organization. Cdc42 in basidiomycete pathogenic yeast, *Cryptococcus neoformans*, is not essential for

establishment of cell polarity and growth at non-stress conditions. However, *C. neoformans* Cdc42 is essential for thermotolerance and dissemination in the murine inhalation model of cryptococcosis. It has been proposed that Cdc42 is involved in adaptation to 37°C by facilitating assembly of the complex composed of the filament-forming GTPases, septins, that contribute to cytokinesis and morphogenesis and are required for growth at 37°C. However, it remains unclear how Cdc42 impacts assembly of the septin complex and how it contributes to growth at 37°C. Here, we constructed a Cdc42-reconstitute strain, in which the activity of Cdc42 is controlled by the blue light-dependent switch. The molecular switch is based on the allosteric changes of a light, oxygen, voltage sensing domain (LOV2) of oat, *Avena sativa*. Blue light triggers allosteric configuration changes of LOV2 and inactivates Cdc42. The effectiveness of this light switch was confirmed. With this tool, we can control the activity of Cdc42 in living cells. Additionally, we are introducing a biotinylation domain (UltraID) to Cdc42 and based on this construct a mass spectrometry analysis of biotinylated proteins will be performed to identify Cdc42 binding partners that may be relevant to high temperature adaptation and virulence of *C. neoformans*.

94 **Allocation of nuclei and growth potential among buds of the multi-budding yeast, *Aureobasidium pullulans*** Alison Wirshing[1], Claudia Petrucco[2], Analeigha Colarusso[1], Daniel Lew[1] [1]MIT, [2]Duke University

A. pullulans is a poly-extremotolerant yeast that thrives in diverse environments (both terrestrial and marine). In its yeast form, *A. pullulans* cells vary greatly in size and nuclear number, and they produce variable numbers of buds in each cell cycle. Here, we ask how equitably an *A. pullulans* mother cell distributes nuclei and growth potential amongst its multiple buds. Using live-cell imaging to track cell growth and nuclear partitioning, we find that a mother cell synchronously builds buds that grow at similar rates and attain similar volumes (~14% variability between daughters). The number of nuclei inherited by each daughter is more variable: most daughter cells inherit one nucleus, while ~10% inherit two. Mother cell nuclear number can increase, remain unchanged, or decrease in a given cell cycle. Daughters that inherit two nuclei have smaller nuclei than daughters that inherit only one, maintaining a relatively constant nuclear to cytoplasmic volume ratio (~10% variability between daughters). Interestingly, multi-budded cells appear to ensure each bud receives at least one nucleus, suggesting that nuclear segregation is coordinated among the daughters. Imaging mitotic spindle dynamics with a fluorescent tubulin probe, we found that metaphase spindles were not pre-aligned towards buds and had few astral microtubules. Anaphase was rapid and coincided with growth of astral microtubules and bending and rotation of the spindles. The fates of individual spindles in multinucleate cells was variable: in some cases the spindle deposited one pole in the mother and one in a bud as in typical budding yeasts, but in other cases both spindle poles remained in the mother, or both spindle poles entered buds. These observations do not indicate any obvious form of coordination between the spindles, and raise the possibility that cells may assure nuclear inheritance to each bud by post-mitotic error correction.

95 **Super-resolution microscopy of the temporal dynamics of septin ring formation during appressorium development by the rice blast fungus** Marisela Garduño-Rosales, Iris Eisermann, Nicholas J Talbot The Sainsbury Laboratory

Magnaporthe oryzae is the causal agent of blast disease and infects rice due to internal turgor of a dome-shaped, melanin-pigmented infection structure called an appressorium. The appressorium then enables the protrusion of a rigid penetration peg to rupture the leaf cuticle. Septins mediate repolarisation of the appressorium into the peg and are necessary for pathogenesis. The four core septins, Sep3, Sep4, Sep5 and Sep6, form a hetero-oligomeric disc-like structure which contracts into a ring at the appressorium pore. Yeast two hybrid analysis core septin interactions and co-localisation is consistent with assembly of *Magnaporthe* septins into hetero-octamers. As formation of septin alternative oligomers, such as hetero-hexamers has been reported in mammalian systems, we set out to investigate *Magnaporthe* septin oligomerisation using live-cell imaging. In this study, we performed super resolution imaging, utilising Structured Illumination Microscopy (SIM) to resolve the temporal dynamics of septin oligomerisation during appressorium development using individual and double core-septin GFP and TagRFP-tagged strains of *M. oryzae*. We have found that septin discs contain hetero-oligomers that organise into a circular mesh, while the mature septin ring follows an internally coiled pattern. Very often, loose septin filaments are observed in the inner part of the ring. Interestingly, Sep6 tagged strains display a very bright punctum at the appressorium base in addition to the ring, which may be linked to a unique function during septin aggregation. We will report the first use of super-resolution imaging of septin dynamics in *M. oryzae* and how this relates to their function in appressorium development.

96 **Cooperation between actomyosin- and microtubule-dependent transport in *Aspergillus nidulans*** Miguel A Penalva[1], Marisa Delgado[1], Paula Polonio[1], Sergio Fandiño[2], Juan Fernández-Carrillo[1], Ignacio Bravo-Plaza[1], Eduardo A Espeso[1], Mario Pinar Pinar[1] [1]CSIC Centro de Investigaciones Biológicas, [2]Animal Health, School of Veterinary

A distinguishing feature of filamentous fungi is their capacity to use microtubules and associated motors for the long distance transport of intracellular vesicles. In Aspergillus, it is widely assumed that kinesin-1 mediates the long-distance transport of secretory vesicles towards the tip, whereas actomyosin mediates the short-distance transport between subapical regions of the tip and the vesicle supply center. In principle, microtubules and actomyosin can act sequentially or in parallel. Null mutations in the genes encoding kinesin-1 and myosin-5 are synthetically lethal, which might indicate that the two pathways act in parallel. However, the fact that secretory vesicles are loaded with three different motor (at least one kinesin, dynein and myosin-5) opens the possibility that the two pathways act sequentially, at least in part. I will discuss in detail current evidence that supports the second possibility. During our studies of crosstalk between actomyosin and microtubule transport we came across accessory factors that help connecting RAB GTPase specificity determinants on vesicles with the cargo binding domain of myosin-5. Some of these studies required the development of novel tools to image actin, which will be compared with current methodology. They are largely based on expression of Lifeact under the control of promoter whose tuning presents significant advantages from the physiological point of view over the state-of-the-art. Using these reporters, we have been able to image endocytosis with unprecedented time resolution, which has served to study novel aspects of endocytic internalization and revisit the role of endocytosis in the hyphal mode of life. An unexpected outcome of these studies is the discovery of a possible mechanism that will help hyphal tip cells to recycle efficiently actin to tip-distant regions.

97 **The role of peroxisome hitchhiking in secondary metabolism in *Aspergillus nidulans*** Livia D Songster[1], Gaurav Kumar[2], Valentin Wernet[2], Patreece Suen[2], Samara Reck-Peterson[1,2,3] [1]Cell and Developmental Biology, UC San Diego, [2]Cellular & Molecular Medicine, UC San Diego, [3]Howard Hughes Medical Institute

In the filamentous fungus *Aspergillus nidulans*, peroxisomes move long distances along microtubules by forming a transient contact with early endosomes through a non-canonical trafficking process termed "hitchhiking". Peroxisome hitchhiking requires the protein PxdA, which is conserved specifically within the Pezizomycotina subphylum of Ascomycota fungi. Peroxisomes are the only organelle demonstrated to hitchhike on endosomes in *A. nidulans*, but it is unclear why. Here, we investigated the physiological function of peroxisome hitchhiking by using bulk RNA sequencing to identify transcriptional changes in peroxisome hitchhiking mutants. Mutants have mislocalized peroxisomes and altered expression of genes related to secondary metabolism (SM). SMs are organic molecules that are not strictly required for fungal growth but confer some ecological advantage. Our preliminary SM extraction results show that peroxisome hitchhiking mutants produce fewer SMs than wild type strains, despite widespread transcriptional upregulation of SM genes. SM pathways are compartmentalized into organelles to protect cells from toxic intermediates and provide regulatory control. While peroxisomes are known to be important to produce some SMs (such as penicillin, siderophores, or sterigmatocystin), the role of peroxisomes in other SM pathways is unknown. We hypothesize that peroxisome hitchhiking might be important for the production and/or secretion of certain SMs. To first estimate the relative importance of peroxisomes in SM, we bioinformatically predicted organelle localization of 1105 SM-associated genes in *A. nidulans* using a deep-learning approach. We found that 57 out of 75 predicted SM gene clusters possess >1 protein with a peroxisomal targeting sequence, supporting the idea that peroxisomes are a major site of SM production. Ongoing work is investigating the mechanism of peroxisome hitchhiking in coordinating SM across multiple organelles. Together, our data support the hypothesis that peroxisome hitchhiking might contribute to the production of some SMs. We also reveal how fungal cells compensate for loss of peroxisome hitchhiking and identify SM production and/or secretion as a potential physiological function for peroxisome hitchhiking across filamentous ascomycete fungi.

98 **Septins Regulate Exocytosis through Physical Interactions with the Exocyst Complex during Fission Yeast Cytokinesis** Davinder Singh, Yajun Liu, Jian-Qiu WU The Ohio State University

Septins are GTP-binding proteins that can function as scaffolds for protein recruitment, membrane-bound diffusion barriers, or membrane curvature sensors. Septins play important roles in cytokinesis, but the nature of these roles is still obscure. In fission yeast, four septins (Spn1 to Spn4) accumulate at the rim of the division site as rings and last until cell separation. The octomeric exocyst complex, which tethers secretory vesicles to the plasma membrane, exhibits a similar localization pattern and is essential for plasma membrane deposition during cytokinesis. We find that the exocyst complex cannot maintain its ring localization without septins. Instead, the exocyst spreads across the whole division plane during furrow ingression in cytokinesis. Loss of the exocyst complex at the rim of the division plane results in mistargeting of secretory vesicles and their cargos at the division site. This contributes to a delayed cell separation in septin mutants. These results suggest that septins and the exocyst may physically interact. Indeed, we predicted several pairs of interactions between septin and exocyst subunits by AlphaFold2 ColabFold, which

are confirmed by co-immunoprecipitation assays. Our results indicate that septins are important in regulating the exocyst localization on the plasma membrane for vesicle tethering during cytokinesis.

99 **Insights into the mating compatibility and sexual communication of *Linnemannia elongata* (Mortierellomycotina) isolates using a novel microfluidic device** Kyle T Mondron[1], Yi-Syuan Guo[2], Scott T Retterer[3], Gregory Bonito[4], Kai Castle[1], Will Gilmour[1], Jessie Uehling[1] [1]Botany and Plant Pathology, Oregon State University, [2]Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, [3]Center for Nanophase Materials Sciences and Biosciences Divisions, Oak Ridge National Laboratory, [4]Dept of Plant, Soil and Microbial Sciences, Michigan State University

Linnemannia elongata (Mortierellomycotina, Mucoromycota) produces sexual zygospores by unknown mechanisms. They also regularly host endobacteria that influence the outcome of sexual interactions between compatible mating partners. One of the major factors limiting our knowledge of Mortierellomycotina hyphal sexual differentiation and interactions between compatible isolates is the resolution available for standard light microscopic approaches. To overcome these challenges, we developed a novel microfluidic device to image *L. elongata* zygospore development in high resolution. Leveraging our growing isolate culture collection of *Linnemannia elongata*, we have typed isolates into mating groups and developed genomic resources to further our understanding of mating type structure, molecular mechanisms of mate recognition, zygospore developmental features, and how bacterial endosymbionts influence fungal mating. We will discuss our zygospore production assays and how our microfluidic device-based mating assays offer insights into the process of sexual differentiation and compatibility.

100 **Interactions between Polyextremotolerant Fungi and Photoautotrophs are Enhanced by Excreted Melanin** Erin C Carr[1], Quin Barton[1], Wayne R Riekhof[1], Steven D Harris[2] [1]School of Biological Sciences, University of Nebraska-Lincoln, [2]Plant Pathology, Entomology, and Microbiology, Iowa State University

Polyextremotolerant fungi are a paraphyletic group of melanized fungi that can withstand multiple extreme conditions simultaneously. *Exophiala viscosa* is a newly characterized polyextremotolerant fungus isolated from a biological soil crust community in the Canadian Rockies. This fungus grows in xeric, nutrient deplete environments implying highly flexible metabolism and the potential to form lichen-like mutualisms with nearby algae and bacteria. However, the exact ecological niche and interactions between this fungus and its surrounding community is not well understood. A combination of whole genome sequencing, analysis of melanin regulation, and microbial interaction experiments have been performed to fully characterize *E. viscosa* and help decipher their fundamental niche within the biological soil crust consortium. Our results reveal that *E. viscosa* excretes melanin into its environment, which can provide increased abiotic resistance, and potentially a carbon source, to the biological soil crust community. We have been able to show that in a carbonless and nitrogenless environment, a tripartite culture of *E. viscosa*, algae, and cyanobacteria are capable of survival and form extensive clumps. However, an *E. viscosa pks1* deletion mutant that cannot produce melanin does not support tripartite interactions, primarily lacking in the essential cell clumping necessary for symbiosis. This study therefore provides new insights into the regulation of melanin production in polyextremotolerant fungi, and novel data about how polyextremotolerant fungi interact with algae and cyanobacteria.

101 **A predatory fungus detects prey pheromones via G-protein-coupled receptors** Chih-Yen Kuo[1,2], Rebecca J Tay[1], Hung-Che Lin[1], Sheng-Chian Juan[1], Guillermo Vidal-Diez de Ulzurrun[1], Jason Hoki[3,4], Frank C Schroeder[3,4], Yen-Ping Hsueh[1,2] [1]Institute of Molecular Biology, Academia Sinica, [2]Molecular and Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Science, National Defense Medical Center, [3]Boyce Thompson Institute, Cornell University, [4]Dept of Chemistry and Chemical Biology, Cornell University

The ability to sense prey-derived cues is essential for predatory lifestyles. Under low nutrient conditions, *Arthrobotrys oligospora* and other nematode-trapping fungi develop dedicated structures for nematode capture when exposed to nematode-derived cues, including a conserved family of pheromones, the ascarosides. *A. oligospora* senses ascarosides via conserved MAPK and cAMP-PKA pathways; however, the upstream receptors remain unknown. Through genomic, transcriptomic, and functional analyses, we identified two families of GPCRs involved in sensing distinct nematode-derived cues. GPCRs homologous to yeast glucose receptors are required for ascaroside sensing, whereas Pth11-like GPCRs contribute to ascaroside-independent nematode sensing. Both GPCR classes activate conserved cAMP-PKA signaling to trigger trap development. This work demonstrates that predatory fungi use multiple GPCRs to sense several distinct nematode-derived cues, enabling robust prey recognition. Furthermore, identification of the ascaroside receptors in *A. oligospora* sheds light on the molecular mechanisms of cross-kingdom communication via conserved pheromones also sensed by plants and animals.

102 **An ER stress regulated signaling network orchestrates fungal-plant communication on multiple levels** Kai Heimel Georg-August-University Goettingen

Communication between organisms depends on the regular exchange and perception of external signals and is the basis of fundamental processes in nature. These include sexual reproduction and community behavior or the establishment of inter-organismic relationships. Biotrophic plant pathogens are in most cases well adapted to their host plants, and the completion of their life cycle requires extensive communication between both organisms. Using *Ustilago maydis* as a model system, we provide mechanistic details on how the unfolded protein response (UPR), a conserved pathway for ER homeostasis, orchestrates the fungal-plant interplay at multiple levels. UPR activation occurs specifically during biotrophy, linking the timing of fungal proliferation within the host plant with effector gene regulation and the ability to secrete a wide variety of effectors. In a comprehensive screening approach, we deleted over 70 UPR-regulated genes and analyzed their contribution to virulence and ER stress resistance. We identified a signal peptide peptidase (SPP)-mediated pathway that is required for plant defense suppression but dispensable for ER stress resistance and effector secretion, suggesting the existence of a novel layer of fungal-plant communication. SPPs are intramembrane cleavage proteases that cleave remnant signal peptides (after initial cleavage by the signal peptidase complex) or type II transmembrane domain-containing proteins targeting cleavage products for degradation via the ER-associated degradation (ERAD) pathway. SPPs have not been studied in plant pathogens and physiological roles known from other organisms (ERAD, hypoxia, heme oxygenase cleavage, ER homeostasis) do not contribute to the virulence function of Spp1. Plant defense suppression by Spp1 depends on its catalytic activity, which is conserved in related smut fungi and humans. Transcriptomic and genomic data suggest that the unusual connection between the UPR and Spp1, and hence its virulence-specific function, is conserved in biotrophic but not hemibiotrophic or necrotrophic fungi.

103 **One signal, two kingdoms: Decoding interkingdom plant signals in fungi** James M. Bradley[1], Michael Bunsick[1], George Ly[1], Bruno Aquino[1], Dario Bonetta[2], Peter McCourt[1], Shelley Lumba[1] [1]University of Toronto, [2]Ontario Tech University

Plants and fungi have been interacting for billions of years and must have evolved an extensive molecular dialogue to coordinate an exchange of nutrients and carbon. This dialogue consists of small molecule signals secreted by plants into the soil to mediate interactions with fungi. Despite the importance of plant-fungal interkingdom communication in agri- and ecosystems, very little is known about the mechanisms by which fungi perceive small molecule signals from plants. To address this knowledge gap, we have developed a novel pipeline centered on transcript profiling of the fungal model, *Saccharomyces cerevisiae* (yeast), treated with a plant small molecule, followed by mutational and structure-function analysis to identify a target. As a proof-of-concept, we put strigolactones (SLs), which act as both hormones in plants and as environmental communication cues for plants and fungi in the rhizosphere, through our pipeline. When plants are starving for phosphate, they produce and exude more SLs into the soil to facilitate symbiotic interactions with fungi. Suprisingly, we discovered that SL causes phosphate depletion in a variety of fungi by inhibiting the high-affinity phosphate transporter, Pho84. SL-regulated phosphate responses are conserved in an endophytic fungus called *Serendipita indica* and the pathogen, *Fusarium graminearum*. Through genetic and structure-function analyses, we have identified a potential binding pocket for SL in the phosphate transporter. Intriguingly, this binding pocket is ubiquitous in phosphate transporters across the fungal kingdom. Our results address longstanding evolutionary questions about the molecular dialogue between plants and fungi.

104 **Mechanism of niche adaptation and defence: beneficial endophytes deploy host-protective antimicrobial effectors** Laura Armbruster[1,2], Ruben Eichfeld[1,2], Lisa K. Mahdi[1], Concetta K. De Quattro[1,2], Asmamaw B. Endeshaw[1], Shingo Miyauchi[3], Margareta J. Hellmann[4], Stefan J. Cord-Landwehr[4], Igor Grigoriev[5], Daniel Peterson[5], Vasanth Singan[5], Kathleen Lail[5], Emily Savage[5], Vivian Ng[5], Gregor Langen[1], Bruno M. Moerschbacher[4], Alga Zuccaro[1,2] [1]Institute for Plant Sciences, University of Cologne, [2]Cluster of Excellence on Plant Sciences, [3]Okinawa Institute of Science and Technology Graduate University, [4]University of Münster, [5]Joint Genome Institute

Associations between plants and beneficial root-endophytic fungi enhance plant performance by improving nutrient uptake, abiotic stress tolerance, and disease resistance. To colonize diverse plant hosts and protect their niche against competing microbes, root endophytes secrete a multitude of effectors. However, the functions, specificity, and transcriptional regulation of effectors remain poorly understood. We analysed the gene expression profiles of two closely related Sebacinales fungi, *Serendipita indica* (*Si*) and *Serendipita vermifera* (*Sv*), in the presence of monocot and dicot hosts and competing microbes. All three host plants triggered extensive transcriptional reprogramming in *Si* and *Sv*, which largely overlapped with their response to the fungal competitor *Bipolaris sorokiniana* (*Bs*). This suggests there are common underlying principles in the interaction of Sebacinales with eukaryotic organisms, including the activation of genes encoding for multifunctional core effectors involved in cell wall degradation

and nutrient acquisition, such as carbohydrate-active enzymes (CAZymes). In addition, Sebacinales expressed distinct effectors in response to host plants (phytosymbiotic effectors) or microbial competitors (antimicrobial effectors). We functionally characterized one of these antimicrobial effectors, a GH18-CBM5 chitinase exclusive to Basidiomycota, and demonstrated that this enzyme hampers the growth of *Bs*, thereby reducing the disease symptoms caused by the plant pathogen in Arabidopsis and barley.

While more fungal effectors are functionally characterized every year, it is unclear how endophytes control effector gene expression. To uncover the regulatory networks underlying the induction of effector gene expression, we screened *Si* transcriptional maps for clusters of co-expressed - and hence likely functionally related - effector genes and transcription factors. Currently, we are generating transcription factor null-mutants and overexpression lines to characterize the biological relevance of promising candidates *in vivo*. In addition, we collaborate with the JGI to perform DNA-affinity purification sequencing on roughly 300 *Si* transcription factors. Understanding the regulatory landscape governing effector gene expression in beneficial fungi might pave the way for sustainable agricultural practices.

105 **Understanding the interconnected microbial life in rhizosphere and its role in shaping vascular wilt disease by *Fusarium oxysporum*** Mugdha Sabale[1], Atharv Ambekar[2], Thorsten Thiergart[3], Stephane Hacquard[3], Antonio Di Pietro[1], Amey Redkar[2] [1]Dept of Genetics, University of Córdoba, [2]National Centre for Biological Sciences (NCBS), [3]Dept of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research

Fungi pose a significant threat to human nutrition and global food security. Fungal pathogens provoke devastating yield losses annually. A significant proportion of these losses are caused by pathogenic fungi belowground, that have evolved strategies to outcompete with their microbial competitors in the rhizosphere and actively suppress the plant immune response. Plants on the other hand, have associated benign microbial communities like the root-associated bacteria which can interact with soil-borne phytopathogens, and determine the fate of microbial interactions towards pathogenic or non-pathogenic outcomes.

The fungal pathogen *Fusarium oxysporum* (Fo) is a soil-borne root infecting fungus that cause vascular-wilt disease in diverse agricultural crops and represents a serious threat world-wide. As this fungus deals with a rich microbial ecosystem to enter plant roots, mechanistic understanding of its behaviour during interkingdom microbial confrontation is important. We aim to understand the molecular mechanisms that determine interkingdom interactions during fungal-plant associations in the rhizosphere.

Using meta-transcriptomics approach, we have defined the compartment specific rhizo-bacterial communities in response to the vascular wilt Fo and have identified bacterial communities that exert an antagonistic effect on pathogen growth. To define such bacterial communities and their interactions, we implement a high throughput pathogen growth reporter assay to determine bacterial isolates with antagonistic activity against *F. oxysporum in vivo.* A semi-sterile gnotobiotic interaction system with these identified bacterial antagonists further revealed their growth promoting and resistance traits on pathogen colonization, showing an interference with the Fo driven mortality. These microbial interaction occurs both at physical and chemical level triggering plant immune activation.

Our work hence demonstrates modulation of rhizosphere microbiome by a root fungal pathogen and the counteraction of beneficial bacterial families which shape outcome of pathogen invasion and plant health in rhizosphere interactions.

106 **Do fungal terpenoids volatiles structure the mycosphere?** Erika Kothe[1], Katrin Krause[2] [1]Friedrich Schiller University, [2]Friedrich Schiller Univresity Jena

Fungi play a major role in structuring their soil habit. In addition to involvement in wood degradation and element cycling, adding structure *via* hyphal growth and excreting carbon sources for the use of bacterial commensals, they release a bouquet of terpenoid volatiles. The effect of such terpenes was investigated for two major habitats in forest soils: rotting wood and ectomycorrhizal symbiosis. Using the wood-rotting basidiomycete, *Schizophyllum commune*, as well as the ectomycorrhizal *Tricholoma vaccinum*, a major impact of fungal terpenes on the microbiome and ectomycorrhizosphere could be established. In an early succession woodland, the effect of basidiomycetes on the bacteriome could be verified.

Here, we show that volatiles of *S. commune* are active against wood-decay fungi and bacteria found in its mycosphere. *Actinobacteria* and *Proteobacteria*,including *Pseudomonadaceae*, *Sphingomonadaceae*, *Erwiniaceae*, *Yersiniaceae* and *Mariprofundaceae*, dominated the microbiome in *S. commune* white rot. With the major sesquiterpenes β-bisabolol, β-

bisabolene and (E)-γ-bisabolene, growth of the competing wood-degraders *Ganoderma lucidum, Flammulina velutipes* and *Kuehneromyces mutabilis* was severely inhibited, while reduced swarming motility and hence reduced distribution was seen with *Serratia marcescens* and *Bacillus subtilis* isolated from the same rotting wood.

In the ectomycorrhizal interaction, *T. vaccinum* terpenes are involved in host specificity. In addition, the ectomycorrhiza fungal community in a woodland determines the bacterial community. Thus, not only can the tree smell an ectomycorrhizal friend, but also soil-dwelling bacteria respond to fungal volatiles. We therefore can conclude that, indeed, basidiomycetes structure their mycobiome in soil providing a major ecosystem service.

107 **Biomolecular condensates in fungi are tuned to function at specific temperatures** Ben Stormo, Amy Gladfelter Duke University

Temperature can impact every reaction and molecular interaction essential to a cell. For organisms that cannot regulate their own temperature, a major challenge is how to adapt to temperatures that fluctuate unpredictability and on variable timescales. Biomolecular condensation offers a possible mechanism for encoding temperature-responsiveness and robustness into cell biochemistry and organization. To explore this idea, we examined temperature adaptation in a filamentous-growing fungus called *Ashbya gossypii* that engages biomolecular condensates containing the RNA-binding protein Whi3 to regulate mitosis and morphogenesis. We collected wild isolates of *Ashbya* that originate in different climates and found that mitotic asynchrony and polarized growth, which are known to be controlled by the condensation of Whi3, are temperature sensitive. Sequence analysis in the wild strains revealed changes to specific domains within Whi3 known to be important in condensate formation. Using an *in vitro* condensate reconstitution assay we found that temperature impacts the relative abundance of protein to RNA within condensates and that this directly impacts the material properties of the droplets. Finally, we found that exchanging Whi3 genes between warm and cold isolates was sufficient to rescue some, but not all, condensate-related phenotypes. Together these data demonstrate that material properties of Whi3 condensates are temperature sensitive, that these properties are important for function, and that sequence optimizes properties for a given climate.

108 **Dynamic Actin Remodeling via Molecular Condensation in Fungal Signaling** Yansong Miao School of Biological Sciences, Nanyang Technological University

The intricate spatial and temporal control of dynamic actin cytoskeleton assembly plays a pivotal role in driving fungal development and resilience under both normal and stress-induced conditions. A key aspect of this regulation involves the adjustable multivalent interactions among actin binding proteins and their macromolecular condensation during signal transduction. Here, we introduce how the multicomponent condensation of polarisome complexes influences the nucleation and crosslinking of the actin cables in yeast polarity and hyphae growth in filamentous fungi. Utilizing a combination of quantitative biochemical and biophysical systems, cell imaging, machine learning, and theoretical modeling, we have discerned a range of outcomes of polarisome condensates affecting fungal physiology and stress response, via regulating their material properties and biochemical activities.

109 **The effects of phase separation on chromatin modifications, transcriptional regulation and virulence in the human fungal pathogen *Candida albicans*** Qing Lan, Zhengqiang Miao, Ruiwen Chen, Pin Wu, Songlin Wu, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau

Candida albicans is an opportunistic pathogen that can live in the human body as a commensal and cause deadly infection when the immune system is compromised. *C. albicans* can colonize different niches and survive the wide range of stresses elicited from the host during infection. These abilities are mediated by rapid and dynamic transcriptional responses, which are tightly controlled at transcription levels (e.g. transcriptional activation, elongation, and termination) and chromatin modification. The phenomenon of liquid-liquid phase separation can rapidly trigger the formation and dissociation of cellular compartments for biomacromolecules like proteins and nucleic acids without physical barriers. The process is reversible and tunable by factors relevant to the infection process, such as pH, temperature, and salinity. Phase separation has been shown to play crucial roles in controlling various biological processes and pathways in many organisms. We hypothesize that phase separation is important for the transcriptional responses of pathogens during adaption to diverse environmental conditions and infection. This study investigated the role of phase separation on the transcription and pathogenicity processes in *C. albicans.*

110 **Alternative splicing regulation in plants by effectors of symbiotic arbuscular mycorrhizal fungi** Ruben Betz[1], Sven Heidt[1], David Figueira-Galan[1], Anna Miucci[1], Thorsten Langner[2], Natalia Requena[1] [1]Karlsruhe Institute of Technology KIT, Joseph Gottlieb Kölreuter Institute for Plant Scienes JKIP, [2]Max Planck Institute for Biology Tuebingen

Most plants in natural ecosystems live in association with beneficial AM (arbuscular mycorrhizal) fungi to survive under poor nutrient conditions and to cope with other abiotic and biotic stresses. To engage in symbiosis, AM fungi secrete effector molecules that, similar to pathogenic effectors, reprogram plant cells. Despite numerous effectors being predicted in the genome of AM fungi, only a few have been functionally characterized. Here we show that the SP7-like family impacts on the alternative splicing program of their hosts. We identified 13 members of this effector family within the genome of the model organism *Rhizophagus irregularis*, including the presence of several effector paralogs. In addition, SP7-like effector sequences were detected in various other symbiotic fungi of the Glomeromycotina phylum, but were absent from fungal species outside this clade. *In planta* expression of SP7-like members revealed their localization at cellular condensates within the plant nucleus and cytoplasm. Biomolecular condensates are often described as the result of phase separation to form micro-compartments in which functional relevant molecules are concentrated, such as mRNA processing related nuclear splicing speckles or P-bodies that serve as cytoplasmic mRNA degradation centers. Indeed, we found multiple components of the plant mRNA processing machinery that physically interacted with the SP7-effector family, most prominently with the splicing factor SR45. Co-expression of SP7-like members and SR45 led to re-localization of the effectors to SR45 occupied nuclear condensates, while co-expression with the plant P-body marker DCP2 additionally demonstrated P-bodies as effector localization target. Furthermore, ectopic expression of two of these effectors in the crop plant potato changed the alternative splicing pattern of a specific subset of SR45 related genes. Cell-type specific expression of SR45 in arbuscule containing cells negatively impacts symbiosis progression, indicating the need for a fine balanced SR45 activity during plant-fungal association. Together our data suggest a scenario where the SP7-like effector family targets the plant mRNA processing machinery, engages in mRNA processing related phase separation events and influences the activity of SR45, ultimately enabling full establishment of symbiosis. Future approaches aim towards identifying specific plant mRNA effector targets using *in planta* RNA immunoprecipitation as well as studying the effector ability to directly bind to RNA molecules.

111 ***Cryptococcus*** **employs alternative translation of a novel regulator *REF1* to produce isoforms with differential capacities for phase separation to control morphogenesis** Nathan K Glueck, Xiaorong Lin Microbiology, University of Georgia

Cryptococcus neoformans causes deadly meningoencephalitis in hundreds of thousands of immunocompromised individuals worldwide. *Cryptococcus* is a polymorphic fungus able to switch between yeast and hyphal growth as part of its sexual cycle, as a way of defending against natural predators, and as a mediator of fungal immunogenicity when interacting with mammalian hosts. The yeast-to-hypha transition is controlled by the transcription factor Znf2. Here, we have identified a new gene that antagonizes Znf2-mediated transcriptional activation and suppresses cryptococcal yeast-to-hypha transition. We named this gene repressor of filamentation 1 or *REF1*. Surprisingly, *REF1* transcription is controlled by Znf2 and is dramatically *increased* during hyphal morphogenesis. We discovered that transcriptional activation of *REF1* in response to filamentation-inducing stimuli produces 5'-truncated *REF1* transcripts. Transcription of these truncated transcripts starts downstream of the 5' splice site of the first intron in the *REF1* ORF, producing a larger Ref1 protein with an alternate N-terminus. Under nutrient-rich conditions favorable to yeast growth, basal level transcription of unmodified *REF1* transcripts results in the translation of Ref1 protein with a diffuse nucleoplasm localization. However, filamentation-inducing nutrient limitation causes a spike in 5'-truncated *REF1* transcripts (outnumbering basal level transcripts >20 fold) that are translated into a Ref1 isoform with a distinct nuclear punctate localization pattern indicative of phase separation. This nuclear punctate Ref1 isoform appears superior to the nuclear diffuse Ref1 in inhibiting filamentation but is more prone to proteolytic degradation. Our evidence points to a model in which Ref1 acts as a built-in negative regulator of Znf2-mediated transcriptional activation during the nascent stages of hyphal morphogenesis both as a bet-hedging strategy to avoid premature commitment of cellular resources to the yeast-to-hypha transition and to exert tight spatiotemporal control over the developmental process.

112 **Exploring RNA thermosensors that drive development and virulence in thermally dimorphic fungal pathogens** Murat Can Kalem[1], Mark Voorhies[2], Anita Sil[2] [1]Microbiology and Immunology, University of California San Francisco, [2]University of California San Francisco

Human body temperature is a key signal sensed by fungi to elicit developmental programs that facilitate pathogenesis. The molecular mechanisms of temperature sensing and thermosensors remain enigmatic. We hypothesized that fungal transcriptomes harbor temperature-responsive RNA structures. We are exploring RNAs as potential thermosensors in the thermally dimorphic fungal pathogens *Histoplasma* and *Coccidioides.* They are the ideal systems to investigate RNA thermosensors since host temperature is the main signal that triggers the reprogramming of morphology and gene

expression. *Histoplasma* and *Coccidioides* grow as multicellular hyphae in the soil but transition to a yeast or spherule form in the host in response to elevated temperature.

RNA is multifunctional due to its ability to fold into complex three-dimensional structures that can interact with other molecules, such as RNA-binding proteins (RBPs). We adopted a global and unbiased RNA structure probing approach, DMS-MaP-seq, to discover temperature-responsive RNA elements and structures. DMS-MaP-seq, along with efficient rRNA depletion, gives us the opportunity to explore the role of RBPs and helicases on thermally regulated structures. RBPs and helicases may be differentially expressed or functional at various temperatures and act on structural elements. Some *Histoplasma* RBPs and helicases have differential translation efficiencies at 22˚C and 37˚C, including the helicase Ded1. We are knocking down Ded1 in *Histoplasma* and investigating its role in regulation of morphology.

In a complementary candidate approach, we are exploring the role of RNA guanine quadruplex (rG4) structures in thermosensing and development. *Histoplasma RYP2,* a transcription factor that is required for yeast-phase growth, has a longer 5' UTR only at room temperature with two putative rG4 structures. The longer 5' UTR correlates with a robust decrease in translation. rG4 structures modulate translation, RNA decay, and phase separation. We utilized carboxy pyridostatin (cPDS), which stabilizes rG4s, to begin dissecting the role of rG4s in morphology. cPDS promoted hyphal growth in *Histoplasma* and altered *Coccidioides* development. This work highlights that RNA structure is crucial for temperature-responsive fungal development. Interrogation of structure-function relationships and effectors contributing to thermosensing will continue to unravel fundamental mechanisms of RNA regulation.

113 **Investigating spatial protein quality control in filamentous fungi** Martin Egan, Audra Rogers, Baronger Bieger Entomology and Plant Pathology, University of Arkansas

The spatial sequestration of damaged and misfolded proteins into specialized quality control compartments represents an important strategy for maintaining protein homeostasis in response to diverse cellular stress. How this process is controlled in time and space and integrated with other protein homeostasis pathways remains unclear. We are exploiting two model filamentous fungi, *Aspergillus nidulans*, and *Magnaporthe oryzae*, to investigate spatial protein quality control within highly polarized hyphal compartments, and during the formation of terminally differentiated infection cells called appressoria, respectively. Here we show that in the absence of Hsp104 disaggregase activity in *M. oryzae*, aggregated proteins are dynamically sequestered into quality control compartments within conidial cells, but not within appressoria, and thus spatial protein quality control pathways exhibit cell type-dependency. We demonstrate that molecular chaperones and misfolded proteins undergo bi-directional microtubule-based motility in highly polarized cells upon heat stress, and associate with motile early endosomes. We explore the role of organelles in aggregate sequestration and retention during cytokinesis, and investigate the cellular fates of model aggregating proteins following proteostatic perturbations using photoconvertible fluorescent proteins. We reveal that impaired aggregate resolution results in a short-term developmental penalty but has no significant impact upon appressorium functionality or pathogenesis. Finally, we show that the bulk autophagy machinery is necessary for the normal formation and compartmentalization of protein aggregates during terminal cellular differentiation. Taken together, our findings provide new insight into spatial protein quality control during cellular differentiation and polarized growth in filamentous fungi, and reveal a new level of interplay between major proteostasis pathways.

114 **How fuzzy molecular interactions can keep strict organismal time.** Jennifer M Hurley Biological Sciences, Rensselaer Polytechnic Institute

Protein macromolecular complexes, the fundamental progenitors of macromolecular condensates, can have profound effects on cellular functions. An archetypical example of this are the complexes that generate circadian rhythms, the highly conserved, 24-hour, oscillations that tune physiology to the day/night cycle. Disruption of proper circadian timing negatively impacts organismal fitness, making understanding the mechanism underlying circadian regulation over cellular physiology critical to appreciating a fundamental rule of life on earth. Circadian rhythms are controlled via a transcription-translation based negative feedback loop, or clock, where an activating arm stimulates the creation of a repressive arm that inhibits the function of the activating arm. The current paradigm for circadian regulation over physiology, termed the clocks "output", is that transcriptional programing generated by the clock drives temporally-specific waves of gene expression. However, our research has revealed that transcriptional programing cannot wholly account for clock output, as we discovered weak correlation between mRNAs and proteins that oscillate with a circadian periodicity. We have shown that intrinsic protein disorder in the repressive complex of the clock may control the formation of fuzzy macromolecular complexes to time clock output post-transcriptionally. This regulation occurs through the circadian modulation of the heterogeneous ensemble of conformations of the repressive complex over the circadian day. Further, we found a repressive arm isoform-specific effect on the formation of these fuzzy complexes. Understanding

the mechanisms that underly the formation of these fuzzy complexes grants insight into both how the circadian clock times biological function across species as well as the principles of IDP functionality. These insights will allow us to understand if, and how, fuzzy complexes could lead to circadianly time condensation events.

115 **Aflatoxin production regulation: a role for volatile and non-volatile chemicals in biocontrol interactions between *Aspergillus flavus* strains** Rebecca R Sweany, Geromy G Moore, Mallika Kumarihamy, Matthew D Lebar Food and Feed Research Unit, U.S. Dept of Agriculture

Aflatoxin is an acutely toxic and carcinogenic compound produced by the fungus *Aspergillus flavus* that contaminates corn, peanuts and tree nuts. Application of non-aflatoxigenic *A. flavus* strains to soils during the growing season is an effective biocontrol strategy to decrease aflatoxin contamination. Historically, the mechanism of biocontrol was reported to be displacement of toxigenic strains by non-aflatoxigenic strains. However, recent evidence suggests that chemosensing is another mechanism whereby both volatile organic compounds (VOCs) and other secreted secondary metabolites (extrolites) reduce fungal growth and/or aflatoxin production. VOCs and extrolites produced by different non-aflatoxigenic strains were screened for their abilities to reduce growth and/or aflatoxin production by multiple toxigenic strains. Despite having little to no impact on growth, VOCs 3-octanone, trans-2-methyl-2-butenal, 2,3-dihydrofuran and decane significantly reduced aflatoxin production by two *A. flavus* and one *A. parasiticus* isolates from Louisiana by 25-95%. Decane consistently resulted in the greatest reduction of aflatoxin by 91 – 95 %. Kojic acid, an extrolite, also reduced aflatoxin production at biologically relevant, although high, concentrations of 16 mg/ml by 20-25%. In some instances, 4 and 8 mg/ml of kojic acid increased aflatoxin production by 30-60%. Non-aflatoxigenic biocontrol isolates produced large quantities of kojic acid (250 to 750 mg/g fungal dry weight). These metabolites, especially the VOCs, likely improve the efficacy of non-aflatoxigenic biocontrol strains by lowering aflatoxin production. Research continues to elucidate how these chemicals genetically regulate aflatoxin production. Since kojic acid is only inhibitory at higher concentrations and highly inhibitory strains produce variable quantities of kojic acid, its role in regulating aflatoxin synthesis during the biocontrol interaction is less clear than VOCs and suggestive that other extrolites are contributing to aflatoxin inhibition. The VOCs, especially decane, hold promise as biofumigants that mimic the presence of biocontrol strains in shipping and storage containers to maintain post-harvest inhibition of aflatoxin contamination.

116 **Survey of lipooxygenase genes in phytopathogenic fungi** Kayla K Pennerman ORISE

Despite their apparent importance across eukaryotic and prokaryotic phyla, lipoxygenases (LOXs) are still relatively understudied. These enzymes catalyze oxidation of polyunsaturated fatty acids to hydroperoxides and is a component of pathways that yield volatile organic compounds involved in signaling processes. Unlike other taxa that only have iron-containing LOXs, fungal LOXs use either iron or manganese as the catalytic metal; fungal LOXs may also be secreted during infection of a plant host. The annotations of 62 assembled genomes of phytopathogenic ascomycetes were retrieved, and their respective proteomic sequences were searched against with hidden Markov model profiles of known LOX amino acid sequences. As a result, 77 sequences in 36 strains of 20 species were identified. The sequences had 1 to 3 isoforms within each proteome. Most sequences were from *Fusarium* spp. and *Zymoseptoria tritici*, both of which included supposed iron and manganese LOXs with differently conserved regions. Such proteins were not identified in 14 species representing genera including *Blumeria*, *Eremothecium* and *Parastagonospora*. Sequence alignments and annotations of surrounding genes revealed an association with ATP binding and nuclear localization functions. With predicted three-dimensional structures, putative metal centers, active sites and oxygen channels were also identified and compared. The functions and selection mechanisms of iron versus manganese LOXs are still undetermined. While LOXs may play roles in disease development, it is not clear why the proteins would evolve to use a different co-factor or how multiple isoforms are beneficial. Foundational knowledge of the types and distributions of LOXs within ascomycetous phytopathogens helps to generate new hypotheses for these questions.

117 **Exploring effect of Ethyl 3-methylbutanoate on fumonisin production and *FUM* gene expression in *Fusarium verticillioides*** Antonia Susca[1], Alessandra Villani[2], Laurie Josselin[3], Vincenzo Lippolis[2], Salvatore Cervellieri[4], Thomas Netti[5], Daria Carella[1], Robert H Proctor[6], Antonio Moretti[1] [1]Institute of Sciences of Food Production (ISPA), National Research Council of Italy, [2]National Research Council of Italy, [3]Gembloux Agro-Bio Tech, Liege University, [4]Institute of Sciences of Food Production (ISPA, National Research Council of Italy, [5]Institute of Sciences of Food Production, National Research Council of Italy, [6]United States Dept of Agriculture, Agriculture Research Service, National Center for Agricultural Utilization Research

Volatile organic compounds (VOCs) are secondary metabolites emitted by organisms and play a role during interactions with other organisms. In the context of fungi, VOCs can serve various purposes, including intraspecies communication, attraction or repulsion of other organisms, and regulation of growth and development. *Fusarium verticillioides* is a major fungal pathogen of maize and produces fumonisins, mycotoxins of worldwide concern to food and feed safety. Therefore, developing innovative control

strategies is essential to reduce the negative impacts of fumonisin contamination in maize. Recently, we found that the VOC ethyl-3-methylbutanoate (E3MB) is emitted by fumonisin-nonproducing mutants of *F. verticillioides* but not by their wild-type progenitor strain. To study the potential of E3MB as a fumonisin inhibitor and understand its mode of action, fumonisins and *FUM* gene expression were monitored in cultures of the mutant and wild type following exposure to E3MB. Two application modes were investigated: a *contact* condition, where E3MB was introduced into the substrate, and a *non-contact* condition, where E3MB was only introduced into headspace of cultures. Although E3MB inhibited fumonisin production in the wild type under both conditions, its effects on *FUM* gene expression varied significantly depending on the application mode. In the *contact* condition, *FUM* genes were overexpressed, while in the *non-contact* condition, most *FUM* genes were down-regulated. These results suggest that E3MB inhibits fumonisin production by different mechanisms in the different application modes. These findings provide insights for potential biocontrol strategies against fumonisin contamination caused by *F. verticillioides*. However, additional investigations, including epigenetic analysis, are needed to fully understand the mechanism by which E3MB inhibits fumonisin production.

118 **"*Daldinia* cf. *concentrica*, its VOCs and their impact on plant pests"** David Ezra, Orna Liarzi Plant Pathology and Weed Research, ARO, The Volcani Institute

Endophytic fungi represent microorganisms that predominantly inhabit plant tissues most of their life cycle, exerting negligible visible harm to their host plants. Numerous endophytes have been observed to produce secondary metabolites, facilitating their survival within the plant and conferring advantageous traits to their hosts, such as heightened growth and resilience to both biotic and abiotic stressors. Certain endophytic fungi possess the capability to release volatile organic compounds (VOCs), some of which exhibit biological activity. The endophytic fungus *Daldinia* cf. *concentrica*, isolated from an olive tree (*Olea europaea* L.) in Israel, emits biologically active VOCs. Among these compounds, *trans*-2-octenal displays antifungal properties against diverse phytopathogenic fungi, nematocide and herbicide activity. Characterized as a linear unsaturated aldehyde with eight carbons and a double bond located at the alpha position of the carbonyl group, *trans*-2-octenal's structural significance was *in vitro* investigated by comparing the effects of various molecules with similar structures on the growth and viability of the fungal soil-borne pathogen *Sclerotium rolfsii* (*Athelia rolfsii*). The findings suggest a structure-function relationship in the antifungal activity of *trans*-2-octenal. Additionally, *trans*-2-octenal exhibited efficacy against *Fusarium oxysporum* under field conditions, as part of soil disinfection product development. Moreover, *D. concentrica* demonstrated activity against the root-knot nematode *Meloidogyne javanica*. To elucidate the mechanism of action of fungal VOCs against nematodes, their impact on the model nematode *Caenorhabditis elegans* was examined. Our study demonstrates that 4-heptanone, another VOC from the endophyte, hinders egg hatching, larval survival and development. A potential mode of action for the VOCs is proposed.

119 **Yeast volatile organic compounds: antifungal and nutraceutical effects on cultivated mushroom species** Alessandra Di Francesco[1], Michele Di Foggia[2] [1]University of Udine, [2]University of Bologna

Yeast volatile organic compounds (VOCs) were investigated comprehensively for their effective biological control against different plant fungal pathogens. In most cases, VOCs produced by yeasts positively affect crops, acting as antifungals or biostimulants. According to results obtained from *in vitro* assays, VOCs produced by *Aureobasidium pullulans* and *Metschnikowia pulcherrima* strains limited green mold without hindering the growth of *Pleurotus ostreatus*, *Lentinula edodes,* and *Cyclocybe cylindracea*, thus indicating beneficial effects of these VOCs on the growth and nutraceutical composition of mushroom crops.

In specific tests of mycelial growth in *Trichoderma atroviride*, *T. pleuroti*, *P. ostreatus*, *L. edodes* and *C. cylindracea*, VOCs produced by the antagonistic yeasts significantly inhibited the growth of *Trichoderma* spp. but did not affect mushroom mycelial growth. Conversely, *M. pulcherrima* VOCs significantly stimulated *L. edodes* mycelial growth. FT-IR spectroscopy on mushroom mycelia exposed to yeast VOCs allowed us to correlate stimulation of their mycelial growth with an increased protein and lipid content and a general decrease in glucans, thus determining interesting nutraceutical differences. Moreover, compounds emitted by *A. pullulans* and *M. pulcherrima* strains were identified by solid-phase microextraction (SPME)–gas chromatography mass spectrometry (GC-MS), revealing that the alcohol class dominated the volatilomes, particularly in *A. pullulans*. In contrast, *M. pulcherrima* was the only yeast producing an ester, isobutyl acetate, which is often recognized as a plant growth promoter. Using different yeasts to produce a composite volatilome could be advantageous for cultivation of edible fungi because a variety of compounds could hinder fungal pathogens present in natural habitats and, at the same time, improve crop growth and nutritional aspects. Further studies will be necessary to better understand the potential application of VOCs and their effect in the context of large-scale mushroom cultivation.

120 **VOC profiles from a chestnut blight fungus *Cryphonectria parasitica* in response to hypovirus CHV1** Yo-Han Ko, Jeesun Chun, Kum-Kang So, Ngoc My Tieu Le, Dae-Hyuk Kim Jeonbuk National University

The chestnut blight fungus, *Cryphonectria parasitica*, its hypovirus comprise useful model system to study fungus-virus interactions. Infection by hypovirus, Cryphonectria hypovirus 1 (CHV1) results in various phenotypic changes in the fungal host including hypovirulence and other associated symptoms such as altered metabolism, retarded development, and reduced sporulation. Although many studies regrading what are the factors affected by hypovirus infection and how these changes occurred, studies on VOC (Volatile Organic Compound) have not been conducted. In this study, we characterized VOC profiles of *C. parasitica* and analyzed the changes in VOCs by CHV1 infection over a 20 days growth period. A total of 65 predominant VOCs with high similarity to the known database were identified. Among these, 51 VOCs were identified from the virus-free EP155/2, 54 VOCs were from the virus-infected UEP1, and 40 VOCs were found from both strains. Among 51 EP155/2-released VOCs, phenylethyl alcohol is the most prevalent VOC but the most predominant VOC from UEP1 changed as culture proceeded, i.e., β-phellandrene (26.4% at 5-day culture), phenylethyl alcohol (32.7% at 10-day culture), and phenylethyl alcohol (51.2% at 20-day culture). Terpenes are most common members of VOCs from *C. parasitica*. Sesquiterpenes were more common in EP155/2 while monoterpenes were major in UEP1. VOC profiles changed greatly depending on the culture period. More importantly, CHV1 infection affected not only the component profiles of VOCs but also the amount of specific VOC released. Transcription analysis of genes responsible for the synthesis of corresponding VOCs revealed that the expression of genes was affected by the CHV1 infection suggesting the presence of hypoviral regulation of fungal metabolic gene expression. Interestingly, olfactory behavioral assay indicated that there was difference in attractiveness between virus-free and -infected strains, i.e., UEP1 showed greater attractiveness to insect than EP155/2, which suggests the difference in efficacy of insect-borne dissemination of this fungus.

121 **Following Fungal Farts: Using random barcoded transposon-site sequencing (RB-TnSeq) bacterial libraries to explore the effects of volatiles from the filamentous fungus Trichoderma atroviride** Catharine Adams[1,2], Jose Manuel Villalobos Escobedo[1,2], Mitchell G Thompson[1,2], Adam M Deutschbauer[1,2], Louise Glass[1,2] [1]UC Berkeley, [2]Lawrence Berkeley National Laboratory

Plant-associated fungi provide their hosts with many important health related benefits, and can even protect the plant from invading microbial pathogens. Trichoderma atroviride IMI is a plant root-associated filamentous fungus with potent antimicrobial effects, and volatile organic compounds (VOCs) from T. atroviride have been shown to discourage growth of a range of pathogenic microbes. However, few studies have explored how these VOCs may impact plant beneficial root-associated microbes. We predict that both volatiles and secreted metabolites will significantly shape fungal-bacterial interactions in the rhizosphere.

To test this hypothesis, we used a co-culture method we call a “Thunderdome” to assess how VOCs from T. atroviride impact the physiology of six Plant Growth Promoting Bacteria (PGPB) selected from across the proteobacteria: Azospirillum brasilense Sp245 and Sinorhizobium meliloti 1021 (alpha-proteobacteria), Burkholderia phytofirmans PsJN and Herbaspirillum seropedicae SmR1 (beta), and Klebsiella michiganensis M5al and Pseudomonas simiae WCS417(gamma). We also tested whether the VOCs significantly impacted 15 additional bacterial species that were isolated from the switchgrass rhizosphere.

We found fungal volatiles inhibited the growth of most tested bacteria, even when HEPES buffer was added to the media to prevent lowering of pH by CO2 or other acidic volatiles. A crystal violet staining assay showed that, in Azospirillum brasilense Sp245, fungal volatiles specifically inhibited biofilm formation. By adding individual VOCs to bacterial media, we have found that sensitivity to each compound varies considerably across species. Using bacterial Random Barcode Transposon Sequencing (RB-TnSeq) libraries, we are further investigating which bacterial genes in the different rhizosphere species are relevant for coping with specific VOCs produced by this fungus.

By elucidating the system wide effects of fungal derived VOCs in the rhizosphere, we can begin to design microbially driven strategies to enhance beneficial plant-rhizosphere relationships, and improve overall plant health.

122 **Spatial variability in bacterial and fungal communities of apples (*Malus domestica*): unexpected patterns of nestedness and co-occurrence from individual fruits to the orchard scale** Justin P Shaffer[1], Rob Knight[2,3], Susan R Whitehead[4] [1]Biology, California State University, Fresno, [2]Dept of Pediatrics, School of Medicine, University of California, San Diego, [3]Dept of Computer Science and Engineering, University of California, San Diego, [4]Biological Sciences, Virginia Tech

Advances in the biological sciences have expanded our understanding of how we define an organism. Similar to the human microbiome contributions to human health and sustainability, terrestrial plants are now best understood as just one component of the **phytobiome** – the plant and its associated microbiome, herbivores, pollinators, and other symbionts. Nearly all plant traits

can be influenced by the phytobiome, including growth rate, nutrient uptake and retention, and responses to environmental stress and disease. Looking forward, it is of utmost importance that current management practices incorporate our understanding of the phytobiome and its effects on plant health, including the links between management practices, phytobiomes, and food quality and yield. Here, we use apples as a model system to improve our fundamental understanding of the fruit microbiome and overall agroecosystem of an apple orchard, including variables that shape and that are impacted by the fruit microbiome. Most studies of the apple phytobiome have focused on broad patterns of microbial community composition across regions, or at the consumer point-of-purchase. However, a critical first step in developing microbially-based management practices is to understand the spatial variability of apple microbial communities and associated changes in fruit quality at scales relevant for local management. We conducted a spatially-explicit survey of microbial communities at multiple scales within a single orchard: across 17 trees within a single orchard block, across 16 fruits within a single tree, and across 22 microsites within a single fruit. For each fruit, we sampled both epiphytes present on fruit surfaces, and endophytes colonizing internal fruit tissues (i.e., pulp). We performed a spatial analysis of bacterial+archaeal and fungal communities, and volatile small molecules from these samples, including analyses of nestedness of microbes and samples across space, as well as co-occurrence of different microbial taxa. Our results highlight unexpected patterns of nestedness and co-occurrence of bacteria+archaea and fungi, and inform best practices for profiling microbes and metabolites for monitoring rapid changes in community composition and metabolic profiles across space. The long-term outcomes of this work include improving human health and increasing the economic sustainability of farms, in part by optimizing pest management via biotechnological applications with the phytobiome.

123 **The ins and outs of *Magnaporthe oryzae* effectors** Barbara Valent[1], Ely Oliveira-Garcia[2], Tyler Suelter[3], Melinda Dalby[3], Sanzhen Liu[3], David Cook[3] [1]Plant Pathology, Kansas State Univ, [2]Dept of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, [3]Dept of Plant Pathology, Kansas State University

Magnaporthe oryzae (synonym of *Pyricularia oryzae*) causes blast diseases on rice, wheat, and other grasses. The blast fungus executes a hemibiotrophic lifestyle in which specialized invasive hyphae grow to consume and fill living plant cells, one after another, to form sporulating eyespot lesions. During host invasion, *M. oryzae* secretes dozens of effectors, small proteins that promote disease development. Live cell microscopy of fungal host leaf sheath invasion has allowed us to track in planta effector dynamics, such as with the cytoplasmic effectors PWL2 (prevents pathogenicity to weeping lovegrass, *Eragrostis* species) and BAS1 (biotrophy-associated secreted protein-1). Compared to normal Golgi-mediated secretion of apoplastic effectors into the extracellular spaces around the fungus, cytoplasmic effectors including PWL2 and BAS1 are secreted via a nonconventional Golgi-independent secretion system by invasive hyphal cells associated with an enlarged interfacial region, the Biotrophic Interfacial Complex (BIC). Recent data supports that these hyphal cells and associated BICs are the sites for cytoplasmic effector secretion and translocation across the plant plasma membrane into the plant cytoplasm. Results using live cell imaging, pharmacological inhibitors, and virus-induced gene silencing indicate that translocation of effectors across plant plasma membrane is mediated by plant clathrin-dependent endocytosis. Once reaching the cytoplasm of the invaded cells, these effectors move through plasmodesmata into surrounding cells before invasion. Understanding the biology of blast effector movements in, around and between host plant cells will ultimately impact blast disease control.

Curiously, the genes encoding effectors PWL2 and BAS1 are located in different core chromosomes in the rice infecting population, but they only occur side-by-side in dispensable wheat pathogen mini-chromosomes (mini-chr). Questions being investigated include conservation of the *PWL2/BAS1* mini-chr segment despite structural instability of the mini-chr, the global distribution of *PWL2/BAS1* mini-chr among the *M. oryzae* lineages adapted to different hosts, as well as the increasing occurrence of the *PWL2/BAS1* mini-chr in present-day aggressive wheat blast field isolates compared to the early strains from Brazil in the late 1980s. Understanding the basic biology and genome dynamics of mini-chr will aid in understanding the origin, evolution, and pathogenicity variation across the *M. oryzae* population.

124 **A fungal abscisic acid-like and botrydial metabolic gene cluster critical for mutualist-pathogen transition in root fungal endophyte *Colletotrichum tofieldiae*** Ren Ujimatsu, Takeshi Higa, Nhi Nguyen Tan Anh, Kei Hiruma The University of Tokyo

Plant-associated fungi show diverse lifestyles from pathogenic to mutualistic to the host; however, the principles and mechanisms through which they shift the lifestyles require elucidation. The root endophytic fungus *Colletotrichum tofieldiae* (Ct) promotes *Arabidopsis thaliana* growth under phosphate limiting conditions (Hiruma et al., Cell 2016). The fungal species are isolated from both dicot and monocot plants grown in various geographical regions. Here, we reveal a Ct strain, designated Ct3, that colonizes *A. thaliana* roots and severely inhibits plant growth during the root colonization, which is very contrast to other beneficial Ct strains promoting plant growth (Hiruma et al., Nat Commune 2023). Comparative transcriptome and genetic analyses between beneficial and pathogenic strains of Ct during root colonization reveal that Ct3 pathogenesis occurs through activation of the host abscisic acid (ABA) pathways via a fungal secondary metabolism gene cluster. This cluster encompasses sesquiterpene putative ABA and botrydial (BOT) biosynthesis genes, in addition to a Zn_2Cys_6 transcription factor. Importantly, the beneficial Ct

strains did not express ABA and BOT genes during the root colonization despite that they have identical ABA-BOT cluster, suggesting that the cluster is tightly transcriptionally suppressed during the beneficial associations. The induction of the putative ABA-BOT cluster in Ct3 during root infection elicits the expression of host phosphate starvation-responsive genes, even in conditions where phosphate is abundantly available. Concurrently, it suppresses several nutrient uptake-related genes, encompassing those linked to nitrate and iron, implying its involvement in modulating the nutritional status of the host. Unexpectedly, despite the negative impacts, Ct3 colonization gives the host benefits when this fungal cluster is genetically disrupted or transcriptionally suppressed at mildly elevated temperatures in a manner dependent on the host phosphate starvation response regulators *AtPHR1*/*AtPHL1*. Our findings indicate that a fungal metabolism cluster provides a means by which infectious fungi modulate lifestyles along the parasitic–mutualistic continuum in fluctuating environments. In this presentation, we will discuss how the fungal flexible lifestyles ranging from beneficial to pathogenic are regulated and as a result affect plant growth positively or negatively under both laboratory and field conditions.

125 **Fungal signaling and plant responses driving fungal accommodation in arbuscular mycorrhizas: from research to application** Andrea Genre[1], Veronica Volpe[1], Matteo Chialva[1], Teresa Mazzarella[1], Andrea Crosino[1], Serena Capitanio[1], Lorenzo Costamagna[1], Wouter Kohlen[2] [1]Life science and systems biology, University of Torino, [2]Wageningen University

Arbuscular mycorrhizal (AM) symbiosis with soil borne glomeromycetes is believed to have developed as early as the first plants started to move from aquatic to terrestrial environment and are found today in the majority of land plants, including most crops. This ecological success derives from the ability of AM fungi to provide their host plants with an additional access to soil mineral nutrients and water, receiving in change a share of the product of photosynthesis.

This exchange of nutrients takes place within the inner cortical tissue of the root. Here, highly branched hyphal structures called arbuscules are hosted inside the living plant cells through a massive process of cell reorganization in coordination with fungal development.

Such an intimate interaction is achieved after an exchange of chemical signals in the rhizosphere, where the fungus releases so-called myc-factors that alert the host plant of fungal vicinity, inducing a number of symbiotic responses.

Myc-factors - which include short-chain chito-oligosaccharides - can be applied exogenously to promote AM development both under controlled laboratory conditions and in the field. Indeed, their perception by the host roots activates the expression of signalling- and cell reorganization-related genes that accelerate fungal accommodation leading to a more efficient and rapid establishment of the symbiosis, with a promising potential for agricultural applications.

126 **Biological roles of a tripeptide exported in cryptococcal extracellular vesicles** Flavia Reis[1], Marcio Rodrigues[2,3] [1]Oswaldo Cruz Foundation (Fiocruz), [2]Carlos Chagas Institute, Oswaldo Cruz Foundation (Fiocruz), [3]Microbiology Institute, Federal University of Rio de Janeiro

Extracellular vesicles (EVs) play a crucial role as carriers for the export of small molecules within the *Cryptococcus*genus. In our investigation of the small molecule composition of EVs produced by *C. deuterogattii*, mass spectrometry revealed the presence of the tripeptide isoleucine-proline-isoleucine (IPI). IPI acts as an inhibitor of dipeptidyl peptidase 4 (DPP4), a serine exopeptidase known for cleaving X-proline or X-alanine dipeptides from the N-terminus of polypeptides. In fungi, DPP4 regulates pathogenesis by interfering with innate immunity. In mammals, DPP4 is the enzyme responsible for cleaving glucagon-like peptide 1 (GLP-1), which, in turn, regulates insulin release and blood glucose levels. In this context, DPP4 inhibitors called gliptins are oral hypoglycemics used to treat diabetes mellitus type 2 and obesity.

In this study, we investigated the potential roles of fungal and host DPP4 in cryptococcal physiopathogenesis. On the microbial side, IPI proved to be an efficient inhibitor of cryptococcal DPP4. Deletion of the gene encoding DPP4 resulted in the absence of enzyme activity and led to significant changes in the EV and cellular proteomes. In mouse infections with *C. neoformans*, a persistent peak of blood GLP-1 was observed, consistent with DPP4 inhibition. Notably, IPI inhibited DPP4 in various host cells, including lung homogenates of mice, macrophages of different origins, and hemocytes of the invertebrate host *Galleria mellonella*. This EV-associated DPP4 inhibitor protected *G. mellonella*against lethal challenges with cryptococci and reduced fungal burden. In mice, infection with the *dpp4*D mutant or with wild-type cells in the presence of IPI resulted in significantly decreased colonization of the lungs. These results indicate that EVs serve as carriers for the export of biologically active small molecules. Furthermore, our findings suggest that EV-associated inhibitors can interfere with both microbial and host DPP4, influencing associated biological processes with potential benefits for the host.

127 **Evolution in overdrive: fungal secreted proteins and innate immune genes** Kyungyong Seong[1], Anne Nakamoto[1], Pierre Joubert[2], Frances Grace Stark[2], Ksenia Krasileva[2] [1]Plant and Microbial Biology, University of California, Berkeley, [2]University of California, Berkeley

Fungi adapt rapidly to new environments, hosts and lifestyles. Fungal genomes often contain slowly evolving regions carrying housekeeping genes, and rapidly evolving regions containing genes involved in host-pathogen as well as microbe-microbe interactions. Using comparative genomics, we described species and lineage specific differences in evolution of genes prone to presence absence variation as well as genes that diversify rapidly through point mutation. These genes include disease-causing effectors, and genes related to antibiotic production, and non-self-recognition. Using Alphafold2 structure prediction across multiple species, we found divergent evolution as a major force in driving evolution of effector proteins encoded by fungal plant pathogens from ancestral proteins shared with non-pathogens. Genes involved in fungal innate immunity have similar evolutionary characteristics and genomic contexts.

128 **Integrated Insights into the Light-Sensing Mechanisms and Transcriptional Responses of *Botrytis cinerea*** Paulo Canessa Centro Biotecnologia Vegetal, Universidad Andres Bello

This study delves into the intricate light-sensing system of *Botrytis cinerea*, revealing a complex network of interactions in the presence of light. The focus is on the BcWCC (white collar complex), a crucial component for photomorphogenesis and circadian regulation. Despite prior knowledge of its components, molecular evidence of light-dependent interactions within the BcWCC and the specific capabilities of BcWCL1, an orthologue of the blue-light photoreceptor WC-1, have remained elusive. Utilizing a yeast two-hybrid system, this work confirms that BcWCL1 and its functional partner BcWCL2 interact in the absence of light and upon blue-light stimulation, primarily through their PAS domains. Deletion of PAS domains severely disrupts this interaction, with the intriguing finding that the truncated BcWCL1PAS-delta protein still exhibits a blue-light response. The study also explores the role of BcWCL1 in the necrotrophic fungus's virulence, particularly in the presence of a biologically relevant light pulse. Through RNA-seq analyses during non-infective and infective growth, global gene expression patterns were examined after a 60-minute light pulse on the wild-type strain and the *bcwcl1* mutant. The results unveil a complex fungal photobiology, with the mutant showing a lack of response to the light pulse during interaction with plants. Upon light exposure during infection, the delta *bcwcl1* mutant exhibited no upregulation of photoreceptor-encoding genes. Differential gene expression analyses highlight distinct responses under non-infective and infective conditions. These findings contribute to a deeper understanding of the interplay between light-sensing mechanisms and virulence, providing valuable insights for future research in plant-fungus interactions.

129 **Genome-wide regulation of mRNA polyadenylation across nutrient environments and over circadian time** Christina Kelliher[1], Jennifer Loros[2], Jay Dunlap[3] [1]Biology, University of Massachusetts Boston, [2]Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth, [3]Molecular & Systems Biology, Geisel School of Medicine at Dartmouth

The circadian clock is a ubiquitous biological process used by nearly every organism on the planet to anticipate daily fluctuations in the environment. Transcriptome profiling studies from cultured mammalian cells, insects, fungi, and many others have demonstrated rhythmic circadian regulation of 10-50% of all genes. Translational and proteomic assays have identified proteins that are expressed rhythmically but are not clock controlled at the level of mRNA abundance, suggesting extensive post-transcriptional regulation.

We identified two mutants in a Cleavage and Polyadenylation Specificity Factor complex through a genetic screen of the *Neurospora crassa* knockout collection. Single mutants Δ*cpsf5* (NCU09014) and Δ*cpsf6* (NCU02152) have a short circadian period length of 16 – 20 hours, depending on the nutrient growth conditions. Using genetic and biochemical approaches, we show that CPSF5 and CPSF6 form a heterodimeric complex in *Neurospora*, indicating functional and evolutionary homology to the mammalian CPSF complex involved in poly(A) tail placement and mRNA cleavage in the 3' UTR. We hypothesized that CPSF is required for normal polyadenylation and expression of a core clock transcript(s), leading to differential regulation and short period length in Δ*cpsf* mutants. Two core clock genes tested (*frequency*, *casein kinase I*) do not explain the short period defect, but two additional downstream genes will be discussed. To test for a pleotropic effect on multiple genes, we performed 3' End Sequencing of the Δ*cpsf5* Δ*cpsf6* double mutant compared to wild-type under various nutrient conditions to map poly(A) tail locations. We find that half of the polyadenylation landscape is altered in the CPSF mutants. Further, we find that hundreds of genes harbor differential polyadenylation depending on carbon source or on circadian time. Post-transcriptional regulation contributes significantly to proper circadian timekeeping, including mRNA polyadenylation.

130 **Rhythmic interaction between ZUOTIN and ribosomes may promote daily rhythms in protein folding and activity** Madhusree Gangopadhyay, Teresa Lamb, Deborah Bell-Pedersen Texas A&M University

Several ribosome-associated chaperones bind nascent polypeptide chains to mediate co-translational folding. Zuotin (ZUO) is a part of the ribosome-associated complex (RAC) that functions as a ribosome co-chaperone. In yeast cells, ~30% of ribosomes associate with the RAC, suggesting some level of specificity. Quantitative mass spectrometry across circadian time in *Neurospora crassa* revealed that the interaction between ZUO and ribosomes is clock-controlled. Thus, we hypothesized that the rhythmic ZUO/ribosome interaction may lead to rhythms in the folding of specific protein targets. Protein aggregation assays confirmed that ZUO promotes proper protein folding in *N. crassa*. Identification of misfolded proteins by mass spectrometry revealed that ZUO-dependent folding targets included copper transporters, and proteins involved in snRNA processing and mRNA catabolic process. Protein aggregates are more likely to form when the ZUO-ribosome interaction is low during the day under control of the clock, supporting that protein folding of select targets is clock-controlled and dependent on ZUO. Interestingly, while the interaction of ZUO with ribosomes is clock-controlled, ZUO protein levels do not cycle. Thus, the daily rhythm in folding of specific protein targets, potentially including ZUO itself, likely contributes to protein activity rhythms in the absence of rhythmic abundance.

131 **On the evolution of clock mechanisms in fungal systems: a case of moonlighting functions of core-clock components?** Carlos Corrial[1], Consuelo Olivares-Yanez[2], Luis F Larrondo[1] [1]iBio, Pontificia Universidad Catolica de Chile, [2]Centro de Biotecnología Vegetal, Universidad Andrés Bello

During the past decade our lab has been studying how light and time shape fungal physiology and organismal interactions, aiming to dissect the molecular mechanisms underpinning circadian clocks and light perception.

Thus, for example, we provided for the first-time evidence of the importance of clock regulation in the interaction between a phytopathogenic fungus and a plant host. However, the relevance of circadian clocks in fungal-fungal interactions remains largely unexplored. We have now characterized a functional clock in the biocontrol agent *Trichoderma atroviride* to assess its importance its mycoparasitic action against the phytopathogen *Botrytis cinerea*. The results highlight the relevance of clock components, as well as dark/light conditions in the way organismal dynamics are established. By utilizing luciferase reporters to monitor the *T. atroviride* core-clock, we confirmed the existence of circadian oscillations that are temperature compensated and can be modulated by environmental cues such as light and temperature. Importantly, the presence of such rhythms appears to be highly dependent on the nutritional composition of the media, which suggest a conditional gating of clock mechanisms. Indeed, while we have identified defined nutritional cues that enable visualizing rhythms, several other ones appear to tamper with *bona fide* circadian oscillations.

Notably, our current evidence (as obtained in both in *B. cinerea* and *T. atroviride*) indicates that the main clock component (FREQUENCY) exhibits extra-circadian roles, particularly in the cross-roads of development, and metabolism, impinging Nitrogen assimilation and secondary metabolism, raising interesting questions about the origin and evolution of clock components in fungi, and suggesting potential moonlighting function for these clock proteins. Current effort concentrates on defining how FRQ converses with other regulators, and on how such interactions have emerged in the evolutionary history of this core-clock component.

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132 **Phytochromes in *Aspergillus fumigatus*: Light, stress and virulence** Reinhard Fischer[1], Kai Leister[2], Yinyang Ma[3], Ling Lu[4], Zhenzhong Yu[3] [1]Microbiology, Karlsruhe Institute of Technology (KIT), [2]Karlsruhe Institute of Technology, [3]University of Nanjing, [4]Nanjing University

Phytochrome serves as red-light sensor in fungi and regulates light-dependent gene expression. In *Aspergillus nidulans* and *Alternaria alternata*, phytochrome also acts as a temperature sensor and performs functions in the absence of light. This study explores the role of two phytochromes, FphA and FphB, in the opportunistic fungal pathogen *Aspergillus fumigatus*. Both proteins were expressed in *E. coli*. FphA, behaved like the *A. nidulans* orthologue and was photoconvertible, while FphB showed no photoactivity. AfFphA complemented *A. nidulans fphA*-deletion strain phenotypes, unlike AfFphB. Co-overexpression of both proteins in *A. nidulans* stimulated asexual development and induced putative pathogenicity-related genes. AfFphA influenced various stress responses in *A. fumigatus*. *fphA* deletion in *A. fumigatus* had no impact on virulence in *Galleria mallonella*, while *fphB* deletion and *fphA/B* double-gene deletion increased virulence. Similar effects were observed

after *fphB* overexpression. RNAseq analyses revealed regulation of mycotoxin genes by FphB, which probably explains its role in pathogenicity. In *A. nidulans*, AfFphA and AfFphB localized exclusively in nuclei, forming a heterodimer. Our results suggest that *A. fumigatus* FphA responds to red light and plays a role in stress responses, while the photoinactive FphB protein controls virulence.

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133 **Phosphorylation of SP sites in WCC determines the phase of the circadian clock of *Neurospora*** Anna Gatz[1], Axel Diernfellner[1], Michael Brunner[2] [1]Heidelberg University, [2]Biochemistry, Univ Heidelberg

Circadian clocks are cell-based molecular oscillators that generate rhythms with a period in the range of 24 hours. In free-running conditions, circadian clocks oscillate precisely in a self-sustaining and temperature-compensated manner, but the period length may deviate from 24 hours. Under most natural conditions, however, recurrent stimuli related to the geophysical cycle of the Earth's rotation, such as light and temperature, synchronize the circadian clock with the day-night cycle. Synchronization not only sets the length of the circadian period to exactly 24 hours, but also creates an internal phase that allows organisms to anticipate environmental changes. The circadian clock of *Neurospora* is based on negative feedback of FRQ on its circadian transcription activator WCC. We present evidence how phosphorylation of specific SP sites in WCC determines the phase of the *frq* transcription rhythm relative to light-dark transitions.

134 **A Case for the Kinases: A Role for CKI in Temperature Compensation of the *Neurospora crassa* Circadian Clock** Elizabeth-Lauren Stevenson[1], Christina M. Kelliher[1,2], Jennifer J Loros[3], Jay C Dunlap[1] [1]Molecular and Systems Biology, Dartmouth College, [2]Biology, University of Massachusetts Boston, [3]Biochemistry and Cell Biology, Dartmouth College

Circadian clocks enable organisms to anticipate the daily environmental cycles that result from the Earth's rotation, so that they may then designate appropriate day to night functions. As such, the clock regulates many physiological processes. The molecular circadian clock in animals and fungi consists of a transcription-translation feedback loop that is regulated post-translationally throughout the circadian day by phosphorylation events. In the classic clock model *Neurospora crassa,* the positive arm of the clock, a heterodimeric complex of transcription factors, activates the transcription of the negative arm of the clock, Frequency (FRQ), which complexes with Casein Kinase I (CKI) to inactivate the positive arm via phosphorylation, thereby inhibiting their own transcription. Several key features define circadian rhythms, including the ability to entrain to external cues, the capacity to continue oscillating in the absence of those cues, and the maintenance of a consistent period across temperatures (known as temperature compensation – TC). TC is an essential clock property which is found in all organisms with circadian clocks, yet its mechanism remains undefined.

We discovered a novel mutation in Casein Kinase I (CKI) that confers a long period and severe undercompensation such that period shortens as temperature increases, suggesting a role for CKI in the TC mechanism of *Neurospora*. We find that reduction in CKI levels or activity causes the clock to be undercompensated, compared to a reduction in Casein Kinase II (CKII) activity conferring overcompensation. Inhibiting CKI in a CKII hypomorph still dose dependently altered its normally overcompensated TC profile, suggesting CKI is downstream of CKII in the TC mechanism. Hypothesizing that TC may be achieved by temperature-dependent differential phosphorylation of clock components, we performed phosphoproteomics in WT and CKI mutant backgrounds across a range of temperatures and identified phosphosites whose phospho-occupancy changes significantly with both temperature and genotype. We find that phosphonull mutations at a subset of these sites on FRQ alter the temperature compensation of the clock. These data provide support for a kinase-based model of temperature compensation.

135 **Light sensing in mushroom-forming fungi: The White Collar regulatory network of *Schizophyllum commune*** Peter Jan Vonk, Zoé Niemeijer, Marieke van der Poel, Robin A. Ohm Biology, Utrecht University

Blue light is an essential signal in the sexual development of many mushroom-forming fungi. It is detected by the White Collar Complex (WCC), composed of WC-1 and WC-2, which promotes transcription in the presence of light. Most of our knowledge on

this complex comes from the ascomycete *Neurospora crassa*. However, due to large structural differences in the WCC in basidiomycetes, it is not known if the WCC has the same function in basidiomycetes and what its role is in initiating fruiting.

We used a combination yeast-2-hybrid, ChIP-Seq and RNA-Seq to identify the direct and indirect roles of the WCC on mushroom development in *Schizophyllum commune*. WC-1 and WC-2 interact with each other both in the light and in the dark. However, WC-2 binds to the promoters of more than 500 genes when grown in the light, but only 3 in the dark. This indicates a different mode of post-translational regulation of the WCC compared to ascomycetes, as the WCC in *N. crassa* is also associated with promoters in the absence of light. Furthermore, the expression profile of a Δ*wc-2* mutant in the light resembled that of the WT in the dark, indicating that a Δ*wc-2* mutant is effectively blind. In the light, WC-2 activates genes related to UV protection, but also genes associated with mushroom development like hydrophobins. Moreover, WC-2 directly activates expression of the C_2H_2 zinc-finger transcription factor *zfc7*, which regulates the progression of primordia development.

Together, these results show that WC-2 directly activates the transcription of fruiting-related genes in *S. commune* in a light-dependent manner that is different from that seen in Ascomycota.

136 **Training a pathogen: uncovering the evolutionary mechanisms of host adaptation in *Cryptococcus neoformans*** Zoe A Hilbert[1,2,3], Joseph M Bednarek[4], Mara JW Schwiesow[2,3], Krystal Y Chung[4], Christian T Moreau[4], Jessica CS Brown[4], Nels C Elde[2,3] [1]Dept of Biology, Boston College, [2]Dept of Human Genetics, University of Utah, [3]HHMI, [4]School of Biological Sciences, University of Utah

For many fungal pathogens, the range of conditions under which they are able to survive and replicate is impressively large. And yet, our understanding of how adaptation to environmental conditions, or wide-ranging hosts, occurs on a molecular level and contributes to pathogenicity in these species is still limited. In this study we use experimental evolution approaches to watch adaptation of the human fungal pathogen *Cryptococcus neoformans* unfold in real time, exploring how interactions with different host species and changing environments shape the evolution of this model fungal pathogen. Using *C. neoformans* environmental isolates, we performed serial passaging experiments exposing fungal cells to relevant environmental and mammalian host cells—amoeba and mouse macrophages, respectively—for many generations to observe how these interactions select for the emergence of fungal populations with an enhanced ability to thrive within host environments. Through this approach, we identify several independent populations that rapidly adapted to their respective hosts, and—via whole genome sequencing—reveal key genetic changes in the populations that underlie these adaptive phenotypes. We find that each evolved *C. neoformans* population acquired a unique set of mutations and took a distinct evolutionary trajectory during the experiment, with some populations being swept early by beneficial mutations and others maintaining more genetic heterogeneity. This suggests that there are many possible routes to host adaptation in this species. We further perform molecular and genetic dissection of these adaptive phenotypes to reveal differences in the function of key signaling pathways across divergent strains of *C. neoformans* and their roles in regulating pathogenicity. Together, this work provides new insight into the evolutionary strategies used by fungi to adapt to different host species and environments, and how these adaptations contribute to the transformation of some fungi, like *C. neoformans*, from environmental microbe to potent pathogen.

137 **Loss of RNA interference in *Cryptococcus neoformans* clinical and environmental isolates: a pathway to hypermutation** Jun Huang[1], Shelby J Priest[2], Fred Dietrich[2], Paul Magwene[2], Vikas Yadav[2], Connor Larmore[2], Sheng Sun[2], Joseph Heitman[2] [1]MGM, Duke University, [2]Duke University

To survive and proliferate in changing environments, microbes have evolved multiple mechanisms for rapid adaptation. While an increased mutation rate typically has negative consequences in multicellular organisms, hypermutation can be advantageous for microbes subjected to significant selective pressures. Previously, we identified two hypermutator *Cryptococcus neoformans* clinical isolates, Bt65 and Bt81, that can rapidly overcome antifungal selection by uncontrolled transposition of a specific retrotransposon, Cnl1. These isolates harbor a nonsense mutation in a novel RNAi component, Znf3, and have accumulated a tremendous transposon burden (~150 copies of Cnl1), and loss of RNAi is responsible for hypermutation. To better understand the adaptation mechanisms in *C. neoformans*, we developed two bioinformatics pipelines to identify additional isolates with RNAi loss-of-function mutations by screening an extensive Strain Diversity Collection. Remarkably, several loss of RNAi isolates were identified but these isolates do not exhibit a hypermutator phenotype and have not undergone transposon amplification. To test if these RNAi loss isolates can become hypermutators, they were crossed with an isolate containing a high Cnl1 burden. F1 hypermutator progeny were identified with distinct mutation spectra. In addition to the Cnl1 insertion, the transposition of a novel gigantic DNA transposon (~11 kb), widely distributed in natural isolates with varying copy numbers, contributed to the hypermutator phenotype

of the progeny. Taken together, our results suggest natural isolates with RNAi defects are not uncommon and many lie on a pathway to hypermutation. Additional passage assays and genome editing of the RNAi loss-of-function mutations are being conducted to determine the connection between RNAi loss and transposon burden in contributing to the evolution of hypermutator isolates.

138 **Avc1 regulates adaptation to high CO_2 levels in the human fungal pathogen *Cryptococcus neoformans*** Benjamin Chadwick, Xiaorong Lin University of Georgia

The opportunistic pathogen *Cryptococcus neoformans* causes fatal systemic disease in immunocompromised individuals worldwide. As an environmental fungus, adaptation to host conditions is key for its survival and pathogenesis. A major difference between the host and ambient environment is the concentration of CO_2. CO_2 makes up ~5% of the air in the human body, which is nearly 125 times higher than in ambient air (~0.04%). The growth of many environmental isolates of *C. neoformans* is inhibited by host levels of CO_2, and the ability to tolerate this high level of CO_2 is correlated with virulence. How CO_2-sensitive environmental isolates have been able to adapt to the higher CO_2 level of the host has remained a mystery. Through experimental evolution and comparative genomics, we found that multiple environmental isolates of *C. neoformans* can adapt to high levels of CO_2 *in vitro* through stable mutation. Loss of function mutations in the gene *AVC1* was responsible for a gain of CO_2 tolerance in multiple environmental isolates. We deleted the *AVC1* gene in multiple CO_2-sensitive isolates and discovered a conserved effect of gain in CO_2 tolerance and gain of fitness *in vivo*. Overexpression of *AVC1* resulted in hypersensitivity to CO_2 and CO_2 dependent cell size enlargement. Taken together, these results implicate a critical role for Avc1 in regulating CO_2 tolerance and a potential mechanism for high CO_2 adaptation of *C. neoformans* environmental isolates.

139 **Rapid gain and loss of an aneuploid chromosome drives key morphology states and virulence in a fungal pathogen of humans** Sarah Heater, Rosa Rodriguez, Mark Voorhies, Anita Sil Immunology and Microbiology, UCSF

Environmental pathogens of humans must rapidly acclimate to thrive as they transition between the disparate conditions of environment and host. Little is known about the molecular regulation of this process in thermally dimorphic fungal pathogens such as *Histoplasma* species, which undergo a drastic transition in morphology and gene expression induced by the temperature of environment (where they form multicellular hyphae) or host (where they form unicellular yeast). Less is known about population genetics throughout these habitat transitions. We made the surprising discovery that a reversible aneuploidy helps *Histoplasma* thrive under environment but not host conditions. This aneuploidy is present in half of 25 clinical isolates tested, indicating that it appears in multiple lineages. It is rapidly gained and lost during laboratory passage, while other aneuploidies are very rare. The aneuploidy biases cells towards the environmental hyphal form: it increases the speed at which yeast transition to hyphae and pushes cells towards hyphal gene expression patterns even in conditions inducing yeast morphology. In a pooled competition, the aneuploidy provides a strong competitive advantage during the shift to environmental conditions. However, the aneuploidy downregulates genes associated with yeast morphology such as virulence factors, and confers a competitive disadvantage during shifts to the host form. Aneuploid *Histoplasma* is considerably less pathogenic than euploid *Histoplasma* in the mouse model of infection. In a competition experiment in mice, the aneuploid strain is at a disadvantage. To understand how the aneuploidy promotes and is advantageous for the environmental form while impeding the host form and decreasing virulence, we performed RNAseq analysis of euploid and aneuploid strains, revealing that the aneuploidy affects expression of key virulence factors and transcription factors (TFs). We identified two previously unstudied TFs within the aneuploid chromosome that, when overexpressed in the euploid strain, are each sufficient to yield phenotypes associated with the aneuploidy, such as hyphal growth. We are currently determining whether these TFs are necessary to promote these phenotypes. Taken together, these data strongly suggest that this aneuploidy benefits *Histoplasma* by rapidly increasing phenotypic diversity, thus helping these populations survive through frequent and abrupt transitions between the environment and mammalian hosts.

140 **Experimental Evolution of *Benniella erionia* and Mollicutes-Related Endobacteria** Reid Longley[1], Aaron Robinson[2], Gregory Bonito[3], Patrick Chain[2] [1]Bioscience Division, Dept of Genomics and Bioanalytics, Los Alamos National Laboratory, [2]Los Alamos National Laboratory, [3]Michigan State University

It has become clear that interactions between diverse bacteria and fungi are common. However, many of these interactions can be short-lived making them difficult to study in laboratory settings. However, diverse Mucoromycota fungi are known to host gram-positive Mollicutes-related endobacteria (MRE) and gram-negative *Burkholderia*-related endobacteria (BRE) which make ideal model systems for studying bacterial-fungal interactions due to the long-term nature of the interactions. Our recent work demonstrated that MRE have highly reduced genomes and metabolic capacity, likely making MRE completely dependent on their fungal hosts. Additionally, MRE genomes are characterized by a high degree of rearrangement leading to low levels of synteny between closely related taxa. To further investigate the evolution of MRE and their fungal hosts, we have carried out a long-term

evolution experiment on *Benniella erionia* GBAus27B and it's MRE. Briefly, fungal hosts containing endobacteria and those cured of endobacteria are transferred every three weeks to fresh 1% Malt Extract Agar (MEA) and 0.1% MEA to assess the impacts of nutrient availability on evolution of fungal hosts and MRE. Endobacterial load is tracked using QPCR and evolution of hosts and MRE is assessed using periodic (every six months) genome sequencing. Preliminary results indicate that *Benniella erionia* has maintained large populations of endobacteria (> 10^5 cells/mg of fungal tissue) for a period of 48 weeks. However, in low nutrient conditions, MRE populations have steadily declined over the experimental period indicating that in stressful low nutrient conditions, MRE populations may not be maintained to due to an expected nutritional burden on the fungal host. Genomic sequencing after six months of experimental evolution indicated that MRE genomes have undergone more insertions/deletions and rearrangements compared to other types of mutations while fungal host genomes remain largely unchanged. This work will give important insights into the role of rearrangement in genome evolution of endosymbionts which are expected to be valid in diverse endosymbiont systems across the Eukaryotic tree of life.

141 **Long-Term Evolution of the Extremely Halotolerant Black Yeast *Hortaea werneckii*: Unraveling Morphological and Genomic Adaptations to High Salinity** Cene Gostinčar[1], Jason E. Stajich[2], Sunita Sinha[3], Corey Nislow[3], Metka Lenassi[4], Farhad Hariri[5], Martina Turk[5], Nina Gunde - Cimerman[5] [1]Biology, University of Ljubljana, [2]Microbiology & Plant Pathology, University of California-Riverside, [3]Faculty of Pharmaceutical Sciences, University of British Columbia, [4]Institute of Biochemistry, Medical Faculty, University of Ljubljana, [5]Biology, Biotechnical faculty, University of Ljubljana

The extremely halotolerant black yeast *Hortaea werneckii* was exposed to experimental evolution for over seven years and more than 800 generations in a medium containing 4.3 M NaCl, significantly above the species' optimal salinity range. Contrary to expectations, the growth rate of the evolved *H. werneckii* strains did not change under extreme salinity or in the absence of salt. However, examinations revealed intriguing morphological and genomic adaptations. At the phenotypic level, evolved strains exhibited a notable reduction in cell width, a decrease in the length of multicellular chains, and altered melanization patterns. Changes in sensitivity to caspofungin, a cell wall synthesis inhibitor, indicated modifications in cell wall composition. Genomic analysis uncovered a remarkable increase in the number of aneuploidies in an otherwise diploid genome, with specific gene groups enriched in these regions. The findings question the assumption that an extremotolerant species, when exposed to extreme conditions it already tolerates efficiently, has limited room for further improvement. The persistence of aneuploidies, observed both in experimental evolution and some wild isolates, suggests potential adaptive value. Similar but shorter-term experiments with the polyextremotolerant black yeast *Aureobasidium pullulans* did not result in aneuploidies, but led to shorter generation times at high salinity and to changes in the management of compatible solutes and intracellular potassium. This suggests that different extremotolerant species may follow different evolutionary trajectories even when exposed to similar selection pressure. This study provides insights into the interplay between morphology, signaling pathways, and genomic adaptations, offering promising directions for future investigations into fungal halotolerance.

142 **Transposons drive environmental adaptation in a clonally evolving fungal pathogen** Cristina López Díaz[1], Dilay Hazal Ayhan[2], Ana Rodríguez López[1], Lucía Gómez Gil[1], Li-Jun Ma[2], Antonio Di Pietro[1] [1]Departamento de Genetica, Universidad de Cordoba, [2]Dept of Biochemistry and Molecular Biology, University of Massachusetts Amherst

The genomes of many fungal pathogens are compartmentalized into core regions and accessory regions, which are enriched in transposable elements (TEs). TEs are widely regarded as drivers of adaptive evolution, but direct experimental evidence remains limited. Here we used an evolve and re-sequence approach to follow environmental adaptation in *Fusarium oxysporum*, a devastating fungal pathogen that attacks more than 150 crops and causes deadly infections in immunocompromised humans. Serial passaging of a clonal isolate through tomato plants or axenic media plates resulted in rapid adaptation and increased fitness under the selection condition. Plate-passaged populations displayed recurrent evolutionary trajectories of sequential loss-of-function mutations that lead to increased proliferation at the cost of reduced virulence. TE insertions accounted for more than half of the variants detected and localized preferentially to sites of histone H3 lysine 27 trimethylation, a hallmark of accessory regions. Our findings reveal that TEs act as the main drivers of adaptation in *F. oxysporum* and reveal fitness trade-offs between developmental programs stimulating proliferation versus invasion.

143 **The effect of facultative heterochromatin on DNA replication fidelity: Do chromatin domains determine evolution rates in fungal chromosomes?** Shay Covo[1], Mohand Adris[1], Faith A Martin[2], Allyson A Erlendson[2], Michael Freitag[2] [1]Hebrew University, [2]Oregon State University

"Mutator" strains, characterized by their high mutation rates, are most frequently uncovered during experimental evolution studies. The explanation for this phenomenon is the high genetic diversity in these strains that allows for selection of mutants that are fittest under the experimental conditions. However, over time mutator strains "crash" and disappear from continuously

growing populations. The explanation for this phenomenon is the accumulation of detrimental mutation that eventually overcome the effect of the beneficial mutations. The evolution rate of several plant pathogenic fungi is not uniform across their genome. In some cases, the rate of evolution of pathogenicity-related genes is higher than other parts of the genome. Putting this in the context of mutators it seems possible that functionally pathogenicity-related parts of the genome are replicated at lower fidelity. One determinant of chromosome territories that may affect replication fidelity is "facultative heterochromatin". In *Fusarium graminearum*, a third of the genome is marked with one mark for facultative heterochromatin, histone H3 lysine 27 trimethylation (H3K27me3). Here, we are testing effect of H3K27me3 on replication fidelity by conducting mutation accumulation experiments in mutants deficient for the catalytic complex generating H3K27me3, Polycomb Repressive Complex 2 (PRC2) compared to wildtype strains, as well as mutants deficient in mismatch repair. As expected, the number of mutations accumulated in WT was very low. In contrast, the number of mutations was increased 20-fold in *mlh1* and to a lesser extent in *mlh3* and *mlh6* mutants. These preliminary data suggest that mismatch repair mutants result in different mutation signatures. We currently study the effect of mutations in the PRC2 complex in WT or mismatch repair deficient backgrounds.

144 **A look into the *Pyrenophora teres* f. *teres* colonization strategies on barley using a transformation-free staining and confocal microscope analysis** Ashley C Nelson[1], Gayan Kariyawasam[1], Nathan Wyatt[2], Janine Haueisen[3,4], Eva H. Stukenbrock[3,4], Pawel Borowicz[5], Zhaohui Liu[1], Timothy L. Friesen[2] [1]Plant Pathology, North Dakota State University, [2]Edward T Schafer Agricultural Center, USDA-ARS, [3]Evolutionary Biology, Max Planck Institute, [4]Environmental Genomics, Kiel University, [5]Animal Sciences, North Dakota State University

Laser scanning confocal microscopy is an invaluable tool in assessing plant microbe interactions at a cellular level. Here we use a transformation-free staining technique with propidium iodide (PI), which stains RNA and DNA, and wheat germ agglutinin labeled with fluorescein isothiocyanate (WGA-FITC), which stains chitin, to visualize fungal colonization of plants. Showcasing this, in tandem with the fungal pathogen *Pyrenophora teres* f. *teres* (*Ptt*) infecting barley, we show how high resolution images shed light on fungal colonization strategies and infection structures of fungal pathogens. In the *Ptt*-barley interaction, intracellular vesicles develop in epidermal cells directly below penetration points and serve as branching points for hyphal growth into the plant's mesophyll layer. Infected plant mesophyll layers are full of deliberate intercellular hyphal growth that maximizes its surface areas to grow around the individual mesophyll cells, exhibiting patterns we characterize as encasement, mesophyll cell trapping, thick layering, and branching. Encasement is the growth of hyphae in the mesophyll where it surrounds the cells on two opposing sides. Mesophyll cell trapping begins as encasement, but the hyphae continue to grow around the whole mesophyll cells surrounding it on all sides. Thick layering is the layered parallel growth of multiple hyphae and branching is the perpendicular growth of hyphae through numerous layers of mesophyll cells. We analyzed morphological differences between avirulent and virulent isogenic strains of *Ptt* and used the growth patterns mentioned above to assess their success in-planta. Hyphae of virulent strains were most intent on growing parallel to the length of the leaf, through the mesophyll layer as rapidly as possible, followed by lateral branching, explaining the net like lesions characteristic of this disease. Cell death was only observed behind the growing point of the fungus, where mesophyll cells were surrounded by the fungal hyphae. Comparatively the avirulent isogenic isolate was able to grow in-planta but had a fitness deficiency that inhibited its quick takeover of the leaf tissue. We believe the pathogen is maximizing fungal biomass to absorb nutrients at a high efficiency while delaying plant defenses before cell death is an advantage to the pathogen. *Ptt* has shown the potential of this technique to relook at the strategies of fungal pathogens and work in tandem with quantitative and molecular analysis.

145 **Integrating microfluidics and biomolecular mapping to advance microbial research** Jayde Aufrecht Pacific Northwest National Laboratory

Microfluidics have enabled tractable experiments in which to study microbial dynamics. Optical imaging has been the primary method for characterizing cells within microfluidic habitats but it is limited to mapping no more than a handful of organisms or biomolecules at a time. To more deeply survey biomolecules (i.e. genes, transcripts, metabolites) and their spatial distributions, requires a new approach to integrating microfluidics with chemical imaging and spatial sampling techniques. Here we describe techniques, including microfluidic methods, for mapping the biomolecular environment around organisms (plants, bacteria, fungi) using various mass spectrometry instruments. As a case study, we demonstrate how these spatial omics results can be used to inform research in environmental microbiology.

146 **Novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa*** Ziyan Wang[1], Bradley M Bartholomai[1], Jennifer J Loros[2], Jay C Dunlap[1] [1]Dept of Molecular and System Biology, Geisel School of Medicine at Dartmouth, [2]Dept of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth

Circadian rhythms are endogenous daily oscillations driven by a molecular clock that helps organisms better coordinate with the environment. Organisms from fungi to animals share a similar phosphorylation-driven transcription/translation negative feedback loop as the core clock mechanism, an oscillator composed of positive and negative elements. Research on the filamentous fungus model organism, *Neurospora crassa*, has provided answers to many fundamental clock-related questions. In *Neurospora crassa*, the transcription factor White Collar Complex (WCC) serves as the positive element driving the transcription of *frequency* (*frq*). The intrinsically disordered protein Frequency (FRQ), with other regulators, forms the negative element complex that inhibits the function of WCC and stops *frq* transcription. Molecular components of the circadian clock have been described over decades of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the subcellular level.

Neurospora crassa grows as a syncytium analogous to muscle cells, thus the subcellular distribution of molecules must facilitate precise temporal control throughout the syncytium. Live-cell imaging has emerged as a valuable tool in circadian research. We implemented novel strategies and microscopy tools for *Neurospora*, including 4-color imaging and microfluidics compatible with multi-day growth, to facilitate live-cell imaging of low-abundance circadian proteins. Through multi-color live-cell imaging in single cells, we tracked the circadian dynamics of the subcellular localization of WCC and FRQ in high spatiotemporal resolution. We also observed *in vivo* highly dynamic liquid–liquid phase separation (LLPS)-like behaviors of FRQ using the super-resolution SoRa microscope. Furthermore, by employing FRAP, we have unraveled the circadian-regulated nuclear import of FRQ and its underlying mechanism. We also optimized photoconvertible fluorescent proteins to facilitate further exploration of the nucleocytoplasmic transport of clock proteins.

Our work showcases the successful application of advanced microscopy techniques in a conventional fungal model organism to gain insights into the intricate subcellular dynamics of circadian proteins, paving the way for a deeper understanding of circadian rhythms.

147 **Microfluidic Approaches In Fungal Research** Alexandra C. Brand[1], Tina Bedekovic[2], Callum Parkin[2], Iana Kalinina[2], Ruben Ramalho[2], Masahiro Abe[2], Vivek V Thacker[3], Peter Cook[2], Duncan Wilson[2] [1]Biosciences, University of Exeter, [2]University of Exeter, [3]University of Heidelberg

Concurrent developments in fungal molecular genetics, microfabrication methods and live-cell imaging have delivered unparalleled opportunities to understand cellular processes as never before. Each research question requires its own experimental system and thought must be given to its design to minimise or mitigate artefacts. Here, we outline exemplars of the microfluidics approaches taken to address 4 specific areas of study within the MRC Centre for Medical Mycology: the properties of *Candida albicans* Goliath cells, *Aspergillus fumigatus* spore uptake by macrophages, calcium dynamics in *C. albicans*, and hyphal responses to electric fields and matrix stiffness. The use of commercially-available chambers vs the requirement for developing bespoke chambers for these applications will be discussed, along with considerations surrounding flow, time-course and imaging. The key findings derived from applying microfluidics approaches to address these 4 research areas will also be presented.

148 **Expanding the fluorescent toolbox in *Aspergillus fumigatus*** Isabelle S R Storer[1], Enrique V Sastré-Velásquez[2], Thomas J Easter[1], Birte Mertens[2], Michael J Bottery[1], Raveen Tank[3], Michael J Bromley[1], Fabio Gsaller[2], Norman van Rhijn[1] [1]Manchester Fungal Infection Group, University of Manchester, [2]Institute of Molecular Biology, Medical University of Innsbruck, [3]Microbial Evolution Research Manchester, University of Manchester

Fluorescent proteins are indispensable tools used to understand the spatio-temporal dynamics of molecular processes in living cells. Even though *Aspergillus fumigatus* causes more deaths globally than any other fungal disease, we lack a well-characterised tool kit of next-generation fluorophores, limiting our ability to probe fundamental biological processes of this critical human fungal pathogen. In this work, we chromosomally transform *A. fumigatus* with 18 fluorescent proteins with emissions covering the visible light spectrum and characterise their practical brightness during the different morphological stages. Through live cell imaging using fluorescence confocal microscopy and imaging flow cytometry, the relative intensity of each fluorophore was measured during hyphal growth and in spores. The fluorescent proteins mTagBFP2, mNeonGreen, Citrine, mKO2, mApple, and Katushka2S - green, yellow, orange, red and far-red respectively - displayed the highest relative fluorescent intensity in germlings. We demonstrate the utility of these reporters as inducible promoter systems, protein tagging, and pathogenicity. Finally, we generate a 4-colour strain by exploiting counter-selectable markers of the pyrimidine salvage pathway. This strain visualises the mitochondria, vacuoles, peroxisomes, and cell membrane to understand the dynamics of these subcellular structures in response to antifungal agents. This new resource will enable the community to conduct advanced live-cell imaging to gain a deeper understanding of subcellular localisation, quantify protein-protein interactions, elucidate novel druggable targets, and visualise host-pathogen interaction models.

149 **FACS-based method streamlines pooled transformations in *Aspergillus oryzae*** Sarah McFarland[1], Jonathan Pham[2], Sandeep Sharma Khatiwada[1], Eric Carter[3], Ceanne Brunton[1] [1]MSE, Novozymes Inc, [2]MDS, Novozymes Inc, [3]MAA, Novozymes Inc

Through precision fermentation, we use genetically engineered microbes including *Aspergillus oryzae* to produce enzymes, proteins, or other compounds in a controlled, sustainable, and animal-free manner. If we can make more protein/enzymes production from our production organisms for the same amount of input costs, we can make our biosolutions increasing cost competitive. Therefore, we often look for ways to improve the productivity of our production strains. Signal peptides are an important contributor to the secretion potential of a candidate protein and a possible route to increasing production strain performance. Here we discuss the how we tested a library of 100+ signal peptides and developed a high throughput method for pooled transformation, FACS (Fluorescence-activated cell sorting), and automated screening to rank *Aspergillus oryzae* strains based on productivity.

150 **Invasiveness and chemotropism of hyphae analyzed by microfluidic devices** Norio Takeshita University of Tsukuba

Filamentous fungi, either infecting or forming a symbiotic relationship with plant roots, intrude from the root surface and extend their hyphae into the root by navigating the spaces between plant cells. The capacity of hyphae to adapt their shape and elongate within confined spaces, smaller than their own size, is crucial for both pathogenicity and symbiosis. To investigate this, we designed microfluidic devices featuring channels as thin as 1 µm and conducted live imaging analyses on seven fungal species. Our findings revealed a trade-off between hyphal elongation rate and the ability to traverse these narrow channels (Fukuda et al., mBio 2021). To further understand the connection between hyphal elongation in micro-spaces and virulence, we examined both the wild type and various gene deletion strains of *Fusarium oxysporum*. These gene deletion strains were previously identified as having reduced virulence. We compared the passage rates through micro channels and the level of virulence, revealing that the cell wall integrity pathway is essential for elongation in micro channels and, consequently, for pathogenicity.

The ability of fungi to search for nutrients and hosts is expected to be important for their role in fungal ecosystems and pathogenicity. However, whether fungal hyphae possess chemotropism towards nutrients has not been clarified to date and requires evaluation at the cellular level. We constructed a microfluidic device to analyze hyphal chemotropism. In this device, hyphae can select their growth direction in a two-layer flow with different compositions that are adjacent but do not mix. This system revealed the chemotropism of hyphae towards nutrients and pH.

151 **Fungi Unleashed – Rapid Ionomic Profiling with Laser-Induced Breakdown Spectroscopy** Tomas A Rush[1], Ann M Wymore[1], Miguel A Rodriguez[2], Sara A Jawdy[1], Rytas J Vilgalys[3], Madhavi Z Martin[1], Hunter B Andrews[4] [1]Bioscience Division, Oak Ridge National Laboratory, [2]BioEnergy Science Centre, Center for Bioenergy Innovation and Biosciences Division Oak Ridge National Laboratory, [3]Biology Dept, Duke University, [4]Radioisotope Science and Technology Division, Oak Ridge National Laboratory

Nutrient acquisition, delivery, and availability dictates microbes' phenotype, lifestyle, and survival. One of the best microbes to study the influence of substrate availability is fungi, because they are heterotrophic, meaning they cannot produce their food from environmental nutrients. Tapping into this biological process by manipulating the substrate available has led to discoveries in identifying how fungi interact with a host or environment or biological products like alternative sources of protein and natural products. Despite the scientific interest and multifaceted roles that nutrient acquisition plays in controlling fungal behavior and producing biological products, the elements that are obtained and how they differentiate across fungal species have remained a largely unexplored area of research. To address this knowledge gap, we used laser-induced breakdown spectroscopy (LIBS) to identify the fungal ionomic profiles of two genetically different fungal species, *Hyaloscypha finlandica* and *Mucor hiemalis*, grown on defined and undefined substrate media. Through Pearson correlation coefficients, we had identified strong positive correlations with the emissions from carbon, zinc, phosphorus, manganese, and magnesium. The positive correlations seen with these elements in both species indicates their vital role in fungi propagation and survival. When the Pearson correlation coefficients of each fungi species are compared to one another a few noticeable differences are seen. Firstly, *H. finlandica* exhibits strong positive correlations between sodium, hydrogen, and the essential element group. This indicates *H. finlandica* has a reliance on sodium that *M. hiemalis* does not exhibit. A similar behavior is seen with potassium in *H. finlandica*, but generally a medium positive correlation exists between the essential elements and potassium. Interestingly, *M. hiemalis* shows a strong negative correlation between potassium and the essential elements. *M. hiemalis* shows stronger positive correlations between silicon, iron, and the essential elements; although, *H. finlandica* shows a positive correlation between silicon and calcium that *M. hiemalis* does not. Taken together, we provide data for the building blocks of what elements are needed for fungal growth and sustainability and how they differ across genetically diverse fungi.

152 **Designing fungal foods for planetary and human health: from traditional fermentation to synthetic biology** Vayu Hill-Maini[1,2,3], Jay Keasling[1,4,5,6,7,8] [1]Dept of Bioengineering, University of California, Berkeley, [2]Miller Institute for Basic Research in Science, University of California, Berkeley, [3]Joint BioEnergy Institute, [4]Dept of Chemical and Biomolecular Engineering, University of California, Berkeley, [5]Joint Bioenergy Institute, [6]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, [7]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, [8]California Institute of Quantitative Biosciences, University of California, Berkeley

Fungi have the potential to radically transform the food system. However, rationally designing fungal foods has been limited by our poor understanding of fungal metabolism and a lack of genetic tools. Here, we describe our multidisciplinary work bridging gastronomy, synthetic biology, and biochemistry, to dissect and engineer fungal food production – from traditional fermentations to contemporary biotechnology. We will present the recent development and application of synthetic biology tools for the edible fungus *Aspergillus oryzae*, the characterization of a traditional food, Oncom, made from food waste in Indonesia, and our collaboration with innovative Michelin-star restaurants to bridge fundamental discoveries from the lab with culinary innovation. This work has uncovered mechanisms for upcycling industrial byproducts into human food, enabled bioengineering of fungal meat for nutritional value and sensory appeal, and unlocked new gastronomic techniques that have already made their way into restaurant kitchens. This work now sets the stage for designing fungal foods for human and planetary health.

153 **From Fungi To High-Tech Cheese: How To Use Precision Fermentation To Rescue Our Food System** Beatrice Bernardi, Bastian Jöhnk Formo Bio GmbH

Our current food system is increasingly discussed as a root cause of climate change and human health (Poore & Nemecek, 2018). The global dairy industry alone is responsible for 4% of global greenhouse gas emission. In addition, livestock production leads to severe losses in biodiversity and fertile farming land each year. Consumer awareness and increased availability of animal-free products on the market is promoting the rise of flexitarian, vegetarian and vegan diets. A recent study on global consumer acceptance of animal-free dairy products, found strong enthusiasm in trying and regularly consuming animal-free dairy products (Zollman & Bryant, 2021). Hence, it is crucial that the food industry responds by enlarging and improving the animal-free dairy market. Plant-based products are a sustainable alternative to animal-based, but plant ingredients can be challenging for the food science industry, especially when emulating taste and texture of traditional dairy products.

The use of microorganisms as food ingredient is usually referred to as processed biomass or to protein purified from it (Graham & Ledesma-Amaro, 2023). For many years microorganisms have been used for drugs and food additives production thanks to the development of precision fermentation technology. Precision fermentation is a refined form of brewing or winemaking, where microbes are fermented to produce a specific molecule, which can be purified from the fermentation broth. This technology can be applied to produce the products of choice at industrial scale, including macronutrients for animal-free replacement products.

At Formo, we are focusing on reimagining dairy products using precision-fermented single protein ingredients. In particular, we are expressing recombinant milk proteins in a variety of expression hosts. Filamentous fungi, due to their ability for high-yield recombinant protein secretion and their capability of post translational modifications, are a promising platform for milk protein production. However, fungi also pose some inherent challenges like limited genetic accessibility, high proteolytic activity or secretion inhibiting pellet-forming morphology. Therefore, we decided to fix these issues by a series of genetic modifications that led to a strain, which demonstrates high efficiency for targeted genomic modifications, low proteolytic activity and a completely dispersed growth phenotype. This modified base-strain showed superior recombinant protein production compared to the WT.

Using fungi as cell-factories to produce food ingredients through precision fermentation technology is a valuable answer for bringing sustainable, fair, and tasty products into our future food system.

154 **Truffles population genomic and associated fungal and bacterial communities - who shapes the true truffles aroma?** Tine Grebenc[1], Nejc Suban[1], Nataša Šibanc[1], Aleksander Mahnič[2], Lidija Strojnik[3], Nives Ogrinc[3], Cene Gostinčar[4] [1]Slovenian Forestry Institute, [2]National Laboratory for Health, Environment and Food, [3]Jožef Stefan Institute, [4]4Biotechnical Faculty, University of Ljubljana

Truffles are the fruiting bodies (ascocarps) of fungi belonging to the genus *Tuber* that are fruiting in the soil and are best known for their aromas. Besides truffles ascocarps' produced aromatic volatiles, associated bacteria and yeast are also recognized to contribute significantly to the truffle. Recently we performed a *Tuber aestivum* and *T. magnatum* whole genomes population re-sequencing, aiming to analyze and correlate the outcome of aroma analysis with the bacterial and fungal communities on surface

and within ascocarps of the same truffle ascocarps. In addition, an extensive studies of truffles aromas in Europe (Strojnik et al. 2020, Šiškovič et al. 2021) were done on same collections. Both, the bacterial and the fungal associated communities were further assessed with site and ecological characteristics of each truffle genotype. Results of the preliminary analysis and statistical assessment of truffle genomes diversity and associated bacterial and fungal communities will be presented.

155 **Koji mold, a traditional Japanese fermentative microorganism, opens up mycoprotein potential** Daisuke Hagiwara University of Tsukuba

Against the backdrop of food shortages caused by the world's population growth and the climate crisis, the establishment of a sustainable food supply, such as protein production with a low environmental impact, has become an urgent issue. While alternative meat products derived from soybeans and other plants are already on the market in Europe, the United States, and elsewhere, "mycoproteins," which utilize mycelia from mushrooms and other fungi, are beginning to attract attention as a new option.

We have focused on koji mold, a traditional Japanese fermentative microorganism, to pursue and develop its potential as a mycoprotein. The cultured biomass (mycoprotein) of koji mold is a nutritionally superior food material with 30-60% protein per dry weight, less fat than meat, and profound dietary fiber and free amino acids. In addition, the complex intertwining structure of mycelium, which is unique to fungi, constitutes a fiber structure similar to the texture of meat. The "taste" of the fungus can be adjusted by changing the composition of the fungus by feeding the culture medium. Such "biomass fermentation" technology makes it possible to efficiently produce high-value koji mold mycelia in a short period of time, and is expected to lead to more tasty and healthier alternative meat.

Research on mycoproteins has progressed in recent years, with data showing that protein production from fungi is superior to beef production in terms of environmental impact. There have also been numerous reports on its contribution to human health. Furthermore, it is possible to cultivate fungi from unused food resources, thus contributing to the recycling of food and agricultural resources through upcycling, and thus providing food that contributes to human and global health.

Koji mold has been used for food production in Japan since ancient times. Therefore, koji mold is one of the microorganisms being intensively researched by many researchers. In other words, it is superior to other mycoproteins in terms of safety and sense of security, which are important for consumer acceptance, and we expect that it will become a food ingredient used in various applications as a major mycoprotein in the future.

156 **From Texture to Taste: Linking fungal genotype to material structure and function** Josephine M Wee Food Science, The Pennsylvania State University

Fungal mycelium, one of the most abundant materials on earth, is naturally composed of chitin, cellulose, glucans, proteins, and other small molecules that can vary considerably between and within different groups of fungi. On its own, mycelium has weak textural properties that are difficult to control for food and biomaterial application. While fungal mycelium materials offer expansive possibilities, little is known about how the genetic capacity of fungal species impact material structure and function. Our rationale is that the genetic capacity of fungi and its evolutionary diversity can help fine tune material properties and function. To gain control of mycelium growth and characterization, the first necessary step is to characterize fungal physiology as it relates to material properties. We compared growth, morphology, chemical composition, and material properties of mycelium from the GRAS fungus, *Rhizopus oligosporus* and the white rot fungus, *P. chrysosporium*. *R. oligosporus* grew at the highest rate between 24-48 h, while rate of *P. chrysosporium* growth is relatively consistent across time with increase carbon:nitrogen supplementation. Although larger median diameter was observed in *R. oligosporus* mycelium compared to *P. chrysosporium*, no significant differences in porosity was observed. We found that *R. oligosporus* mycelium had higher lipid:protein ratio compared to *P. chrysosporium* for all treatments ($p<0.01$) and *R. oligosporus* mycelium had higher protein:polysaccharide ratios for all treatments ($p<0.01$). As proof of concept, we present two applications for use of mycelium as an alternative protein source and as scaffolding material for cell-cultivated meat.

157 **Regulation of sugar metabolism under abiotic stress in various yeasts and filamentous fungi** Elisabeth Tamayo[1], Pedro Tomaz da Silva[2], Julien Gagneur[2], J. Philipp Benz[1] [1]Fungal Biotechnology in Wood Science, Technical University of Munich, [2]Computational Molecular Medicine, Technical University of Munich

Fungi are important for biotechnological and medical reasons. Additionally, fungal biotechnology can offer solutions to ensure food supply for a growing human population with little impact on the environment, since mycoprotein is an interesting meat

substitute with a high nutritional value and much lower carbon footprint. Fungi have developed elaborated strategies to cope with abiotic stress, as they often experience both in nature and during biotechnological fermentations. Several studies have been conducted to better understand the regulation underlying this adaptability in certain fungi, and e.g. genes related to glycerol transport were found to be important in acidic pH-tolerant yeast strains or under salt stress conditions. However, less is known regarding these processes from an evolutionary point of view, particularly considering the involvement of sugar metabolism in this regulation.

With the aim to identify both conserved and species-specific routes of sugar regulation in abiotic stress tolerance across Ascomycete fungi, we subjected several distantly related fungal species, including eight yeasts and three species of filamentous fungi of the genus *Neurospora*, to five abiotic stress conditions and analyzed their response by transcriptomics. Taking advantage of our previous study of the sugar transportome of *Neurospora crassa*, we focused on the transcriptomic changes of sugar transporters, sugar metabolism-related enzymes and transcription factors. The influence of the different stress conditions was explored in detail for *Neurospora* sp. candidate genes and compared with that in orthologues in the yeast species, to find out which regulatory features are specific or conserved. Several sugar transporters belonging to the major facilitator superfamily were identified as candidates involved in salt stress tolerance, some carbohydrate-active enzymes were found to be upregulated at acidic pH, and nine uncharacterized transcription factors were identified to have a putative role in carbon starvation, pH or ethanol stress.

Our data will help to better understand the mechanisms of sugar regulation in response to abiotic stress in fungi. This knowledge could then be used to develop biotechnological strategies to improve fungal nutritional values under stress conditions in fungal species that could serve to feed the human population in an environmentally friendly manner. Our new findings in this regard will be presented and discussed.

158 **Characterization of acid phosphatases in *Aspergillus oryzae* strain with reduced "umami" degradation activity** Kanae Sakai[1], Tadahiro Suzuki[2], Yuichiro Horii[3], Yutaka Wagu[4], Ken-Ichi Kusumoto[5] [1]engineering, Osaka University, [2]National Agriculture and Food Research Organization, NARO, [3]Food Research Center, Niigata Agricultural Research Institute, [4]Bio'c Co., Ltd., [5]Osaka University

Miso, fermented soy bean paste, is a traditional Japanese seasoning. It is made from soybeans, salt, water, and *koji* (solid-state culture of *Aspergillus oryzae* on rice, soybean, or barley). To fit the demand of modern busy lifestyle, production of dashi (broth) containing miso has been increasing in recent decades. In the manufacturing process of dashi containing miso, heat treatment of miso is needed before adding dashi. Since acid phosphatase secreted by *A. oryzae* degrades one of the dashi component ribonucleotides yielding no taste ribonucleosides and phosphoric acid, acid phosphatase should be inactivated by heat treatment. However, heat treatment requires energy and special equipment and it reduces the quality of miso. In this study, we attempted to obtain a strain with low acid phosphatase activity to avoid heat treatment, and analyzed the characteristics of acid phosphatase for breeding purpose.

Through the screening of 503 practical *A. oryzae* strains stocked in Bio'c Co., we found a strain with greatly reduced acid phosphatase activity while maintaining protease and amylase activities sufficient for miso fermentation and named KBN-p [Food Sci. Technol. Res. (2012) 83-90]. Among 13 putative extracellular acid phosphatase genes (*aphA-M*) in *A. oryzae* genome, AphC was considered to be one of the main causes of low acid phosphatase activity in KBN-p strain based on the results of transcriptional analysis and activity test. When AphC amino acid sequence of KBN-p was compared to RIB40 and practical strain for miso koji (No.6020), 5 and 1 amino acid substitutions were found, respectively. So, we analyzed the properties of AphC with three different amino acid sequences. At first, 3 kinds of *aphC* genes were expressed under the *tef1* promoter in each *A. oryzae* strain and found that AphC (KBN-p) have some problem in secretion. However, it was difficult to determine the reason whether the secretion defect of AphC (KBN-p) in KBN-p host lies in the AphC sequence or the host strain itself. Three kinds of AphC were expressed in a unified *aphC* null mutant host and tested the properties of AphC activity in response to heat and NaCl which thought to be related to dashi containing miso making process. As a result, it was found that AphC (KBN-p) was less stable than the other AphCs (RIB40, No.6020). This property is thought to be advantageous for producing dashi containing miso without heat treatment.

159 **Making fungal biology easier to engineer for sustainable food production** Peter J Punt[1,2] [1]Ginkgo Bioworks, [2]Leiden University

Fungal Biotechnology started off more than hundred years ago with the discovery of the starch degrading activities of fermentation broths originating from the fungus *Aspergillus oryzae*. Since then both classical and later also molecular engineering approaches

for fungi have been developed. The multicellular filamentous growth behavior of fungi has provided challenges in developing these approaches, but they can now be considered at par with those for single cell microorganisms like bacteria and yeasts. In particular in the last two decades technological developments in the fields of genomics, transcriptomics, proteomics and metabolomics, have been instrumental to develop new fungal strains and production processes, but have also indicated fields for further development. The next step of making fungal biology easier to engineer relies on onboarding new high throughput technologies like genome editing, smart biological screens based on lab-automation and high throughput process development equipment. Again here filamentous fungi provide new challenges requiring fungal specific solutions, in particular when producing functional food proteins or developing fungal biomass as a food ingredient. Examples on how these challenges can be addressed based on a large biological codebase, unique fungal host strains and a state-of-the-art foundry instrumentation, will be presented.

160 **Fungi with diverse lifestyles employ antimicrobial proteins to mediate niche establishment** Anton Kraege, Fantin Mesny, Valentina Wolf, Bart Thomma Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne

Recently, several plant pathogenic fungi were shown to use antimicrobial proteins to manipulate the plant microbiota and promote host colonisation. For example, during various stages of the infection cycle, the soil-borne fungus *Verticillium dahliae* secretes various proteins with antimicrobial activity to supress the growth of diverse antagonistic microbes. However, it is unclear how many antimicrobials are encoded in the *V. dahliae* genome to mediate host colonization, nor how widespread the use of antimicrobial proteins among other fungal plant pathogens is to promote host colonisation. To discover novel antimicrobial proteins encoded in fungal genomes we developed a machine-learning based predictor that can recognize antimicrobial activity based on protein sequence and (predicted) structural properties. Surprisingly, thirty percent of the predicted secretome of *V. dahliae* is a predicted antimicrobial. Intriguingly, a similar proportion is not only encoded by other plant pathogens, but also by fungi with other lifestyles. To investigate how conserved such antimicrobial proteins are, we predicted the antimicrobial activity of the most conserved secreted protein families in a set of 150 phylogenetically diverse fungi. Remarkably, many protein families that are widely conserved in fungi have predicted antimicrobial activity, suggesting that they are used for microbiota manipulation in diverse microbial ecosystems. We propose that fungi with diverse lifestyles have co-opted antimicrobial proteins that evolved in ancestor fungi to act in current-day niche establishment by targeting microbial niche competitors.

161 **Mechanisms of bacterial-fungal interactions and their environmental roles** Leah Johnson, Reid Longley, Julia Kelliher, Buck Hanson, La Verne Gallegos-Graves, Aaron Robinson, Patrick Chain Los Alamos National Laboratory

Soil microbes perform important functions in their environmental niche, such as nutrient cycling, carbon sequestration, and contributing to overall ecosystem health and resilience. As two dominant constituents of the soil microbiome, bacteria and fungi play important roles in these ecosystems, and interactions between these organisms can shape the function of the microbial community overall. While there is some knowledge on how bacteria and fungi impact each other, it is a burgeoning field and the impacts of bacterial-fungal interactions (BFIs) on their communities and ecosystems are not well understood, particularly in the face of a changing climate. Our group aims to systematically characterize interactions between bacteria and fungi and their underlying genetic and functional mechanisms to understand how BFIs impact their roles in their environments and hosts under changing climate conditions. To this end, we have isolated bacteria and fungi from the stress-tolerant plant, Bouteloua gracilis (blue grama), across three desert grassland sites in New Mexico. These field sites undergo climate change-relevant stresses such as drought and warming, and provide a valuable model for predicting how these stresses will impact the resident microbes. We have screened bacterial-fungal interaction phenotypes, such as pigmentation and growth inhibition, of blue grama endophytic bacteria (e.g. Pseudomonas, Bacillus, Streptomyces) and fungi (e.g. Darksidea, Fusarium, Monosporascus), under a relevant soil warming stress temperature for our field sites. These isolates have been sequenced to enable a multi-omics approach towards characterizing the underlying molecular mechanisms of these BFI phenotypes. We will additionally characterize how these BFIs impact their blue grama host, such as impacts on root colonization by these endophytes and plant stress responses. Together, this work will elucidate how BFIs impact their environments and plant hosts under changing climate conditions.

162 **Comparative genomics of *Basidiobolus* isolated from the herptile gut microbiome** Lluvia B Vargas[1], Daniel Farthing[1], Connor Dooley[1], Andrii P. Gryganskyi[2], Stephen Mondo[3], Igor V. Grigoriev[3], Kerry L. McPhail[4], Donald M. Walker[5], Jason E. Stajich[6], Joseph W. Spatafora[7] [1]Dept of Botany and Plant Pathology, Oregon State University, [2]Division of Biological & Nanoscale Technologies, UES, Inc., [3]Dept of Energy (DOE), Joint Genome Institute (JGI), Lawrence Berkeley National Lab, [4]Dept of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, [5]Dept of Biology, Middle Tennessee State University, [6]Dept of Plant Pathology & Microbiology, University of California, Riverside, [7]Botany and Plant Pathology, Oregon State University

Basidiobolus is a zygomycete fungus that possesses a complex life cycle. It produces multiple spore types adapted to different ecological niches. This fungus is best known for being associated with the gut microbiome of herptile (amphibians and reptiles) species. Two *Basidiobolus* species have been sequenced as part of the 1000 Fungal Genomes project revealing the genus is characterized by relatively large genomes of ~100 MB with approximately 50% repetitive DNA, and an unusual genome architecture compared to most eukaryotes (e.g. hundreds of chromosomes). Compared to other zygomycete fungi of Mucoromycota and Zoopagomycota, *Basidiobolus* genomes also possess a higher count of genes functioning in secondary or specialized metabolism. Many of these genes are hypothesized to be acquired by horizontal gene transfer (HGT) from bacteria, a finding that supports *Basidiobolus* as an example of animal-associated gut fungi adapting to their environment through HGT. We will present results of the analysis of six new *Basidiobolus* isolates, obtained from different herptile species, focusing on genome organization, comparative genomics of *Basidiobolus* species, the genomic diversity of secondary metabolite gene clusters, and the impact of HGT from co-occurring gut bacteria.

163 **Distribution of endobacteria associated with Mortierellomycotina and Mucoromycotina fungi across coastal and desert eco-regions of South Africa** Mmanoko Napo[1], Alicia Kock[1], Kazeem Alayande[1], Jessie Uehling[2], Teresa Pawlowska[3], Rasheed Adeleke[1] [1]Microbiology, North West University, [2]Botany and Plant Pathology & Plant-Microbe Biology, Oregon State University, [3]Plant Pathology & Plant-Microbe Biology, Cornell University

Endobacteria-fungal interactions have been reported worldwide. However, information concerning these interactions in South African plant-associated communities is limited. In the present study, fungal species of Mortierellomycotina and Mucoromycotina were isolated from rhizospheric soil samples collected from the Fynbos and Nama Karoo biomes in South Africa. We identified potential symbiotic interactions by screening for putative endosymbiotic bacteria using the polymerase chain reaction (PCR) for 16S rDNA in fungal DNA extractions. We then identified endobacterial symbionts using phylogenetic analysis. We found that species of Mucoromycota with endobacterial symbionts were significantly more diverse in the Nama Karoo eco-region when compared to that of the Fynbos eco-region. A total of 437 Mucoromycota fungi were screened for endobacteria in this study, from which 41 were confirmed to harbour symbiotic interactions. Out of the symbiotic interactions, 68% belonged to *Rhizopus,* 15% to *Linemmania elongata*, and 5% to *Cunninghamella echinulata.* Conversely, only 7% of the interactions were identified in *Mortierella* and 5% in *Podila* species isolated from the coastal eco-region. Phylogenetic analysis revealed that endobacteria identified were closely related to *Burkholderiaceae.* While the most prevalent Mucoromycota genus was *Rhizopus* in the desert eco-region, with 65% prevalence, followed by *Mortierella* with 14% and *Cunninghamella* with 14%, the most predominant fungal genus found in the coastal eco-region was *Mucor,* with 47% prevalence, followed by *Mortierella* with 37%, with less symbiotic interaction with endobacteria. The least prevalent fungal genus found in both eco-regions was *Actinomucor,* contributing only 0.7% and 1.1% of the overall diversity in the desert and coastal eco-regions, respectively.

164 **The molecular mechanisms of toxocyst development in oyster mushroom** Yi-Yun Lee[1,2], Sheng-Chian Juan[1,2], Yen-Ping Hsueh[1,2] [1]Institute of Molecular Biology, Academia Sinica, [2]Molecular and Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Sciences, National Defense Medical Center

Pleurotus ostreatus, the oyster mushroom, utilizes lollipop-shaped structures, toxocysts, to rapidly paralyze and kill nematodes under starvation. We have conducted forward genetic screens in *P. ostreatus* and isolated 22 *loss-of-toxicity* (*lot*) mutants that were incapable of paralyzing *C. elegans*. Whole genome sequencing and genetic mapping were employed to pinpoint the causative mutations in *lot1*, *lot2*, and *lot3* mutants to be pho85-cyclin 1 (*PCL1*), *HIS1*, and a transcription factor for toxocyst development 1 (*TTD1*), respectively. First, we explored the connection between Pho85, a cyclin-dependent protein kinase, and Pcl1. Through yeast two-hybrid assays, we demonstrated a physical interaction between Pho85 and Pcl1, suggesting that the Pho85-Pcl1 complex may influence the cytoskeleton and septin organization, leading to toxocyst formation. Subsequently, we developed a congenic strain, PC9.15, derived from the original mutagenized PC9 strain, and conducted bulked segregant analysis to map the remaining 19 *lot* mutants. Our analysis revealed that the *lot5*, *lot9*, *lot12*, *lot15*, and *lot21* mutants have mutations in tetrahydrofolate synthase (*MIS1*), *HIS4*, and *HIS5*. Furthermore, histidine supplementation in the growth medium successfully restored the

deficiency in toxocyst development observed in these *lot* mutants, indicating the histidine biosynthetic pathway is required for toxocyst development.

165 **The nematode-trapping fungus *Arthrobotrys flagrans* small-secreted protein NipA interferes with cuticle integrity in *Caenorhabditis elegans*** Jennifer Emser, Reinhard Fischer Karlsruhe Institute of Technology

The primary line of protection for animals often lies in their surface structures, which are frequently reinforced by polymeric proteins. Nematodes, such as *Caenorhabditis elegans*, rely on a robust cuticle as their first defense against external threats, necessitating pathogenic microorganisms to surmount this barrier during assaults via unconventional entry points. Nematode-trapping fungi have evolved specialized hyphal structures designed to capture and immobilize live nematodes. *Arthrobotrys flagrans* employs adhesive trapping networks to lure and capture the nematode prey. It subsequently breaches the nematode cuticle, establishing an infection bulb beneath the epidermis, from which it proceeds to colonize and digest the entire nematode organism. While lytic enzymes play a significant role, small-secreted proteins (SSPs) have emerged as crucial effectors in this intricate process. Here, we characterized NipA (nematode induced protein A), a key SSP in this context. *nipA* was transcriptionally upregulated in fungal traps, with the protein accumulating at the penetration site. The absence of NipA resulted in delayed penetration compared to wild type. Moreover, expression of *nipA* within the epidermis of *C. elegans* led to aberrant regulation of specific pathways and the formation of characteristic blisters. NipA cysteine residue 23, not involved in intramolecular disulfide bond formation, was required for blister formation. These findings shed light on the multifaceted role of NipA in the complex interaction between nematode-trapping fungi and the nematode preys.

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166 **Inducing Novel Endosymbioses by Bacterial Implantation into Fungi** Gabriel H. Giger[1], Chantal Ernst[1], Ingrid Richter[2], Thomas Gassler[1], Patrick Kiefer[1], Christoph G. Gäbelein[1], Orane Guillaume–Gentil[1], Kirstin Scherlach[2], Miriam Bortfeld-Miller[1], Tomaso Zambelli[3], Markus Künzler[1], Christian Hertweck[2,4,5], Julia A. Vorholt[1] [1]Institute of Microbiology, ETH Zurich, [2]Leibniz Institute for Natural Product Research and Infection Biology, HKI Jena, [3]Institute for Biomedical Engineering, ETH Zurich, [4]Institute of Microbiology, Friedrich Schiller University Jena, [5]Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena

Endosymbioses, marked by the intimate partnering of cells within cells and their metabolisms, have profoundly influenced life and have driven evolutionary transitions and innovations, including the emergence of eukaryotes. Furthermore, endosymbioses play an important role in the interactions across kingdoms, including those between fungi and bacteria. Uncovering new endosymbiotic relationships is particularly challenging because it mostly relies on retrospective analysis of systems that have naturally evolved. By combining atomic force microscopy, optical microscopy and nanofluidics, we developed a FluidFM-based approach for bacteria implantation to follow the fate of artificially induced endosymbiosis in fungi. As a model system, we use the wide-spread filamentous fungus *Rhizopus microsporus*. The injection of *Escherichia coli* and pre-adapted endosymbiont bacteria of the Burkholderia family into *R. microsporus* resulted in striking differences in host response. While injected *E. coli* was not transmitted to fungal spores, *Mycetohabitans rhizoxinica*, known for forming endosymbiotic relationships in a different *R. microsporus* strain, reached the spores in this non-host fungus and was vertically transmitted. This bacterium, which naturally synthesizes rhizoxin congeners to aid the host in acquiring carbon and defending against predators, maintained its metabolic functions within the new host. Vertical transmission of novel endosymbionts impacted host fitness, however, positive selection mitigated these fitness constraints and stabilized the endosymbiotic relationship during adaptive laboratory evolution. The approach provides an experimental framework to investigate the initial stages of endosymbiosis and to empirically test cost-benefit trade-offs.

167 **Do Fungi have an Immune System? The Neurospora crassa and Pseudomonas syringae pathosystem reveals an initial cellular reaction to bacterial proximity** Frances G Stark, Mari Torii-Karch, Ksenia Krasileva Plant and Microbial Biology, University of California, Berkeley

Recent comparative genomics and evolutionary analyses provided multiple lines of evidence for the fungal immune system and proposed hypotheses for mechanistic interactions. Fungi possess complex non-self, or "xeno-recognition", surveillance systems similar to protective, non-self surveillance systems in plants, animals, and bacteria. Understanding similarities of how different organisms across kingdoms respond to non-self requires leveraging existing model systems and their genetic toolkits. We leveraged two model systems, Neurospora crassa and Pseudomonas syringae DC3000 (pstDC3000) to dissect fungal response to bacteria. PstDC3000 preferentially surrounds N. crassa germlings on a solid surface, causing Propidium Iodide (PI) vital dye uptake, indicative of a cell death response, as early as ten minutes post bacterial proximity. Inoculating N. crassa with heat-killed pstDC3000 abolished the PI uptake. Deletion mutants of common or proposed cell death regulating genes in N. crassa and pstDC3000 did not abolish PI uptake including; multiple HET genes, VIB1, PhcA, T3SS, and eleven of the seventeen proposed NLR-like genes in N. crassa. To try and dissect initial cellular signaling events, we performed transcriptomics on N. crassa after pstDC3000 inoculation at ten minutes and one hour. Our study provides insight into an early transcriptional response in filamentous fungi exposed to bacteria alongside surveying fungal NLR-like deletion mutants.

168 **Spatiotemporal regulation of peroxisome and endoplasmic reticulum dynamics during *Podospora anserina* sexual development** Leonardo Peraza-Reyes, Beatriz Aguirre-Lopez, Sebastian Palacios-Martinez, Matias Ramirez-Noguez Institute of Cellular Physiology, National Autonomous University of Mexico (UNAM)
Sexual reproduction is a complex developmental process that requires precise coordination between the differentiation of multiple cell types and nuclear progression through karyogamy and meiosis. This process entails important changes in cellular function and architecture. In the model fungus *Podospora anserina*, sexual development involves precise regulation of peroxisome dynamics, which includes changes in its morphology and intracellular distribution. During this process, peroxisome remodeling requires the activity of Dnm1 and Fis1 fission proteins, which also mediate mitochondrial fission. These proteins promote peroxisome segregation at key meiotic differentiation processes and their elimination affects the progression of these processes. Sexual development also involves remodeling of the endoplasmic reticulum (ER), which depends on the membrane-shaping protein Rtn1. This protein defines spatially distinct ER domains along meiotic development, and is required for meiotic spindle function and nuclear segregation. These findings suggest that the processes that regulate the remodeling and distribution of these organelles are coupled. The transport and distribution of peroxisomes and the ER in filamentous fungi depends on early endosomes, with whom they interact to be co-transported by microtubule motor proteins. While the nature of the early endosome-ER association is unknown, endosome-peroxisome interaction in *Aspergillus nidulans* is mediated by the adapter protein PxdA. In *P. anserina*, in addition to peroxisome motility, Pxd1(PxdA) is required for ER remodeling. The elimination of this protein affects hyphal growth and morphogenesis, as well as meiotic differentiation, and these defects are exacerbated upon simultaneous elimination of ER-shaping proteins, such as Rtn1. Altogether, our findings support the idea that sexual development requires the concerted regulation of peroxisome and ER dynamics. This research was supported by grants CONACYT-DFG 277869 and PAPIIT, DGAPA, UNAM IN227823.

169 **Molecular determinants of *Cryptococcus neoformans* pleiotropic morphologies in response to host-relevant conditions** Elizabeth Ballou MRC Centre for Medical Mycology, University of Exeter

In response to a variety of host-relevant signals, the human pathogenic yeast *Cryptococcus neoformans* undergoes a programmed morphological switch. Uniform haploid yeast convert to a heterogenous population of pleiotropic morphologies, including large, polyploid titan cells, haploid yeast-like cells, and small titanide cells. Each form contributes to disease progression by driving phagocyte evasion, dissemination, drug resistance, and aneuploidy underpinning genomic diversity. However, the nature of these different morphologies and the molecular mechanisms by which heterogeneity emerges remain poorly understood.

Here, we more fully describe key *in vivo* relevant morphologies, including titanide cells (2-3 um oval cells), "seed cells" (4-6 um *in vivo*-relevant cells), yeast-like cells (4-6 um), and titan cells (>10 um). To further explore this, we take a high throughput approach to identify genetic determinants of heterogenous size across populations. We identify signaling mechanisms that integrate environmental signals with second messenger dynamics to enable entry into cell cycle arrest and subsequent exit resulting in a variety of cellular fates. Then, using genetic, genomic, and cell biology approaches, we identify and validate key determinants of population heterogeneity. Overall, our approach reveals molecular determinants of the cellular response to exposure to the host environment by a major human fungal pathogen.

170 ***Candida albicans*** **morphogenesis at different scales** Antonio Serrano, Emily Plumb, Charles Puerner, Robert A. Arkowitz Institute of Biology Valrose, University Côte d'Azur/CNRS/INSERM

We have been focusing on the yeast to hyphal transition, as well as subsequent filamentous growth, both that of the main filament apex and filament branching, in the human fungal pathogen, *Candida albicans*. Filament branching results in an increase in the number of growth sites and may play a role in virulence in this fungal pathogen [1,2]. We have been using live-cell microscopy to investigate these processes in *C.* albicans and to analyze morphological, as well as molecular, changes associated with these transitions. Our results indicate that filament branching is a distinct growth state. Specifically, we observe differences between branch and main filament growth, together with distinct distributions and/or levels of reporters for exocytosis, endocytosis and critical lipids. In contrast, the distribution and level of the key polarity small GTPase Cdc42 are similar between branch and main filament growth. We have also been investigating the biophysical characteristics of the cytoplasm using a micro-rheological probe, and observed a striking difference between these growth processes with respect to cytoplasmic crowding/viscosity. In summary, at the cellular scale, our results reveal that filament branching is a distinct growth process. Furthermore, at the sub-cellular scale, our results reveal differences in the cytoplasm during morphogenesis and response to stress.

[1] Barelle CJ *et al*. **Eukaryot. Cell** 2003 **2:**398-410.
[2] Veses V *et al*. **Mol Microbiol**. 2009 **71**:505-19.

171 **Modeling Asynchronous Nuclear Division in *Ashbya Gossypii*** Grace McLaughlin[1,2], Jay Newby[3], Veronica Ciocanel[4], Lauren Melfi[5], Benjamin Stormo[4], Therese Gerbich[6], Marcus Roper[7], Alexander Mayer[7], Timothy Elston[8], Amy Gladfelter[4] [1]Cell Biology, Duke University, [2]Biology, University of North Carolina at Chapel Hill, [3]University of Alberta, [4]Duke University, [5]Wentworth Institute of Technology, [6]Harvard University, [7]UCLA, [8]University of North Carolina at Chapel Hill

Multinucleate cells are common in biology, with examples including muscle cells, placenta, and fungi. Despite this, many aspects of their cell biology are not well understood. Dividing nuclei residing in a common cytosol would be expected to synchronize, as the oscillating levels of cell cycle regulators from each nucleus should in theory entrain neighbors. However, in the multinucleate fungus *Ashbya gossypii*, spatially neighboring nuclei have been observed to divide out of sync. Here we mathematically model *Ashbya* nuclei as a dynamically growing system of coupled phase oscillators to determine possible mechanisms that could lead to asynchronous division. Our goal is to study the effects of nuclear mobility, cytosolic compartmentalization, inhibitory signals, and noise on transient phase dynamics. To compare the model with experimental results, we develop a nuclear tracking pipeline with the aim of tracking nuclei during bypassing events, identifying nuclear division, and linking nuclei into hyphae. Initial results suggest a combination of locally and globally acting mechanisms are at play leading to the observed asynchrony in *Ashbya*.

172 **Peroxisome hitchhiking in the Pezizomycotina** Samara L Reck-Peterson[1,2], Livia Songster[1], Valentin Wernet[1], Gaurav Kumar[1], Swetha Mahesula[1], Patreece Suen[1] [1]UC San Diego, [2]Howard Hughes Medical Institute
Organelle positioning in many eukaryotic cells is driven by the microtubule cytoskeleton. Previously, we discovered that peroxisomes move by 'hitchhiking' on early endosomes in the filamentous fungus *Aspergillus nidulans*. Using a genetic screen, we identified an endosome-associated protein that is required for peroxisome hitchhiking, which we named PxdA. PxdA is found exclusively in the Pezizomycotina subphylum of the Ascomycota fungi. This subphylum of fungi is notable for its many pathogenic and industrially important species, as well as the large number of secondary metabolites they produce. Secondary metabolites in these fungi, which are encoded in gene clusters, include antibiotics such as penicillin, toxins such as aflatoxin, and metal harvesting siderophores. We are investigating the role of peroxisome hitchhiking in two members of the Pezizomycotina: *Aspergillus nidulans* and *Alternaria alternata*. Our data suggest that the majority of secondary metabolite biosynthesis pathways use peroxisomes. Furthermore, our preliminary data suggest that peroxisome hitchhiking on endosomes is important for the production and/ or secretion of secondary metabolites. Finally, I will discuss our ongoing work to determine if peroxisome hitchhiking is required for virulence in virulent species of the Pezizomycotina.

173 **A conserved oxylipin alarm blocks the fungicidal effects of echinocandins in pathogenic aspergilli** Dante G Calise[1], Sung Chul Park[1], Jin Woo Bok[1], Gustavo H Goldman[2], Nancy P Keller[1,3] [1]Dept of Medical Microbiology & Immunology, University of Wisconsin - Madison, [2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, [3]Dept of Plant Pathology, University of Wisconsin - Madison

Humans readily inhale spores of the ubiquitous mold *Aspergillus fumigatus*. Small enough to reach the alveoli of the lung, these spores are rapidly cleared by a healthy immune system without development of disease. However, in immunocompromised individuals, germination and tissue invasive hyphal growth can lead to a life-threatening infection termed invasive aspergillosis

(IA). The recommended first line treatment of IA is with triazole antifungals, but in cases of poor clinical response to these membrane targeting drugs, salvage therapy with the cell wall active echinocandins is crucial for effective treatment. The echinocandin antifungals—caspofungin, micafungin, and anidulafungin—are limited in that they are only fungistatic against aspergilli due to their inability to kill established hyphae. However, *in vitro,* treatment of *A. fumigatus* with inhibitory concentrations of caspofungin results in the death of approximately fifty percent of germinating conidia by fungicidal lysis of their growing tips. Surviving germlings are further inhibited fungistatically and display severely stunted hyphal growth developing into highly branched chitin rich microcolonies. As they grow, caspofungin treated hyphae continue to undergo tip lysis, but the fungicidal effect is limited to the apical most hyphal compartment by the blocking of septal pores. Our lab recently found that the fungal oxylipin 5,8-diHODE, produced by *A. fumigatus* and related aspergilli, induces hyphal growth reminiscent of echinocandin treatment with increases in lateral branching, septation, and cell wall chitin. Here, we uncover an endogenous mechanism of antifungal tolerance in aspergilli whereby 5,8-diHODE activates echinocandin tolerant growth. We found that treatment of wild type *A. fumigatus* with echinocandins induced robust production of 5,8-diHODE by the enzyme PpoA. Further, we found that cotreatment with 5,8-diHODE blocked the fungicidal lysis of germinating conidia by caspofungin and micafungin. This protection against echinocandin tip lysis was also conserved in the related species *A. flavus* and *A. nidulans*. Lastly, we found that the transcription factor ZfpA was required for both induction of PpoA by caspofungin and full protection by 5,8-diHODE. Together, our findings reveal 5,8-diHODE to be an inducible and protective signal to activate echinocandin tolerant growth programs among pathogenic aspergilli.

174 **A fitness landscape instability determines the morphological diversity of tip growing organisms** Branka Zivanovic[1], Enrique Rojas[2], Maxim Ohairwe Ermoshkin[2] [1]Institute for Multidisciplinary Research, University of Belgrade, [2]Biology, New York University

Cellular morphology affects many aspects of cellular and organismal physiology. This makes it challenging to dissect the evolutionary basis for specific morphologies since various cellular functions may exert competing selective pressures on this trait, and the influence of these pressures will depend on the mechanisms of morphogenesis at play. To tackle this problem, we combined experiment and theory to investigate the mechanistic basis for morphological diversity among tip-growing cells from across the tree of life including fungal, plant and oomycete organisms. We discovered that an instability in the widespread mechanism of “inflationary” tip growth leads directly to a bifurcation in the common fitness landscape of tip-growing cells, which imposes a strict global constraint on their morphologies. We were able to test the predictions of shape instability on the highly tapered hyphae of the oomycete Achlya bisexualis. This result rationalizes the morphology of an enormous diversity of important fungal, plant, protistan, and bacterial systems. More broadly, our study describes a novel principle by which strong evolutionary constraints on complex traits, like biological form, may emerge from emergent instabilities within developmental systems.

175 **The *Neurospora crassa* JSN-1 protein binds multiple transcripts, including mRNA species required for proper conidiation** Anne Yenewodage[1], Zheng Wang[2], Jeffrey P Townsend[2], Oded Yarden[3] [1]Hebrew Univ of Jerusalem, [2]Yale University, [3]The Hebrew University of Jerusalem

RNA-binding proteins (RBPs) play crucial roles in cellular processes such as RNA transport, degradation, and translation. They are essential for the regulation of post-transcriptional gene expression and constitute hundreds of proteins in the eukaryotic genome. JSN-1 (NCU06199) is a member of the highly conserved Pomillio RBP family and is a member of the COT-1-GUL-1 complex, a kinase and RBP involved in the regulation of cell polarity, in *N. crassa*. The Δ*jsn-1* strain exhibits a block in the minor constriction phase of conidiation and phenotypically resembles the mutants *fl* and *acon-3*. To profile the repertoire of transcripts bound by JSN-1, we employed high-stringency RNA immunoprecipitation using a JSN-1::GFP-expressing culture, coupled with RNA sequencing. JSN-1 was found to bind a total of 1515 transcripts. 71 of them were also found to be bound by the co-complexing RBP GUL-1. Gene Ontology (GO) analysis revealed enrichment of organelle- and membrane-related genes. We also found enrichment in transcripts related to protein and nucleic-acid binding and a variety of protein kinases (e.g., *stk-32* and *stk-43*). Interestingly, JSN-1 exhibited binding affinity to several conidiation-related transcripts, including *con-6* (NCU08769), *con-8* (NCU09235), *con-10* (NCU07325), *acon-3* (NCU07617), *acon-4* (NCU03043), *fl* (NCU08726) and *fld* (NCU09739). To identify which genes exhibited altered expression in a Δ*jsn-1* mutant during conidiation, we profiled the transcriptome of wild-type and the Δ*jsn-1* strains at four time points (0, 4, 8, and 14 hours) after induction of conidiation. The major differences in the expression of genes involved in conidiation included a three-fold increase in the expression levels of *con-6* in the mutant 4 hours after induction of conidiation, a 60% reduction in the mRNA abundance of *acon-3* at the 8- and 14-hour time points, and a two-fold increase in the expression of *con-8* in the mutant at 14 hours post-induction. In addition, *csp-1* (NCU02713) expression was reduced two-fold at 8 hours post-induction. Furthermore, we found that expression of genes encoding for the hypothetical proteins NCU05529 and NCU09952 was completely abolished in the Δ*jsn-1* mutant. While JSN-1 is required for proper conidiation, the multiple mRNA species found to

bind to this protein indicate its involvement in multiple cellular processes. It is likely that other RBPs (like GUL-1) can provide at least partial functional compensation when JSN-1 is impaired.

176 **Evolution of outbreak potential and pathogenesis via a novel fungal adhesin** Teresa O'Meara University of Michigan

Candida auris is an emerging fungal pathogen responsible for healthcare-associated outbreaks driven by persistent surface colonization, including on catheters and human skin. We functionally characterized the arsenal of adhesins used by *C. auris* for surface association and discovered that instead of using conserved and canonical adhesins, *C. auris* relies on *Surface Colonization Factor (SCF1)*, a novel adhesin that appears to be unique to the *C. auris* lineage. The sequence of the *SCF1* protein is highly conserved across all isolates of *C. auris* from all five clades; however, expression of *SCF1* varied widely among *C. auris* isolates and was tightly correlated with adhesive capacity. Unlike canonical adhesins which mediate surface attachment via hydrophobic interactions, *SCF1* relies on cation-substrate interactions in a manner analogous to marine bivalve attachment proteins, potentially suggesting convergent selective pressures during ocean growth. We also demonstrate that *SCF1* is a major contributor to both colonization and pathogenesis. Overexpression of *SCF1* was sufficient to drive biofilm formation in the rat central venous catheter and high levels of skin colonization in both murine models and human skin explants. Importantly, *SCF1* is also critical for systemic disease in the neutropenic mouse model of invasive candidiasis, while having no impact on *in vitro* growth, suggesting that *SCF1* is the first true virulence factor for this emerging fungal pathogen. We speculate that the presence of this novel adhesin protein may have facilitated the movement of *C. auris* from a currently unknown environmental reservoir into hospital and patient-associated outbreaks.

177 **Host adaptation mechanisms in fungal pathogens: harnessing GWAS to explore host associated genomic traits in natural infections of fungal pathogens** Cecile Lorrain[1], Alice Feurtey[1,2], Julien Alassimone[1], Bruce A McDonald[1] [1]Plant Pathology, ETH Zurich, [2]Laboratory of Evolutionary Genetics, University of Neuchatel

Plant-pathogenic microbes, including the wheat fungal pathogen *Zymoseptoria tritici*, need to adapt to their host environment[1]. Despite extensive research efforts, the mechanisms underlying specific host environment adaptation of fungal pathogens are still largely unknown. In plants, genome-wide association studies (GWAS) have been extensively used to uncover the complexity of local adaptation and disease resistance genetic architecture. However, the application of GWAS in deciphering fungal pathogenicity and host adaptation is trailing behind**.** The main limitation for large-scale GWAS in plant pathogens remains the phenotyping of several hundreds to thousands of strains in multiple hosts. Here, we leverage the power of GWAS to identify host-associated genomic traits in *Z. tritici* using natural infection data and whole-genome sequencing of 900 fungal strains from twelve different host cultivars. For this we compared one group of strains from a focal wheat cultivar against randomly sub-sampled groups of strains from different hosts, likewise correcting for unbalanced group sizes with a bootstrapping approach. We identified from one to twelve candidate genes associated with specific wheat cultivars. Among these, we found the effector *Avr3D1*, one of the few *Z. tritici* characterized effectors, which provides a proof-of-concept for our host-associated GWAS approach. Additionally, we identified a diversity of gene functions from predicted effector candidates to transcription factors, highlighting the complexity of the genetic basis underlying natural infections. By tapping into natural infection data, our study provides a novel outlook for GWAS in fungal plant-pathogens, transcending the limitations imposed by traditional phenotyping methods.

1. Feurtey, A. *et al.* A thousand-genome panel retraces the global spread and adaptation of a major fungal crop pathogen. *Nat. Commun. 2023 141* **14**, 1–15 (2023).

178 **Genomic insights into recurrent vulvovaginal candidiasis** Abdul-Rahman Adamu Bukari[1], Javier San Juan[1], Yana Syvolos[1], Rebekah Kukurudz[1], Vanessa Poliquin[2], Aleeza Gerstein[1,3] [1]Microbiology, University of Manitoba, [2]Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, [3]Statistics, University of Manitoba

Vulvovaginal candidiasis is one of the most common vaginal and fungal infections. The majority of symptoms are successfully treated with antifungal drugs, but in ~9% of cases, symptoms return even with treatment. Although there are some known risk factors for recurrence, many cases are idiopathic. We sought to examine the genotypic diversity of yeast populations that are present during a symptomatic infection to gain insight into the evolutionary processes that function during these chronic infections. We collected a total of 116 yeast isolates from vaginal swabs of 12 participants (6-12 isolates each) with a history of symptomatic recurrent vulvovaginal candidiasis. Ten of the participants had a *Candida albicans* infection while two had *Nakaseomyces glabrata*. To precisely quantify the standing genetic variation and genetic relatedness of the isolates colonizing each participant, we conducted phylogenetic analyses placing the *C. albicans* and *N. glabrata isolates* into global phylogenetic trees, consisting of 413 and 526 isolates respectively. Our phylogenetic analyses revealed that all isolates from an individual are highly clonal. The average nucleotide diversity among isolates from the same participant was < 0.003, with between 596 to 5,977 single nucleotide

polymorphisms differentiating contemporary isolates. *C. albicans* isolates from seven (of 10) participants clustered closely within clade 1. Examining the entire tree, clade 1 was statistically overrepresented for vaginal isolates compared to other clades. To determine whether individuals were colonized by a unique genotype in the vagina than other body parts, rectal isolates from four participants and an oral isolate from one participant were compared with the vaginal isolates. In all cases, the other-site isolates were phylogenetically overlapping with vaginal isolates, indicative of frequent migration between sites. Isolates from the same participant exhibited a consistent loss of heterozygosity (LOH) profile, but one individual had isolates with two different LOH profiles on the left arm of chromosome 1. Specifically, 11 out of 24 isolates had a ~1Mb LOH region containing 461 genes, most of whose functions are unknown. Notably, this LOH was present in both rectal and vaginal isolates, but it was more prevalent in the vaginal isolates. This study highlights the within-host isolate variation during a yeast infection and shows the similarity between isolates within individuals with recurrent vulvovaginal candidiasis.

179 **Evolutionary significance of fungal hypermutation** Johanna Rhodes[1,2], Norman van Rhijn[3], Yinggai Song[1], Michael Bottery[3], Xinyi Wang[2], Hugh Gifford[4], Rodrigo Leitao[2], Amelie Brackin[2], Duncan Wilson[4], Rhys Farrer[4], Alireza Abdolrasouli[5], Matthew Fisher[2], Paul Verweij[1], Darius Armstrong-James[2] [1]Radboudumc, [2]Imperial College London, [3]University of Manchester, [4]University of Exeter, [5]King's College Hospital

Elevated spontaneous mutation rates in pathogenic fungi, similar to their bacterial cousins, result in 'hypermutators'. Whilst deleterious mutations may result in a fitness defect, the accumulation of beneficial mutations can confer antifungal drug resistance and host adaptation. This could potentially lead to a serious public health threat when hypermutator infections occur in the clinic.

Defects in DNA mismatch repair (MMR) genes result in hypermutator states. We have previously shown that nonsense mutations in MMR genes result in truncated MSH2, MSH5 and RAD5 proteins in *Cryptococcus neoformans*. These nonsense mutations caused an elevated mutation rate, an antifungal drug resistant phenotype, and likely contributed to a relapse infection.

We are now observing (hyper)mutator states in more human pathogenic fungi. Non-synonymous mutations conferring amino acid substitutions in *mre11* in a drug-susceptible background of *Aspergillus fumigatus* also result in a mutator state; over time this could result in spontaneous mutations conferring antifungal drug resistance. Similarly, we have observed non-synonymous and nonsense mutations in MMR genes in major lineages of *C. auris*, resulting in an elevated mutation rate. In some isolates this mutation switches to encode a stop codon, resulting in hypermutation. It is possible that this (hyper)mutator state in *C. auris* is responsible for the rapid evolution of its pan-drug resistance profile, and swift host adaptation and worldwide dispersal.

180 ***Verticillium dahliae* Vta3 promotes *ELV1* virulence factor gene expression in xylem sap, but tames Mtf1-mediated late stages of fungus-plant interactions and microsclerotia formation** Isabel Maurus[1], Rebekka Harting[1], Cornelia Herrfurth[2], Jessica Starke[1], Alexandra Nagel[1], Lennart Mohnike[2], Ying-Yu Chen[1], Kerstin Schmitt[1], Emmanouil Bastakis[1], Marian T. Süß[1], Miriam Leonard[1], Kai Heimel[1], Oliver Valerius[1], Ivo Feussner[2], James W. Kronstad[3], Gerhard H. Braus[1] [1]Dept of Molecular Microbiology and Genetics, University of Goettingen, [2]Dept of Plant Biochemistry and Service Unit for Metabolomics and Lipidomics, University of Goettingen, [3]Dept of Microbiology and Immunology, University of British Columbia

Verticillium transcription activator of adhesion 3 (Vta3) is required for plant root colonization and pathogenicity of the soil-borne vascular fungus *Verticillium dahliae*. RNA sequencing identified Vta3-dependent genetic networks required for growth in tomato xylem sap. Vta3 affects the expression of more than 1,000 transcripts, including candidates with predicted functions in virulence and morphogenesis such as Egh16-like virulence factor 1 (Elv1) and Master transcription factor 1 (Mtf1). The genes encoding Elv1 and Mtf1 were deleted and their functions in *V*. *dahliae* growth and virulence on tomato (*Solanum lycopersicum*) plants were investigated using genetics, plant infection experiments, gene expression studies and phytohormone analyses. Vta3 contributes to virulence by promoting *ELV1* expression, which is dispensable for vegetative growth and conidiation. Vta3 decreases disease symptoms mediated by Mtf1 in advanced stages of tomato plant colonization, while Mtf1 induces the expression of fungal effector genes and tomato pathogenesis-related protein genes. The levels of pipecolic and salicylic acids functioning in tomato defense signaling against (hemi-) biotrophic pathogens depend on the presence of *MTF1*, which promotes the formation of resting structures at the end of the infection cycle. In summary, the presence of *VTA3* alters gene expression of virulence factors and tames the Mtf1 genetic subnetwork for late stages of plant disease progression and subsequent survival of the fungus in the soil.

181 **Segmental duplications drive the evolution of accessory genomic regions in the major fungal plant pathogen *Fusarium oxysporum*** Anouk C. van Westerhoven[1,2], Einar Martinez de la Parte[1], Gert H.J. Kema[1], Michael F. Seidl[2] [1]Laboratory of Phytopathology, Wageningen University, [2]Theoretical Biology and Bioinformatics, Utrecht University

Fungal plant pathogens are in a constant evolutionary arms races to overcome host resistance. Many fungal plant pathogens carry a compartmentalized genome, containing conserved core and variable accessory regions. The variable accessory regions often contain pathogenicity genes essential for host colonization, and the variability of these regions is thought to enable rapid adaptation helping to overcome host resistance. The fungal plant pathogen *Fusarium oxysporum* carries large accessory regions that can span entire chromosomes and encode many essential pathogenicity genes. The presence of specific accessory regions can influence the host range, and horizontal transfer of accessory regions can modify the pathogenicity of the recipient strain. Although the accessory regions in *Fusarium oxysporum* play a role in host pathogenicity, it remains unclear how variable these regions are between isolates infecting the same host and how these accessory regions emerge and evolve. Here, we define the pangenome of 69 banana infecting *Fusarium* isolates that cause Fusarium wilt of banana, a devastating disease that poses a significant constraint to global banana production. We analyzed the diversity and evolution of the strains and identified extensive variation of accessory regions and pathogenicity genes, even between *Fusarium* strains that can infect the same banana variety. Moreover, we demonstrate that segmental duplications and chromosomal aneuploidy are associated with accessory regions and show that these duplications drive the evolution and expansion of accessory regions not only in *Fusarium oxysporum* but also in related phytopathogenic *Fusarium* species. This provides novel insights into the origin and evolution of accessory genomic regions in *Fusarium*, which helps to understand how pathogenicity and host-specificity are established in this major plant pathogen.

182 **Ryp transcription factors link temperature sensing and morphogenesis in *Histoplasma*** Anna Morrison, Mark Voorhies, Anita Sil UCSF

Temperature plays a critical role in altering the developmental program and virulence of thermally dimorphic fungal pathogens, including *Histoplasma* species. In the environment, *Histoplasma* grows as a multicellular hyphal form that produces vegetative spores. Upon inhalation by a mammalian host, spores undergo a developmental change to a parasitic yeast form that secretes virulence factors and causes disease. This morphological transition can be recapitulated in the laboratory, where temperature is a sufficient signal to shift cultures of *Histoplasma* between hyphal and yeast forms, which grow at 25°C and 37°C, respectively. To understand how *Histoplasma* links temperature to changes in morphology and gene expression, we performed RNA-Sequencing on samples grown at 37°C versus those transitioned to 25°C for two hours to capture early changes in transcript levels. Expression of the majority of acutely temperature-responsive transcripts is dependent upon the Ryp (Required for Yeast Phase) transcription factors, which were previously identified in our laboratory to be key regulators of the morphogenesis program in *Histoplasma*. To identify temperature-sensitive changes in Ryp DNA-binding activity that might account for transcriptomic changes, we then performed Ryp Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) on samples grown at 37°C versus those transitioned to 25°C for two hours. The majority of Ryp2 association events were not differential under these conditions. However, we observed Ryp2 binding events at heat shock elements in the promoters of key heat shock protein (HSP) genes, including HSP90 and HSP70, only at 37°C. Strikingly, Ryp2 association at these promoters was lost within two hours at 25°C, indicating that Ryp2 association is temperature-dependent. Loss of Ryp2 association was correlated with a greater than two-fold drop in transcript levels of many HSP genes. This study strongly suggests that Ryp2 is upstream of HSP gene expression, and highlights for the first time a link between the heat shock pathway, which is intrinsically temperature-responsive, and the Ryp transcription factors, which control morphology in *Histoplasma*. This work thus provides insight into how thermally dimorphic fungi sense temperature and transduce this signal into downstream pathways involved in morphogenesis and virulence.

183 **Pathogenicity is associated with population structure in a fungal pathogen of humans** Anne Hatmaker[1], Amelia E Barber[2], Milton T Drott[3,4], Thomas J.C. Sauters[1], Ana Alastruey-Izquierdo[5], Dea Hermoso-Garcia[6], Oliver Kurzai[7], Antonis Rokas[1] [1]Biological Sciences, Vanderbilt University, [2]Friedrich Schiller University, [3]University of Minnesota, [4]USDA-ARS, [5]Instituto de Salud Carlos III, [6]Institut Pasteur, [7]Leibniz Institute for Natural Product Research and Infection Biology

The saprotrophic fungus *Aspergillus flavus* is a clinically and agriculturally important species responsible for devastating human infections and contamination of seed crops. To examine population structure and the pan-genome of *A. flavus*, we collected genomes from 250 (95 clinical and 155 environmental) isolates from 9 countries, including 70 newly sequenced clinical isolates. The core genome of *A. flavus* consisted of over 10,000 protein families present in at least 95% of isolates. Of these, 3,375 were single-copy orthologs present in all strains. Using over 900,000 single nucleotide polymorphisms, we identified five *A. flavus* populations; the five populations mostly corresponded to distinct clades in the genome-wide *A. flavus* phylogeny of the 250 isolates. Accessory genes, including genes previously associated with virulence and genes within biosynthetic gene clusters, were

distributed unequally across the five populations. Strikingly, although clinical isolates were present in all but one population, we found that over 75% of all clinical isolates were from a single population. These results suggest that, in contrast, to the cosmopolitan major pathogen *Aspergillus fumigatus*, *A. flavus* pathogenicity is associated with population structure, highlighting the value of whole genome sequencing of geographically diverse clinical and environmental isolates of clinically relevant fungi.

184 **Shaping of the structure and composition of microbiomes by natural products** Axel Brakhage Molecular and Applied Microbiology, Leibniz-HKI

In all known habitats on earth microorganisms form consortia with a multitude of prokaryotic and eukaryotic microorganisms. New data concerning the composition of microbiomes are published almost daily. They impressively demonstrate the diversity of microorganisms in various ecosystems that provide services critical for life. What has been missing until today is the identification of widespread universal communication molecules in nature that govern bacterial-fungal interactions across kingdoms and the structure and composition of microbiomes. Recently, we have discovered such molecules that are represented by microbial natural products, specifically ubiquitous bacterial arginine-derived polyketides. This was concluded from the discovery of an unprecedented tripartite interkingdom microbial consortium consisting of the bacterium *Streptomyces rapamycinicus* (or *S. iranensis*), the fungus *Aspergillus nidulans* and the green alga *Chlamydomonas reinhardtii* involving NPs. *The streptomycete produces the arginoketide azalomycin F that triggers the expression of the otherwise silent ors* gene cluster of *A. nidulans* resulting in the production of orsellinic acid and derivatives. In this way, the bacterium re-programs the epigenetic machinery of the fungus leading to acetylation of histones located in the *ors* gene promoters. Azalomycin F is also released in presence of *C. reinhardtii*. As a response, the alga swims to the mycelia of the fungus and is thereby protected from the toxic activity of azalomycin F. Furthermore, sublethal concentrations of azalomycin F trigger the formation of a protective multicellular structure by *C. reinhardtii*, which we named gloeocapsoid, suggesting that NPs may have contributed to the evolution of multicellularity. Together, the algae survive lethal NPs by forming a multicellular structure and of an alliance with a fungus. The ubiquitous distribution of biosynthesis gene clusters for the biosynthesis of arginine-derived polyketides in bacteria on all continents on earth except Antarctica and the ease with which we were able to isolate both arginoketide producers and fungal responders to the signal underlines the universality of this communication system. Arginoketides impact surrounding microorganisms both directly and indirectly, by inducing the production of fungal NPs that further influence the composition of microbial consortia. PNAS 2009; 2011; eLife 2018; 2020; ISME J 2020; PNAS 2021; Nature Microbiology 2023

185 **Small RNA-mediated gene expression regulation: a new knowledge on the mechanisms of biocontrol interactions** Edoardo Piombo[1], Ramesh Vetukuri[1], Anders Broberg[1], Dan Funck Jensen[1], Magnus Karlsson[1], Mukesh Dubey[2] [1]Swedish University of Agricultural Sciences, [2]Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences

Fungal biocontrol agents from the genera *Trichoderma* and *Clonostachys* can occupy diverse environmental niches and closely interact at inter-species and intra-species levels for nutrients and space. In addition to directly antagonizing fungal plant pathogens, some of these species can colonize plant roots and establish mutualistic associations with host plants, thereby promoting health and inducing immune responses against pathogens. For this association, biocontrol fungi and their fungal and plant hosts reprogramme their genetic machinery and establish a molecular dialogue determining the degree of interactions from fungal parasitism on other fungi to fungal mutualism on plants. Small RNAs (sRNAs) can play a crucial role in such interactions by mediating gene expression regulation at endogenous and cross-species levels through a mechanism known as RNA silencing. We investigated the role of sRNA-mediated gene regulation in biocontrol interactions by generating deletion mutants of Dicer genes (*dcl1* and *dcl2*) in the biocontrol fungus *Clonostachys rosea*. Deletion of *dcl2* resulted in *C. rosea* mutant with reduced specialized metabolite (SM) production, antagonism towards the plant pathogenic mycohost *Botrytis cinerea*, and reduced ability to control foot rot disease on wheat caused by *Fusarium graminearum*. However, the root colonization ability of the Δ*dcl2* strains was increased compared to *C. rosea* wildtype (WT). By comparing the sRNA and transcriptome of the WT and Dicer deletion strains during interactions with fungal hosts *B. cinerea* and *F. graminearum* and wheat root, we identified fungal and plant host-responsive microRNA-like RNAs (milRNAs) in *C. rosea* and their endogenous gene targets putatively involved in antagonism and induction of plant stress responses. At the cross-species level, these milRNAs were predicted to target *B. cinerea* and *F. graminearum* genes involved in fungal virulence, SM production and cell wall biogenesis, which showed an increased expression during interaction with the Δ*dcl2* mutant incapable of producing the corresponding milRNAs. Transcriptome analysis of wheat roots during interactions with *C. rosea* strains revealed transcriptomic reprogramming of genes involved in stress response, metabolism, and growth during the interactions with Δ*dcl2* compared to the WT. Our study expanded the understanding of underlying mechanisms of biocontrol interactions with essential implications for the biological control of fungal plant diseases.

186 **Hijacked! Investigating the strategies used by a zombie-making fungus to manipulate carpenter ant behavior** Charissa de Bekker Biology, Universiteit Utrecht

The evolutionary arms race between parasites and their hosts can culminate into complex extended phenotypes that benefit disease progression and transmission. The fungus-adaptive changes in behavior as seen in *Ophiocordyceps*-infected carpenter ants are a prime example. These "zombie ants" demonstrate a suite of behaviors that are thought to circumvent the social immune responses of the colony. Subsequently, the hijacked ant climbs and attaches itself at an elevated position that benefits fungal spore development and dispersal. These fungus-induced behaviors are not unique to this particular infection as parallel behaviors have also been observed in invertebrate infections by other parasite taxa. The precise mechanisms that are involved in this behavioral manipulation and others are unknown. To begin to unravel these mechanisms, we have conducted extensive fieldwork and developed the *Ophiocordyceps*-ant interaction into an integrative model system that allows us to study parasitic behavioral manipulation in greater detail in the lab. By combining fungal culturing and lab infections with behavioral assays and multiple omics approaches, we propose several comprehensive mechanistic hypotheses about the fungal proteins and ant receptors involved in this phenomenon. These hypotheses include specific fungal "manipulation" effectors of interest and their potential binding to ant proteins involved in light perception, biogenic amine binding and daily rhythms. To test these hypotheses we are currently, for the first time in this model, beginning to integrate functional genetics assays to determine the function of presumed fungal "manipulation" effectors, the host behaviors they elicit, and the host pathways that underly those phenotypes. Our results will provide detailed insights into fungus-animal interactions in general while giving some of the first insights into parasitic hijacking of animal behavior in particular.

187 **Cross-kingdom predator-prey interactions from two sides of a coin** Yen-Ping Hsueh Academia Sinica

Nematodes are the most abundant animal in the soil ecosystem, where they cohabitate with an array of natural predators including insects, other nematodes and nematophagous fungi. Considering the prevalence and abundance of nematodes, it is not surprising that multiple groups of fungi have independently evolved the ability to switch to a predatory lifestyle on nematodes under nutrient deprivation. Among these carnivorous fungi, the nematode-trapping fungus *Arthrobotrys oligospora* is one of the most ubiquitous and well-studied species. This emerging model species develops adhesive nets as a trapping device to capture its nematode prey. Multiple conserved signaling pathways, such as the cAMP and the MAPK pathways, have been shown to play a role in prey-sensing and trap morphogenesis. However, little is known about how the traps capture nematodes. To dissect the molecular basis of the robust adhesion between nematodes and fungal traps, we conducted forward genetic screens in *Caenorhabditis elegans* to identify mutants capable of escaping from *A. oligospora* traps. Genetic mapping and whole genome sequencing of seven such mutants revealed that loss-of-function mutations in a nuclear hormone receptor gene, *nhr-66*, resulted in resistance to *A. oligospora* predation. Since nuclear hormone receptors are transcription factors that function as master regulators, we conducted RNAseq analysis on wild-type and *nhr-66* mutant animals and found that more than 60 collagen genes were down-regulated in the *nhr-66* mutants. Overexpression of the down-regulated collagen genes in the *nhr-66* mutant abolished the escape phenotype, revealing that collagens play a crucial role in mediating adhesion between fungal traps and nematode prey. Interestingly, we have not identified loss-of-function *nhr-66* variants in wild *C. elegans* populations. This result suggests that there is a fitness trade-off for possessing an *nhr-66* mutation and that the adhesins on the fungal traps likely recognize multiple targets on the nematode surface, showcasing the evolutionary arms race between nematode-trapping fungi and their nematode prey.

188 **Development of a vaccine against *Coccidioides*, the Valley fever pathogen** Marc J Orbach[1], M. Alejandra Mandel[2], Lisa F Shubitz[2], Devin J Seka[3], Daniel A Powell[2], Mana Ohkura[4], Thomas M Tomasiak[3], John N. Galgiani[2] [1]School of Plant Sciences, University of Arizona, [2]Valley Fever Center for Excellence, University of Arizona, [3]Chemistry and Biochemistry, University of Arizona, [4]Oregon State University

Coccidioides spp. are mammalian pathogens endemic to the Southwest US, and the Americas. Climate change suggests an expanding range for this pathogen and its disease. To counteract this pathogen, a live-attenuated vaccine against coccidioidomycosis has been developed by deletion of the *CPS1* gene in *Coccidioides posadasii* and is undergoing regulatory approval for commercial use in dogs.

The *Δcps1* mutant fails to propagate in host animals and is avirulent in all mouse strains tested, including severely immunodeficient mice, indicating a high degree of safety. Vaccination provides a high level of protection in mice and dogs. To understand why the *CPS1* deletion is so debilitating to *Coccidioides*, structural studies of the Cps1 protein and functional and expression studies of *CPS1* are being used to define the role of the gene in *Coccidioides* growth and spherulation. Domain deletion derivatives indicate

that either of two adenylate-forming domains are critical for parasitic spherule maturation with single domain deletions reiterating the whole gene deletion phenotype of avirulence and failure to persist in the host. Modeling and physical characterization using purification and cryo-EM predict Cps1 to be a globular peripheral membrane protein. The catalytic activity of Cps1 is being defined by *in vitro* assays of purified protein. An understanding the molecular and biochemical role of *CPS1* in spherulation is critical to support the safety of this live-attenuated fungal vaccine.

The route to commercialization of the *Δcps1* canine vaccine is a public-private partnership with Anivive LifeSciences and requires approval by the USDA Center for Veterinary Biologics. The path to licensing requires the demonstration of the vaccine's protection against an experimental infection model in the target species which has been done. It also requires a series of proscribed safety tests for reversion to virulence, lack of shed/spread of the vaccine strain and injection site tolerance. Based upon the biology, there is no reason a *Δcps1* vaccine could not also be developed to protect humans, and this opportunity is actively being pursued.

189 **Diversity and characterization of filamentous fungi isolated from sediments of Basque estuaries** Ainara Otamendi[1], Ziortza Agirrezabala Urkia[2], Carla Perez-Cruz[3], Raquel Liébana[3], Laura Alonso-Sáez[3], Maria Teresa Dueñas[1], Anders Lanzén[4,5], Oier Etxebeste[1] [1]Laboratory of Biology, Dept of Applied Chemistry, Faculty of Chemistry, University of the Basque Country (UPV/EHU), 20018 San Sebastian, [2]Applied Chemistry, Laboratory of Biology, Dept of Applied Chemistry, Faculty of Chemistry, University of the Basque Country (UPV/EHU), 20018 San Sebastian, [3]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Sukarrieta, [4]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Pasaia, [5]IKERBASQUE, Basque Foundation for Science, Bilbao

Fungi and bacteria within marine ecosystems contribute to ecological balance by playing critical roles in nutrient cycles and by shaping food webs. In this context, marine microbes developed genetic mechanisms to adapt and survive in marine environments and stress conditions such as, *e.g.*, high salt concentrations and nutrient scarcity, or to degrade complex polymeric substrates. These features make marine microorganisms a valuable source for the development of new biotechnological tools. However, marine environments and mainly marine fungi are still underexplored. Research on marine microorganisms is mainly focused on bacteria, with a couple of hundreds of fungal species retrieved from marine environments, despite the fact that the kingdom fungi is composed of millions of species. Here, we focused on the isolation of filamentous fungi, using sediment samples collected in estuaries of the Basque Country, Bay of Biscay. Their phenotypic characterization led to the identification of strains potentially able to grow on minimal culture medium supplemented with recalcitrant algal polysaccharides or to produce secondary metabolites. Two isolates belonging to the order Hypocreales were selected for genome sequencing (Illumina and Nanopore technologies) and analysis: 1) *Marquandomyces marquandii* due to its ability to secrete a yellow pigment described in the literature as urea sorbicillin and 2) *Albophoma yamanashiensis* for its apparent ability to grow in minimal culture medium supplemented with commercial fucoidan. Analysis and comparison of their CAZyme and secondary metabolite gene cluster repertoires with those of other species of the order Hypocreales, in combination with RNA-seq results, suggest that these isolates could be used as a source of new enzymatic activities and secondary metabolites.

190 **The myco-ecology of the *Stylophora pistillata* holobiont: a case study with two associated fungi - *Cladosporium halotolerans* and *Stachybotrys chlorohalonata*** Lior Granit[1,2], Nofar Lifshitz[1,2], Britt Ronen[2], Koren Karp[3], Shmuel Carmeli[3], Maoz Fine[1,2], Oded Yarden[1,2] [1]Hebrew Univ of Jerusalem, [2]The Interuniversity Institute for Marine Sciences, [3]Tel-Aviv University

Coral reefs are pivotal ecosystems sensitive to climate change. *Stylophora pistillata*, a Red Sea Scleractinian coral, accommodates repeatedly-isolated fungal genera, suggesting the presence of a coral-acclimated mycobiome that may have potential implications on coral well-being. *S. pistillata* nubbins were collected between 2018 and 2023 in the Gulf of Aqaba. 173 different fungal strains from 25 genera were isolated, 61% of which were found to be either *Aspergillus*, *Alternaria*, or *Cladosporium* spp. The abundance of other genera varied and included the isolation of at least two new species. To assess the fungi's impact on coral well-being, *S. pistilata* colonies were inoculated with conidia from prevalent or rare fungal species (*Cladosporium halotolerans* or *Stachybotrys chlorohalonata*, respectively). Inoculation with *C. halotolerans* yielded no visible effects within 24 hours, despite conidial adherence to the coral tissue surface. Transfer of the coral from ambient (25^{o}C) to elevated (33^{o}C) sea water temperature conferred a short-term detrimental effect, as evident by the reduced maximum photosynthetic yield of the algal symbionts, based on chlorophyll fluorescence. This effect was less pronounced in coral inoculated with *C. halotolerans*. In contrast, Inoculation with *S. chlorohalonata* led to visible coral bleaching after 24 hours. Conidial germination of *C. halotolerans* in culture was not affected by the presence of 0.66M NaCl (typical of the northern Red Sea) with ~60% of the tested conidia reaching the first branching stage within about 24 hours. However, hyphal growth was significantly enhanced by the presence of salt, as the colony area in the presence of 0.66M NaCl was 36% larger after 14 days of growth in comparison to medium lacking the NaCl amendment, suggesting the coral-derived isolate is well adapted for proliferation under saline conditions. The bleaching-inducing strain of *S.*

chlorohalonata was found to secrete metabolites that significantly inhibited the lag phase of several tested gram-positive (yet not gram-negative) bacteria. We propose that at least some of the anti-bacterial activity observed was due to the production of saturated fatty acids by this strain. Taken together, we conclude that isolating the cultural components of the coral mycobiome can progress our ability to identify and dissect the potential contribution of fungal community members to coral well-being.

191 **Fungal diversity in deep-sea sunken plant substrates** Yuriko Nagano, Yoshiyuki Ishitani, Noriyuki Isobe, Ryota Nakajima, Shunichi Ishii, Hiroyuki Kashima, Hidetaka Nomaki JAMSTEC

Fungi are the main decomposers of plants on land, and it is also presumed that they play an important role in the decomposition of sunken plants in deep-sea environments. However, the diversity, distribution, and ecology of deep-sea fungi associated with sunken plant substrates are still largely unknown and only six species of obligate deep-sea fungi that form fruiting bodies on sunken woods have been reported to date.

In this study, 21 samples (19 deep-sea sunken plants, 1 deep-sea sunken seaweed, and 1 shallow water sunken plant) were collected from 5 different sites (shallow water depth: 5m, deep water depths: 720–5,707m) in the Western Pacific Ocean off Islands of Japan, to investigate the diversity and distribution of fungi related to deep-sea sunken plant substrates. Fungal amplicon analysis was performed on the samples by targeting the ITS rRNA gene region.

As a results, fungal fruiting bodies were observed on one of the sunken wood samples which was collected at a water depth of 5,707m, and morphological and phylogenetic analysis suggested this fungus as novel sp. closely related to *Oceanitis scuticella,* one of the obligate deep-sea fungi. No fungal ascomata was observed in other samples, but amplicon analysis detected sequences highly homologous to *O. scuticella* in 8 samples collected from different depths and locations. These results indicated that *O. scuticella* and its relatives commonly exists in sunken plant substrates in deep-sea environments. Furthermore, sequences with highly homologous to *Ceriosporopsis halima,* a cosmopolitan shallow marine fungus, were predominantly detected in the sample collected from the shallow water. Interestingly, sequences which showed homologous to *C. halima* but presumed to be a different species (approximately 86% homology) were detected in 4 samples from deep-sea environments. This suggests that a novel species closely related to *C. halima* inhabit in deep-sea environments.

Our results indicated that there are many undiscovered deep-sea fungi and some obligate deep-sea fungi, such as *O. scuticella*, are distributed in a wide range of water depths, from bathyal to abyssal zones. Further comprehensive investigations on their diversity, distribution, physiology and genomics will provide key insights into the adaptation, evolution and ecology of deep-sea fungi.

192 **Ecology of marine fungi in the South Pacific Ocean off Chile** Marcelo H Gutiérrez[1,2], Silvio Pantoja-Gutiérrez[1,2], Karina Fuentes-Cruz[2] [1]Oceanography, Universidad de Concepción, [2]Center for Oceanographic Research COPAS Coastal, Universidad de Concepción

Located at the eastern border of the South Pacific, the coastal ocean off Chile displays strong geographic and oceanographic heterogeneities that impact the distribution, diversity, and functioning of marine microorganisms. In northern and central Chile, coastal upwelling supports high autotrophic production, assuring the availability of organic matter that sustains elevated rates of heterotrophic activity. In addition, large rivers in central and southern Chile and anthropogenic activity provide allochthonous substrates that guarantee a wide range of microbial niches in coastal areas. In the southern region, the large fjord ecosystem with glacial influence in the Chilean Patagonia is a focus of interest for studying novel aspects of the adaptability of marine microbes to changing conditions in the coastal ocean. In these contrasting marine environments, we have tackled questions on the community structure and the role of marine fungi, focused on the influence of physicochemical variability, biological interactions, and their consequences for marine carbon cycling. Our findings include results from spatial and temporal surveys of fungal biomass, with seasonal changes allowing high contribution to microbial carbon following phytoplankton blooms during austral spring in the upwelling ecosystem. Peaks of fungal biomass during the productive period were also associated with elevated rates of degradative activity on organic macromolecules, which are higher than those of prokaryotes. Exploring the diversity and abundance of fungi in the fjord region evidenced patterns of beta-diversity coupled with water masses distribution and spatial gradients associated with fresh and meltwater influence in the continental shelf. Regarding biological interactions, we demonstrated the effect of fungal parasitism on the ecology of marine diatoms, and we evidenced the concerted action of fungi-bacteria associations on degradative capability and its effect on the fate of terrestrial organic matter in surface sediments of coastal environments. Biochemical characterization of marine-derived fungi indicates a distinctive pattern of lipids and proteins and a nutritional value comparable with other planktonic components of the marine pelagic food webs. Their elemental and stable isotope compositions are consistent with consumption of marine organic matter through heterotrophic uptake of phytoplankton-derived substrates.

193 **Prevalence, succession, and activity of marine fungi in particle-associated communities** Syrena Whitner[1], Samantha Gleich[2], Jennifer Beatty[3], Brittany Stewart[3], Anthony Amend[1], David Caron[2] [1]Marine Biology, University of Hawai'i at Manoa, [2]Biological Sciences, University of Southern California, [3]University of Southern California

The biological carbon pump (BCP) plays a crucial role in regulating Earth's climate, and sustaining marine ecosystems by sequestering and transporting atmospheric carbon dioxide to the deep ocean. The BCP's energy transfer occurs primarily through the gravitational sinking and subsequent decomposition of POM. While previous studies have characterized the diversity, metabolic activity, and community succession of free-living and particle-associated bacteria in pelagic ecosystems, the role of marine fungi in POM decomposition remains understudied. Here, we used metatranscriptomics to characterize the metabolically active fungal communities of free-living and particle-associated communities at 150m depth in the North Pacific Subtropical. Our study shows that the dominant metabolically active fungal communities changed over time as POM degradation occurs. Our study tests that the composition of dominant free-living fungal taxa is driven by substrate availability, with Chytridiomycota associated with fresh biological substrates (such as diatoms), and Dikarya more strongly associated with biological substrate scarcity. In contrast, we hypothesize that saprotrophic fungi, specifically Ascomycota dominate particle-associated fungal communities at all times. Further, we predicted that fungal abundance will decrease over time as POM decomposition occurs. Fungal metabolic activity is expected to be highest during earlier time points due to their ability to degrade complex macromolecules inedible to other microorganisms, followed by a decrease in their metabolic activity and abundance replaced by other heterotrophic grazers. Together, our results show that marine fungi play an important role in POM degradation in pelagic systems due to their early dominance of metabolic activity.

194 **Transcriptional landscape of the salinity-driven physiology and biotechnological potential of the halophile model *Aspergillus sydowii*** Yordanis Perez Llano[1], Eya C. Rodríguez-Pupo[1], Heydi Peidro-Guzman[1], Jorge Luis Folch-Mallol[2], Nina Gunde-Cimmerman[3], Ramon A. Batista-García[1], María del Rayo Sánchez-Carbente[2] [1]Center for Research on Cell Dynamics, Autonomous University of the State of Morelos, [2]Center for Research on Biotechnology, Autonomous University of the State of Morelos, [3]University of Ljubljana

Halophiles have been widely studied for their unique physiology and potential biotechnological applications. However, fungal adaptations to salinity have been commonly drawn from halotolerant strains or conducted in settings where cells are subjected to stress, either hypo or hyperosmotic, which can be a confounding factor in describing physiological responses to salinity. Transcriptomic analyses reveal upregulated genes related to cell wall modification and cation transporters under hypersaline conditions. This fungus exhibits a unique ability to accumulate osmolytes such as trehalose, arabitol, mannitol, and glycerol, showcasing its adaptability to osmotic stress. Establishing *Aspergillus sydowii* as a model marine fungus, we highlight its distinctive physiological responses to salinity and concluded that most adaptation mechanisms described in fungi are a consequence of saline stress and might not occur under the optimal salinities preferred by halophiles, underlining a methodological shortcoming that is widely spread within the field. Additionally, *A. sydowii* demonstrates potential in lignocellulose degradation, presenting a sustainable option for biofuel production. The fungus's robust enzymatic activities at high salt concentrations further suggests a potential for bioremediation in heavily polluted marine environments. Forthcoming research will focus on its pathogenicity to corals, sponges, and other members of marine ecosystems to assess its possible use in bioremediation. The combination of physiological resilience and biotechnological potential positions *A. sydowii* as a promising candidate for further exploration in both marine and industrial contexts.

195 **Eukaryotic metagenome-assembled genomes recovered from seagrass leaves include a novel chytrid in the order Lobulomycetales** Cassandra L Ettinger[1,2], Jonathan A Eisen[3,4,5], Jason E Stajich[1,2] [1]Dept of Microbiology and Plant Pathology, University of California, Riverside, [2]Institute for Integrative Genome Biology, University of California, Riverside, [3]Genome Center, University of California, Davis, [4]Dept of Evolution and Ecology, University of California, Davis, [5]Dept of Medical Microbiology and Immunology, University of California, Davis

Fungi play pivotal roles in terrestrial ecosystems as decomposers, pathogens, and endophytes, yet their significance in marine environments is often understudied. Seagrasses, as globally distributed marine flowering plants, have critical ecological functions, but knowledge about their associated fungal communities remains relatively limited. Previous amplicon surveys of the fungal community associated with the seagrass, *Zostera marina* (ZM) have revealed an abundance of potentially novel chytrids. In this study, we employed deep metagenomic sequencing to extract metagenome-assembled genomes (MAGs) from these chytrids and other microbial eukaryotes associated with ZM leaves. Our efforts resulted in the recovery of five eukaryotic MAGs, including a single fungal MAG in the Chytridiomycota (75% BUSCO completeness), three MAGs representing diatoms in the Bacillariophyta (95%, 88% and 44% BUSCO completeness) and one MAG representing a haptophyte algae in the Prymnesiophyceae (61% BUSCO completeness). Whole-genome phylogenomic assessment of these MAGs suggests they all largely represent under sequenced, and

possibly novel eukaryotic lineages. Of particular interest, the chytrid MAG was placed within the order Lobulomycetales, consistent with the identity of the dominant chytrid from previous ZM amplicon survey results. Annotation of this MAG yielded 5,650 gene models of which 77% shared homology to current databases. With-in these gene models, we predicted 121 carbohydrate-active enzymes and 393 secreted proteins (103 cytoplasmic effectors, 30 apoplastic effectors) paving the way for in-depth ecological exploration of the role of this chytrid within the ZM ecosystem. Exploration of orthologs between this MAG and existing Chytridiomycota genomes is currently ongoing and promises further insights into its evolutionary and ecological adaptations. Overall these five eukaryotic MAGs represent substantial genomic novelty and valuable community resources. Ongoing and future work will continue to unravel their evolution and ecology, contributing to a deeper understanding of the roles of fungi and other microbial eukaryotes in the larger seagrass ecosystem.

196 **Sustainable uses of marine fungal biodiversity: the Flensburg strain collection of marine fungi** Antje Labes Energy and Life Science, Flensburg University of Applied Science

Marine fungi are well known for their ability to produce natural products. Fungal marine natural products include a large diversity of structural classes and a wide range of substituent patterns resulting in some remarkable antibacterial and other bioactivities. As other microbes, filamentous fungi have the advantage to be cultivable in controlled scalable production systems, allowing sustainable biotechnological production of molecules and enzymes of interest [Kramer and Labes, 2020].

Nevertheless, only a very small portion of marine microorganisms is available to technological application using the common isolation and cultivation strategies. Estimations on the accessible, marine microbial diversity result in less than 0.1% [Himaya *et al.*, 2013]. Strain collections do play therefore a significant role in the discovery of new natural product, as well as in exploration of the microbial diversity itself. The “Flensburg strain collection of marine fungi” contains around 16.000 fungal isolates of marine origin. Up to date, only a few of them have been characterised. The role of culture collections in preservation of the diversity and making it accessible to other researchers for research, teaching and for biotechnological exploitation is obvious [Reich and Labes, 2017]. We present the resource and illustrate possible paths for its application. Based on a number of examples, the challenges and future prospects of harnessing the full potential of marine fungi for sustainable industrial development will be described.

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197 **Convergent genome expansion in fungi linked to evolution of root-endophyte symbiosis** Yi-Hong Ke[1], Gregory Bonito[2], Hui-Ling Liao[3,4], Brian Looney[1], Alejandro Rojas-Flechas[2], Jake Nash[1], Khalid Hameed[1], Christopher Schadt[5], Francis Martin[6], Pedro W Crous[7], Otto Miettinen[8], Jon K Magnuson[9], Jessy Labbé[5], Daniel Jacobson[5], Mitchel J Doktycz[5], Claire Veneault-Fourrey[6], Alan Kuo[10], Stephen Mondo[10], Sara Calhoun[10], Robert Riley[10], Robin Ohm[10], Kurt LaButti[10], William Andreopoulos[10], Jasmyn Pangilinan[10], Matt Nolan[10], Andrew Tritt[10], Alicia Clum[10], Anna Lipzen[10], Chris Daum[10], Kerrie Barry[10], Igor V Grigoriev[10,11], Rytas Vilgalys[1] [1]Duke University, [2]Michigan State University, [3]North Florida Research and Education Center, [4]University of Florida, [5]Oak Ridge National Laboratory, [6]Interactions Arbres-Microorganismes, INRA Centre de Nancy, [7]Westerdijk Fungal Biodiversity Institute, [8]University of Helsinki, [9]Pacific Northwest National Laboratory, [10]U.S. Dept of Energy Joint Genome Institute, [11]University of California Berkeley

A highly diverse community of fungal endophytes exist within the intercellular apoplast of plants, yet genomic features associated with endophytism remain largely unknown. To identify genomic features associated with evolution of endophytic symbiosis, we sequenced genomes of 23 fungal root endophytes from a single host tree genus (*Populus*) representing 19 phylogenetically diverse lineages across the fungal tree of life. Compared with closely related fungi belonging to other ecological guilds, poplar root endophytic fungi had significantly greater genome size and gene counts, including higher numbers of CAZymes, protease, lipase, small secreted proteins, and antibiotic resistance genes. We identified orthogroups, Pfam domains and CAZymes that discriminate poplar root endophytes from other fungi. Pectinase GH88 was consistently found enriched associated with endophytism across

different annotation methods. Other pectinases were also enriched as has been observed in root endophyte communities of the model plant species *Arabidopsis thaliana*. Other discriminative genes included proteins functioning in transporters, carbon metabolism, and transcription factors. Contrasts comparing multiple genomic features indicate the intermediate placement of pathogenic fungi between endophytes and saprobic fungi genomes, suggesting a distinctive state of endophytism. Our observations suggest that the expanded genomic content of fungal endophytes enables them to exploit a wider range of nutritional sources and symbiosis-related genes which are required for growth in-planta. The identification of key genomic features associated with endophytic lifestyle in poplar root endophytes provides new insights into the universal genetic components in endophytism and offers abundant genome resources for further studies of their functional significance in plant-fungal symbiosis.

198 **Origin and evolution of fungal secondary metabolism** Jerome Collemare Westerdijk Fungal Biodiversity Institute
Studies on the origin and evolution of life have suggested that secondary metabolism (SM) genes likely originate from the duplication and divergence of genes involved in primary metabolism (PM), and that such duplications occur independently at different times during evolution of life under the continuous selective pressure imposed by ecological competition between organisms. In fungi, SM genes are organized in biosynthetic gene clusters (BGCs) which evolution is mostly explained by mutations, gene duplications, gene losses, and lateral transfers. However, the origin of the genes recruited in SM BGCs remains poorly understood. Here, the phylogenetic analyses of fungal fatty acid synthases and citrate synthases reveal how genes from PM are recruited into BGCs after gene duplication. Determination of selection acting on different paralogues provides hints about variation of selection over time after gene duplication. Based on these examples with clear relationship to PM genes and inferred episodic positive selection, the origin and evolution of other SM genes found in unrelated BGCs is reconsidered. Altogether, the results suggest that BGC duplication and fluctuation in natural selection across time and species contribute to the observed discontinuous distribution of BGCs in the fungal tree of life and corresponding fungal chemical diversity.

199 **Ectomycorrhizal *Suillus* fungi represent hot-spots of metabolic diversity, structured by gene presence/absence variation and significant horizontal gene transfer** Lotus Lofgren[1], Steven Ahrendt[2], Sameer Mudbhari[3,4], Paul Abraham[4], Sara Branco[5], Hui-Ling Liao[6], Nhu Nguyen[7], Peter Kennedy[8], Kerrie Barry[2], Alan Kuo[2], Igor Grigoriev[2], Rytas Vilgalys[1] [1]Biology, Duke University, [2]Joint Genome Institute, [3]University of Tennessee, [4]Oak Ridge National Lab, [5]University of Colorado Denver, [6]Soil and Water Science, University of Florida, [7]University of Hawai''i at Manoa, [8]University of Minnesota

The ectomycorrhizal genus *Suillus* is speciose and widespread. *Suillus* fungi display a gradient of partner specificity responses, possess unique traits with high ecological relevance, and are tractable to laboratory manipulation, making the genus an ideal model for studying ectomycorrhizal ecology and evolution. Leveraging 46 whole-genome sequencing projects, considerably more than exist for any other ectomycorrhizal fungal taxa, we conduct a comprehensive analysis of *Suillus* fungi using a combination of phylogenetics, pan-genus comparative genomics, gene ancestry analysis, and machine learning to identify genomic elements associated with important ecological traits. We present evidence for significant gene presence/absence variation across the genus, horizontal gene transfer, and mitogenome diversity. With a particular focus on primary and secondary metabolic capacity, we follow up this in-silico analysis with LC/MS based metabolomics with the goal of linking genome-based predictions to realized metabolic diversity.

200 **Insights into the biology and ecology of the Ceratocystidaceae emerging from their genomes** Brenda Wingfield BGM, University of Pretoria

The Ceratocystidaceae accommodates fungi with diverse lifestyles, including saprophytes and some of the world's most aggressive plant pathogens. During the course of the last decade the genomes of all the genera and many species have been sequenced. The availability of these genomes has given rise to a substantially more robust taxonomy including the definition of genera and species boundaries, which broadly align to different ecological niches. Importantly these data have facilitated research considering the differences between genera and the ecology of the species they accommodate. Our studies have shown that species in Ceratocystidaceae have diverse mating strategies, and the genomic data has made it possible to describe the genes linked to these mating strategies as well as structural characteristics of the mating type locus. Overall, the Ceratocystidaceae genomes have emerged as smaller than those of many other ascomycetes. We have also found that in some cases gene duplication is linked to lifestyle and that some genes are duplicated in the pathogenetic species. Most members have been found to have a diverse number of biosynthetic gene clusters and it has emerged that transposable elements have played a role in shaping their genomes. We now also have access to a large number of genomes for some species making it possible to describe patterns of genetic diversity between and within a species. This is enhancing our understanding of inter-species diversity and making it possible to consider intra-species variation. Broadly, comparative genomic studies in the Ceratocystidaceae are increasingly providing a foundation to

better understand the complex interactions between pathogenicity, speciation and secondary metabolism in a remarkably diverse, important and fascinating group of fungi.

201 **Genomic Architecture of Fungal Metabolism Involved in Host and Ecological Specialization** Rodrigo Olarte[1], Dean K Malvick[2], Kathryn E. Bushley[3] [1]Plant and Microbial Biology, University of Minnesota, [2]Plant Pathology, University of Minnesota, [3]Emerging Pests and Pathogens Unit, USDA-ARS

In fungi, host and ecological specialization is shaped both by the products of secondary metabolism (i.e., host-selective toxins) and those from primary metabolism involved in utilizing specific classes of host carbohydrates or proteins. Secondary metabolite genes synthesizing toxins are among the fastest evolving genes classes in fungi and are often localized to unstable regions of the genome such as subtelomeres and other transposable element (TE) and repeat rich regions. They respond to selective pressures imposed by either the host or the environment, enabling fungi to rapidly adapt to changing conditions. Additionally, secondary metabolite genes, as well as genes involved in virulence, are often found clustered within fungal genomes, which may facilitate efficient epigenetic regulation. Genes involved in ecological adaptation may also be localized to small "accessory" chromosomes, which like bacterial pathogenicity plasmids, may facilitate their horizontal transfer among fungi. Yet the mechanisms by which these genes and clusters evolve remain elusive. Using a dataset of six nearly chromosomal-scale assemblies of the insect pathogenic fungus *Tolypocladium inflatum*, as well as examples from several plant pathogenic fungi, we examine evidence for genetic processes such as transposition, inversions, microdeletions, and homologous recombination or gene conversion on the ends of chromosomes for driving the diversification of metabolite clusters and gene families involved in host and ecological adaptation. In particular, the role of transposable elements in rearrangement or mobilization of clusters within fungal genomes is addressed. We also examine how these types of structural rearrangements impact the expression of metabolite clusters or lead to loss of function through pseudogenization mutations that may also serve as the basis for host and ecological adaptation.

202 **Extensive and independent evolution of secondary metabolism genes across the early diverging fungal genus *Basidiobolus*** Jasper Carleton, Liam P Cleary, Emily Newman, Madison Hincher, Javier F Tabima Biology, Clark University

Secondary metabolism is a hallmark of fungal species and plays fundamental roles in fitness like survival, competition, and resource acquisition. Most fungal secondary metabolytes have been reported in Dikarian fungi. The paucity of secondary metabolism has been reported in early divergent fungi such as zygomycetes. However, recent genomic and functional approaches show that secondary metabolism is present in the zoopagomycete genus *Basidiobolus.*

Basidiobolus is a microfungus predominantly found in the intestinal tracts of amphibians. Previous studies have shown high rates of secondary metabolites in *Basidiobolus* with predicted functions of antibiosis*,* metal acquisition in anoxic environments and other functions. In addition, these secondary metabolism genes are apparently derived from horizontal gene transfer (HGT) from bacteria that coinhabit the amphibian gut tract. There are limitations with these hypotheses, as only three published genomes are available. More information is needed to determine the secondary metabolite richness in this early diverging fungal species.

Here, we present the result of the genome sequences of 35 samples of *Basidiobolus* across different locations, hosts and environmental sources to test the hypothesis of prevalent secondary metabolite genes as a hallmark of the genus. Our sequences show a high richness of genic families such as non-ribosomal peptide synthetases, polyketide synthase and terpene cyclase genes. We confirm the hypothesis of bacterial HGT as the source of these genes, as well as finding genes with dikaryan origins. Interestingly, our results suggest that each SM acquisition may have occurred independently, and further investigation in the sources of SM transfer needs to be studied across these early diverging fungi.

203 **Starship elements drive genome evolution dynamics in a model eukaryotic microbe** Emile Gluck-Thaler[1], Adrian Forsythe[2], Charles Puerner[3], Jason E Stajich[4], Daniel Croll[5], Robert A Cramer[3], Aaron Vogan[2] [1]Plant Pathology, University of Wisconsin-Madison, [2]Uppsala University, [3]Geisel School of Medicine, Dartmouth University, [4]University of California, Riverside, [5]University of Neuchatel

Microbial genomes are colonized by diverse mobile genetic elements (MGEs) whose activities shape their hosts' biology in profound ways. Yet for many of the microbes in which they are found, much remains unknown about the mode and tempo of MGE-mediated evolution. We recently described a new superfamily of fungal MGEs called Starships that are capable of mobilizing host genes but whose impact on their fungal hosts remains unquantified. Here, we investigate the extent to which Starships act as a mechanism of eukaryotic microbe evolution by systematically characterizing their activity and expression in the model organism Aspergillus fumigatus. Supplementing a global sample of 509 genomes with 12 newly sequenced long-read isolates, we find that A. fumigatus harbors 20 distinct Starships whose presence/absence varies in 154 regions distributed along all 8 chromosomes,

including a biosynthetic gene cluster hotspot. At least 4.8% of genes in the A. fumigatus pangenome are Starship-mobilized and many are differentially expressed under antifungal- and infection-related conditions. Starships carry diverse molecular functions, including secondary metabolite and biofilm pathways previously known to contribute to stress tolerance and pathogenicity. Together, our results suggest Starship-mediated evolution must be taken into account when investigating ecologically- and clinically-relevant variation in fungi.

204 **Genomic and Phenotypic variation in *Rhodotorula* species sampled from Extreme Environments** Xin-Zhan Liu[1,2], Eva Ottum[2], Cene Gostinčar[3], Benedetta Turchetti[4], Claudia Coleine[2,5], Laura Selbmann[5], Ian Wheeldon[2], Nina Gunde-Cimerman[3], Jason E Stajich[2] [1]Institute of Microbiology, Chinese Academy of Sciences, [2]Microbiology & Plant Pathology, Univ California, Riverside, [3]Biotechnical Faculty, University of Ljubljana, [4]Dept of Agriculture, Food and Environmental Sciences & DBVPG Industrial Yeasts Collection, University of Perugia, [5]Dept of Ecological and Biological Sciences, University of Tuscia

Rhodotorula are basidiomycete yeasts in the Pucciniomycotina subphylum, which are characterized by production of carotenoids and can be cultured from a broad range of temperature and harsh environments. They are found in spoiled food, in freshwater lakes, ocean and brackish waters, the human built environment, and from soils or rocks in arid lands including cold arctic/antarctic and hot deserts. Some lineages have been characterized exclusively from rock surface or endolithic samples. The species *Rhodotorula mucilaginosa* is capable of causing disease in mammals through skin and blood-borne infections. We characterized phenotypic traits of 288 strains grown in a range of laboratory conditions, including temperatures from 4 to 37 °C, carbon sources glycerol and xylose, pH and high and low salinity conditions. The results establish a phenotyping dataset consisting of survival and growth rates, carotenoid production, and morphological traits based on image analyses of colony growth on solid media. Using short-read Illumina sequencing, we have assembled and annotated draft genomes of 400 isolates of *R. mucilaginosa* and 100 across 15 other species in the genus collected from a diversity of environments from our collections and public genome datasets. The work reveals additional cryptic species and within *R. mucilaginosa*, a range sub-populations admixture. Several strains have copy number amplification of some chromosome and a few diploid strains are detected among the predominantly haploid isolates. We assembled reference genomes for type strains for 9 *Rhodotorula* species with Oxford Nanopore long reads to support comparative analysis of the *Rhodotorula* genus pangenome and focused population genomics study of *R. mucilaginosa*. The reference genomes, population genomics, and phenotyping support deep investigations of evolution, adaptation, link genotypes to phenotypes of these ubiquitous basidiomycete yeasts and test hypotheses about gene-level adaptation to cold, hot, and saline environments.

205 **Unearthing Nature's Hidden Arsenal: Mining Fungal Genomes for a New Class of Natural Products** Grant R Nickles[1], Natalia Sayuri Wassano[2], Sung Chul Park[1], Mira Syahifriena Binti Amir Rawa[1], Milton T Drott[3], Nancy P Keller[1] [1]Medical Microbiology and Immunology, University of Wisconsin-Madison, [2]Dept of Biochemistry and Tissue Biolofy, Institute of Biology at University of Campinas, [3]Cereal Disease Lab, USDA

Isocyanides (also called isonitriles) are a chemical class of SM produced by bacteria and fungi. These compounds are produced by non-canonical BGCs that prior to our research were not detected by existing genome-mining software. They are characterized by the presence of the highly reactive isocyanide functional group (RºN+-C-), which is formed by the conversion of the amino group on select amino acids. Possessing potent bioactivities, isocyanides can engage in unique chemical reactions due to their affinity for chelation to metals, with some isocyanides targeting specific transition metals and metalloproteins. We sought to enable research into this class of compounds by characterizing the biosynthetic potential and evolutionary history of these genes across the Fungal Kingdom. Here, we present the first genome-mining pipeline, assembled from preexisting tools, to identify fungal ICS BGCs. We discovered 3,800 ICS BGCs in 3,300 fungal genomes, establishing ICSs as the fifth-largest class of SMs when compared to canonical BGC classes found by antiSMASH. Our results create a roadmap for future research into ICS BGCs. This work lays the foundation for further exploration of ICS BGCs, bolstered by our dedicated website (https://isocyanides.fungi.wisc.edu/), which offers comprehensive access to all identified fungal ICS BGC files. In addition, I will present updates on our targeted investigations into specific fungal ICS BGCs that our laboratory has been investigating.

206 **The peroxisome trafficking protein PxdA is required for secondary metabolite production and infection in the plant pathogenic fungus *Alternaria alternata*** Valentin Wernet, Livia D Songster, Gaurav Kumar, Swetha Mahesula, Patreece Suen, Samara Reck-Peterson University of California San Diego

The evolutionary success of fungi is partially based on their ability to produce secondary metabolites. Biosynthetic genes of these secondary metabolites are typically organized in gene clusters, and their production is often compartmentalized in different organelles (for example in peroxisomes) to protect the host from either toxic precursors or aid with complex biosynthesis. However, the role of organelle trafficking during secondary metabolite production remains largely unexplored. This is due to the specific conditions under which they are produced. In filamentous fungi, organelles are primarily moved long distances by the

microtubule cytoskeleton and their associated motor proteins. In the mold *Aspergillus nidulans*, peroxisomes move along microtubules by hitchhiking on motile endosomes, a process that requires the endosome-associated protein PxdA (peroxisome distribution mutant A). However, the physiological reason for peroxisome hitchhiking is unclear. Based on our preliminary data we propose a role in secondary metabolite production.

Here we studied the role of peroxisome trafficking in the filamentous fungus *Alternaria alternata*, which produces toxic secondary metabolites during plant infection. To investigate the role of peroxisome trafficking during the fungal-plant interaction we generated a mutant strain lacking the *A. alternata pxdA* gene using CRISPR-Cas9. The *A. alternata* Δ*pxdA* strain produces less aerial mycelium and has increased black pigmentation. Analysis of secondary metabolite production by thin-layer chromatography revealed the absence of at least two secondary metabolites in the Δ*pxdA* mutant, one being the mycotoxin Alternariol. Infection assays on tomatoes revealed a reduced virulence of the *pxdA* deletion strain. Investigating peroxisome localization in wild-type and the Δ*pxdA* mutant strain under varying conditions will unveil insights into trafficking mechanisms crucial for secondary metabolite production.

207 **A novel reporter system to identify arginoketides in soil that mediate cross-kingdom microbial interactions** Maira Rosin[1], Mario K. C. Krespach[1], Maria C. Stroe[1,2], Nils Jaeger[3], Kirstin Scherlach[4], Volker Schroeckh[1], Thorsten Heinzel[3], Christian Hertweck[4], Axel A Brakhage[1] [1]Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), [2]Dept of Microbiology, Karlsruhe Institute of Technology (KIT), [3]Dept of Biochemistry, Friedrich Schiller University Jena, [4]Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI)

In all habitats on earth microorganisms form consortia with many different species closely living together in the soil. Interspecies communication in these communities are decisive for function of microbial communities and further lead to the induction of otherwise silent natural product biosynthesis gene clusters. One prominent example is the interaction of the fungus *Aspergillus nidulans* and the bacterium *Streptomyces rapamycinicus*. Upon co-cultivation, the streptomycete is able to reprogram the epigenetic machinery of the fungus by activation of the histone acetyltransferase GcnE which leads to the induction of the otherwise silent *ors* biosynthesis gene cluster in *A. nidulans* [1,2]. By inhibitor studies with the pan-sirtuin inhibitor nicotinamide and analyses of several histone deacetylase mutants, we identified the silent information regulator SirE as the histone deacetylase terminating the induction of the *ors* BGC by *S. rapamycinicus* [3]. Furthermore, we discovered that the compound family of arginoketides including azalomycin F produced by *S. iranensis* and *S. rapamycinicus* serve as the long sought-after bacterial signals for this induction [4]. To estimate the induction of silent gene clusters, we developed a fungal reporter system encoding the gene for the green fluorescence protein (GFP) coupled to the nanoluciferase gene and the gene of interest. Thus, enabling the qualitative and quantitative measurement of the transcriptional activation of genes. Here, this construct was translationally fused to the *orsA* gene of the orsellinic acid biosynthesis gene cluster of *A. nidulans.* Transformants showed fluorescence and luciferase activity upon addition of *S. iranensis*, azalomycin F or the pan-sirtuin inhibitor nicotinamide to the culture. Interestingly, extracted soil also led to an increased nanoluciferase activity and green fluorescence indicating that arginoketides are indeed present in the soil. Further, with this reporter we were able to identify several bacterial strains, isolated from a random soil sample, that induce green fluorescence in the fungus [4]. This indicates that arginoketides can be found around the world and playing an important role in mediating microbial interactions in the soil.

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208 **On the role of natural products as virulence factors in fungi with a predatory lifestyle** Maria C. Stroe[1], Xi Yu[2], Xiaodi C. Hu[1], Reinhard C. Fischer[1] [1]Institute for Applied Microbiology, Karlsruhe Institute for Technology, [2]Karlsruhe Institute for Technology

An intriguing and fascinating inter-kingdom interaction occurs between nematodes and fungi that trap and prey on them. Nematode-trapping fungi are soil microbes which can switch from a saprotrophic lifestyle to a predatory behavior in the presence of nematodes[1,2]. Among them, *Arthrobotrys flagrans* is a typical member of the soil microbiome and is able to form adhesive, three-dimensional trap networks to catch nematodes. Using *Caenorhabditis elegans* as model, we previously showed that secondary metabolites produced by *A. flagrans* are crucial for attracting the nematodes into fungal colonies and traps and for controlling trap formation, together with nematode-derived ascarosides[3]. Bioinformatic analysis has revealed that *A. flagrans* only encodes 3 PKSs, 3 NRPSs and 3 NRPS-like biosynthetic gene clusters, which is a relatively small collection of clusters compared to other fungi. Therefore, the *A. flagrans - C. elegans* interaction offers the perfect system and opportunity to elucidate the entire secondary metabolism of *A. flagrans,* as well as the importance of the produced compounds for nematode predation. Here we

focus on the two uncharacterized PKS-encoding genes of *A. flagrans*. We show that the two genes are expressed exclusively in the fungal traps and in trophic hyphae inside the nematode. Chemical analysis revealed that the two PKSs work in a concerted fashion and biosynthesize volatile natural products with synergistic effects, increasing the attraction of nematodes into the hyphal traps. The molecular nematode targets are currently under investigation.

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209 **The evolution of fungal secondary metabolism and its regulation; lessons from *Aspergillus* fungi** Antonis Rokas Vanderbilt University

Fungi produce a remarkable diversity of secondary metabolites: small, bioactive molecules not required for growth, but which are essential to their ecological interactions with other organisms. Genes that participate in the same secondary metabolic pathway typically reside next to each other in fungal genomes and form biosynthetic gene clusters (BGCs). We have a established a long-term, tripartite collaboration with Nicholas Oberlies' lab at the University of North Carolina-Greensboro and Gustavo Goldman's lab at the University of Sao Paulo to characterize the evolutionary mechanisms that generate fungal chemodiversity, using *Aspergillus* fungi as our model. Organisms in the filamentous fungal genus *Aspergillus* have diverse lifestyles, including as plant and animal pathogens, which are partially mediated through their ability to biosynthesize some of the most iconic secondary metabolites, including penicillin (the world's first antibiotic), lovastatin (lipid-lowering blockbuster drug), aflatoxins (cancer-causing mycotoxins), and gliotoxin (genetic determinant of virulence in the opportunistic pathogen *Aspergillus fumigatus*). In my talk, I will synthesize the latest research from our team that takes advantage of genomic, transcriptomic, molecular genetic, natural products chemistry, phylogenetic, structural biology, and machine learning approaches to gain insights the regulation and evolution of secondary metabolism in *Aspergillus* fungi.

210 **A new mediator of nitrogen metabolite repression in *Aspergillus nidulans*** Richard B. Todd Plant Pathology, Kansas State Univ

Nitrogen utilization is a highly regulated process in fungi. In *Aspergillus nidulans*, the GATA transcription factor AreA activates the expression of genes for uptake and breakdown of nitrogen nutrients. AreA activity is highly regulated in response to nitrogen availability by multiple mechanisms, including autogenous transcriptional regulation, differ rential *areA* transcript stability, interaction of AreA with the corepressor NmrA, cleavage of NmrA by the protease PnmB, repression by the negative-acting GATA transcription factor AreB, and regulated AreA nuclear accumulation. When a preferred nitrogen source is available, nitrogen metabolite repression of the uptake and metabolic genes for utilization of alternative nitrogen nutrients occurs via reduced AreA activity. We have previously shown that overexpression of NmrA from a xylose-inducible promoter prevents AreA-dependent gene expression and leads to inhibited growth on alternative nitrogen sources. Here, I will focus on our efforts to understand the mechanism of action of NmrA. We have analyzed *nmrA* loss-of-function mutants, selected for full derepression in a partially derepressed strain defective in regulation of *areA* mRNA stability. One of these mutants affects the NADP-binding motif in NmrA. We have also analyzed a spontaneous extragenic suppressor mutant selected for suppression of the NmrA overexpression phenotype. Whole-genome sequencing revealed a frameshift truncation mutation in a Mediator complex subunit gene and a missense mutation in a putative beta-glucosidase gene, suggesting that one or both genes are needed for NmrA function. We have deleted both genes. Deletion of the Mediator complex component gene, but not the beta-glucosidase gene, phenocopies the truncation mutant with respect to suppression of the NmrA overexpression phenotype. Therefore, the Mediator complex plays a role in mediating NmrA-dependent repression.

211 **Identifying interconnected carbohydrate sensing pathways in oleaginous yeast** Lori B Huberman[1], Joshua D Kerkaert[1], Brandon Reyes-Chavez[2] [1]Plant Pathology and Plant-Microbe Biology, Cornell University, [2]Microbiology, Cornell University

Sensing available nutrients and efficiently utilizing them is a challenge common to all organisms. The oleaginous yeast *Rhodosporidium toruloides* is capable of utilizing the carbohydrates found in lignocellulose hydrolysate, including the cellulose building block, cellobiose. We screened for genes involved in cellobiose utilization and identified a transcription factor, which we named *CBR1*. Cells lacking *CBR1* are unable to utilize cellobiose as a carbon source but grow as well as wild type cells when exposed to the building blocks of hemicellulose and pectin. Intriguingly, Cbr1 is not only required for cellobiose utilization but also for utilization of tricarboxylic acid cycle intermediates. Our work suggests an interplay between utilization of the

carbohydrates in the plant cell wall and the citric acid cycle in oleaginous yeast. This finding may be important in better engineering oleaginous yeast for production of biofuels and other bioproducts during growth on lignocellulose hydrolysate and other plant derived carbohydrates.

212 **Deciphering ploidy transitions of titan cells in *Cryptococcus neoformans*** Zhuyun Bian, Sheng Sun, Joseph Heitman Duke University School of Medicine

Cryptococcus neoformans is a prominent global fungal pathogen affecting primarily immunocompromised individuals and causing life-threatening meningoencephalitis. During the initial stage of pulmonary infection, cryptococcal cells produce large polyploid titan cells that exhibit heightened resistance to host immune defenses and potentially contribute to the persistence of cryptococcal infections. These titan cells divide to produce haploid and aneuploid (1N+1, 1N+2) daughter cells, potentially contributing to systemic infections. However, both the factors involved in triggering polyploidization in cryptococcal cells and the mechanisms mediating ploidy reduction remain elusive. In this investigation, we focused on diploid BH strains (fusion products of strains H99 and Bt63) as well as the AI187 strain (fusion product of strains M001 and JF99) to examine titan cell functions. Our findings reveal that titan cells that originate from diploid cells produce diploid daughter cells in most cases. Interestingly, we did identify several haploid daughter cells based on flow cytometry analysis. Moreover, distinct drug resistance profiles compared to the original diploid parental cells were observed among diploid daughter cells. Whole genome sequencing and comparison of both daughter and progenitor diploid cells revealed genetic variation, attributable to Loss of Heterozygosity (LOH) events or aneuploidy (2N+1) in the daughter cells. We are further exploring the possible involvement of meiosis-specific genes in titan cell biology. To this aim, we generated both haploid and diploid strains with deletions in the *SPO11* and *DMC1* genes, known for their central roles in canonical sexual reproduction. These studies will provide insight into whether meiotic genes, beyond their conventional functions in sexual reproduction, may also contribute to the adaptation of eukaryotic cells undergoing substantial genome changes in response to genotoxic stress.

213 **Variation in parasexual recombination between *Aspergillus niger* and *Aspergillus fumigatus*** Ben Auxier[1], Eveline Snelders[2], Alfons J.M. Debets[2], Jianhua Zhang[3] [1]Wageningen University, [2]Wageningen University & Research, [3]RIVM
In many fungi a parasexual cycle is described as the sorting of haploid genetic variation through a mitotic diploid intermediate. This is often thought to play a potentially important role in shaping genetic variation in many fungi, acting as a form of horizontal gene transfer. Particularly in fungi where sexual reproduction is rare or totally absent, parasex may provide a mechanism to escape deleterious mutations and negative epistatic effects. Here, we examine the genetic outcome of parasex in both *A. fumigatus,* a species with a regular sexual cycle with an exceptionally high meiotic crossover rate, and *A. niger*, which lacks a known sexual stage. Using two heterokaryon compatible *A. fumigatus* siblings, we produced a highly heterozygous diploid with 7,822 usable markers. Using isogenic strains of *A. niger*, we produced a diploid with 145 markers. When cultivated with only a brief diploid phase, 2% of *A. fumigatus* chromosomes were observed to be involved in a mitotic crossing over, while for *A. niger* 6% of chromosomes had crossovers observed. However, with ten serial diploid transfers the crossovers increased to 4% for *A. fumigatus* and 29% for *A. niger*. Crossovers were unevenly distributed across chromosomes, but not found to be associated with previously identified hotspots. Calculations with the r̄ metric show that chromosomal shuffling is responsible for approximately 90% of genetic diversity that occurs during parasex. These results suggest that while short diploid phases will efficiently segregate variation between chromosomes, extended diploid phases are necessary to segregate variation within chromosomes. Consistent with the hypothesis of Pontecorvo (1958) that parasex would be an alternative to sex for asexual fungi, we find that the mitotic recombination rate in the asexual *A. niger* is indeed higher than in the sexual *A. fumigatus.* Further studies on the environmental prevalence of diploids in Ascomycete fungi are needed to understand the biological relevance of parasexuality.

214 **Discovery of plant- and algal-derived plastids in diverse fungi** Julia Kelliher[1], Aaron Robinson[1], Demosthenes Morales[1], La Verne Gallegos-Graves[1], Karen Davenport[1], Guillaume Cailleau[2], Saskia Bindschedler[2], Gregory Bonito[3], Pilar Junier[2], Patrick Chain[1] [1]Los Alamos National Laboratory, [2]University of Neuchatel, [3]Michigan State University

Fungi can form complex and close associations with plants and algae as well as plant- and algal-associated chloroplasts and other plastids. These interactions can affect organismal functioning in a variety of ways ranging from fungal modulation of photosynthesis to the stimulation of chloroplast-derived defenses against fungal pathogen invasion. However, little is known regarding the nature and extent of more intimate associations between fungi and plastids, nor what these relationships may mean in the broader context of fungal interactions. In filamentous fungi, new endohyphal relationships with bacteria are being continuously discovered, but the broader endohyphal microbiome and its potential inhabitants (e.g. archaea, viruses, other fungal cells, etc.) remain largely underexplored. Herein, we present the discovery of a new form of kleptoplasty, where we demonstrate that phylogenetically diverse fungi can internalize plant and algal-derived plastids that can then persist inside fungal cells. Plastome sequences were

initially identified when screening a fungal culture collection for bacterial associates. Further investigations utilizing fluorescence *in situ* hybridization imaging, phylogenetic analyses, probe-based chloroplast enrichment sequencing from fungi, screens of tens of thousands of publicly available fungal sequencing datasets, and chloroplast internalization experiments confirmed our findings, and supported the notion that phylogenetically diverse fungi harbor plastids derived from various plants and algae. Supported by a Joint Genome Institute (JGI) Community Science Program (CSP), internalization experiments coupled with time-course transcriptome sequencing on both the fungal and plastid sides provide insights into the mechanisms associated with fungal internalization of endohyphal microbiome components. While the functional implications of such interactions are not yet known, our discovery of fungal kleptoplasty has far reaching implications for fungal biology and fungal evolution, plant-fungal and algal-fungal interactions, and bioengineering.

216 **Increased genetic diversity of clonal rice blast fungus lineages through multiple mini-chromosome transfers** Cristina Barragan[1], Sergio M. Latorre[2], Angus Malmgren[1], Adeline Harant[1], Joe Win[1], Yu Sugihara[1], Hernan A. Burbano[2], Sophien Kamoun[1], Thorsten Langner[1,3] [1]The Sainsbury Laboratory, [2]Centre for Life's Origins and Evolution, Dept of Genetics, Evolution and Environment, University College London, [3]Max Planck Institute for Biology

Crop disease pandemics are often driven by clonal lineages of plant pathogens that reproduce asexually. How these clonal pathogens adapt to their hosts despite harboring limited genetic variation is poorly understood. Here, we show multiple instances of horizontal chromosome transfer involving clonal lineages of the rice blast fungus Magnaporthe (Syn. Pyricularia) oryzae. We identified the horizontally transferred chromosome as a 1.2Mb supernumerary mini-chromosome, mChrA, which is remarkably conserved across blast fungus isolates from lineages infecting the wild grass Eleusine indica (Indian goosegrass) and rice. Further analyses revealed mChrA was acquired by clonal rice blast fungi through parasexual-mediated horizontal transfer, with evidence of at least eight distinct transfer events over the past four centuries. These findings establish horizontal mini-chromosome transfer as a mechanism facilitating genetic exchange among blast fungi infecting different hosts. We propose that blast fungus populations infecting wild grasses act as genetic reservoirs that contribute to the evolvability of pandemic clonal lineages that afflict crops.

217 **Innovation, constraint, and the evolution of genetic networks in major eukaryotic lineages** Jacob L Steenwyk[1], Maxwell C Coyle[2], Noah Bradley[3], Xiaofan Zhou[4], Yuanning Li[5], Xing-Xing Shen[6], Chris Hittinger[7], Antonis Rokas[8], Nicole King[2] [1]UC-Berkeley / HHMI, [2]UC-Berkeley/HHMI, [3]Northwestern University, [4]South China Agricultural University, [5]Shandong University, [6]Zhejiang University, [7]University of Wisconsin - Madison, [8]Vanderbilt University

Genetic networks depict the intricate relationships among genes, their pathways, and cellular functions. Here, we infer genetic networks using coevolutionary information across 26 major lineages of animals and fungi. Analysis of network features uncovers both analytical and biological factors influencing their structural properties, including evolutionary rate and signal-to-noise ratios. Ancestral reconstructions of complex gene-gene relationships uncover patterns of gain and loss, mirroring gene families, but substantially more dynamic. Guided by these findings, we construct individual genetic networks for Animals and Choanoflagellates and identify complex gene relationships shared in both lineages, thus likely predating animal origins. Shared hubs of genes encode ancient cellular functions, such as cell cycle regulation, DNA replication, and ciliary processes. The principle of 'guilt-by-association' emerges as a promising approach for uncovering genotype-to-phenotype relationships. These findings uncover innovation and constraint in genetic network evolution and suggest that gene-gene association changes are a dynamic and underexplored mode of genome evolution.

218 **Frequent horizontal chromosome transfer between asexual fungal insect pathogens** Michael Habig[1], Anna V. Grasse[2], Judith Müller[1], Eva H. Stukenbrock[1], Hanna Leitner[2], Sylvia Cremer[2] [1]Kiel University, [2]Institute of Science and Technology Austria
Entire chromosomes are normally only transferred vertically from one generation to the next. Horizontal transfer of entire chromosomes has long been considered unlikely, but has recently gained support in several pathogenic fungi where it may affect fitness or host specificity. So far, it is unknown how these horizontal transfers occur, how frequent they are and whether they can occur between different species. In this study, we show several independent instances of a horizontal transfer of the same accessory chromosome between two different strains of the asexual entomopathogenic fungus *Metarhizium robertsii* during experimental co-infection of its insect host, the Argentine ant. In particular, only one chromosome was transferred from the donor strain to the recipient strain, and no other. The recipient strain, now carrying the accessory chromosome, showed a competitive advantage under certain host conditions. Using phylogenetic analysis, we further show that the same accessory chromosome was horizontally transferred in a natural environment between *M. robertsii* and another congeneric insect pathogen, *M. guizhouense*. Thus, horizontal chromosome transfer is not limited to the frequent intraspecific events observed during experimental infections, but also occurs naturally between species. The transferred accessory chromosome contains genes that may be involved in its preferential horizontal transfer, encoding putative histones and histone-modifying enzymes, but also putative virulence factors

that may support its establishment. Our study shows that both intra- and interspecies horizontal transfer of entire chromosomes is more common than previously thought and may represent a not uncommon mechanism for gene exchange.

219 **Mobile elements on mobile chromosomes in *Fusarium oxysporum*** Like Fokkens Wageningen University & Research

Fusarium oxysporum (Fo), the causal agent of Fusarium wilt, is an economically important fungal pathogen. Closely related lineages infect different host species, making this an excellent model for studying evolution of host switches. Previous research has shown that horizontal transfer of particular transposon-rich chromosomes can result in a host switch. Using comparative genomics, we have identified footprints of past horizontal transfer events and found that, while some lineages with the same host-range share the same mobile chromosome, others mix-and-match chromosomes. This is reflected in differences in their repertoire of small, secreted effector proteins, but also in differences in their transposon content. Here, we study genomic processes following horizontal chromosome transfer, including loss of (partial) chromosomes and proliferation of transposable elements that hitchhike on mobile chromosomes.

220 **Azole resistance mechanisms, multifungicide resistance and population structure of *Aspergillus fumigatus* from agricultural environments and retail plant products in the United States** Marin Brewer[1], Caroline Wang[2], Brandi Celia-Sanchez[1], Michelle Momany[1] [1]University of Georgia, [2]University of Texas Southwestern

Aspergillus fumigatus is a ubiquitous saprotroph and human-pathogenic fungus that is life-threatening to the immunocompromised. Triazole-resistant *A. fumigatus* was found in patients without prior treatment with azoles, leading researchers to conclude that resistance had developed in agricultural environments where azoles are used against plant pathogens. Azole-resistant *A. fumigatus* has been isolated from patients in the Unites States (US), but little is known about the distribution in US agricultural environments or retail plant products, which is essential to understanding the epidemiology of aspergillosis. Our objectives were to determine the distribution of azole-resistant *A. fumigatus* in agricultural environments and retail plant products produced in the US, as well as to identify the resistance mechanism(s) and population genetic structure of these isolates. We collected isolates from retail plant products and diverse agricultural sites in the east and west coast regions of the US and tested them for sensitivity to azoles. We found azole-resistant *A. fumigatus* in agricultural environments in 7 states showing that it is widespread in the US. Approximately 5% of the isolates collected from retail compost, soil, flower bulbs, and raw peanuts were pan-azole-resistant. We sequenced 135 environmental isolates representing the range of US sample sites and compared them with publicly available environmental and clinical US and worldwide isolates in phylogenetic analyses. We found worldwide isolates, as well as isolates from retail products in the US, fell into three clades and that TR-based resistance to azoles was largely in a single clade that was strongly associated with resistance to multiple agricultural fungicides. This is consistent with previous studies detecting three clades of *A. fumigatus* and identifying most pan-azole-resistant isolates with TR alleles in a single clade. In resistant isolates from retail plant products we identified the TR_{34}/L98H, TR_{46}/Y121F/T289A and H147Y *cyp51A* alleles, all known to underly pan-azole resistance, as well as the Y46F/V172M/T248N/E255D/K427E allele, which is associated with resistant isolates but does not likely underly resistance. We found pan-azole resistance in US retail plant products, particularly compost and flower bulbs, which indicates risk of exposure to these products for susceptible populations and that highly resistant isolates are likely distributed worldwide on these products.

221 **The role of environmental fungicides in triggering antifungal resistance in Cryptococcus spp** Daniel A Santos Dept of Microbiology, Universidade Federal de Minas Gerais

Antifungal resistance in yeasts often arises in patients undergoing prolonged azole therapy. Interestingly, resistant isolates are also found in individuals without prior azole treatment, suggesting alternative pathways contributing to antifungal resistance. Notably, resistant strains have been discovered in plant materials, soil, and decomposing matter—environments where crucial human fungal pathogens reside. Environmental yeasts like *Cryptococcus neoformans* and *C. gattii*, exposed to agrochemicals, exhibit cross-resistance with azoles in a temperature-dependent manner. In vitro adaptation to azole (tebuconazole) and non-azole (benomyl and pyraclostrobin) agrochemicals leads to cross-resistance with fluconazole and altered virulence, both *in vitro* and murine-induced cryptococcosis. Genomic alterations in adapted strains affect protein structures and gene regulation mechanisms, influencing fungal resistance and virulence. Increased efflux pump expression and genome copy numbers in the presence of agrochemicals indicate resistance mechanisms unrelated to the drug target. Chemical-genetic screening with a transcription factors mutant library suggests various genes regulating fungal metabolism during agrochemical exposure. Exploration of available databases, unrelated to antifungal resistance, identified genes potentially linked to drug inactivation, which are currently under investigation. Although limited, evidence of an environmental route influencing resistance in *Cryptococcus* spp. should not be

overlooked. Comprehensive fieldwork comparing resistant strain isolation in azole-containing versus azole-free environments remains essential to uncover the molecular mechanisms of fungicide-induced resistance.

222 **How accurately can experimental evolution predict fungicide resistance mechanisms?** Nichola J Hawkins National Institute of Agricultural Botany

The repeated emergence of resistance to fungicides is an example of parallel evolution, with resistance evolving in multiple pathosystems and against multiple fungicide classes. For site-specific fungicides, parallels can also be seen at the genotypic level, with resistance evolving by mutations in the same, target-site-encoding gene, and sometimes the same point mutations recurring, in multiple pathogen species. However, the repeatability of specific point mutations varies widely between fungicide classes. For QoIs, a single mutation, conferring high levels of cross-resistance, is found in the majority of fungal species, and for MBCs, most cases of resistance are due to changes at one of two codon positions. In contrast, for azoles and SDHIs, many different mutations have been reported, between and within species; resistance is quantitative, cross-resistance is partial, and multiple mutations can combine in one individual.

I am using experimental evolution under different selective scenarios for fungicide classes that have shown contrasting levels of genotypic repeatability in the evolution of resistance in field populations, to assess whether the predictability of evolution in the field is reflected in the lab, and whether the reliability of resistance mechanism predictions can be assessed in advance for new fungicide groups.

223 **Elevated mutation rates in multi-azole resistant *Aspergillus fumigatus* drive the rapid evolution of antifungal resistance** Michael J Bottery[1], Norman van Rhijn[1], Harry Chown[2], Johanna Rhodes[3], Brandi Celia-Sanchez[4], Marin T Brewer[4], Michelle Momany[4], Matthew Fisher[2], Christopher G Knight[1], Mike Bromley[1] [1]University of Manchester, [2]Imperial College London, [3]Radboud University Medical Centre, [4]University of Georgia

The evolution of antifungal resistance is a global problem. Of particular concern is the widespread emergence of azole resistance within *Aspergillus fumigatus*, a globally prevalent environmental mould that causes around 1 million life-threatening invasive infections in humans. It is becoming increasingly evident that the environmental use of azoles has led to selective sweeps across multiple genomic loci resulting in the rapid expansion of a genetically distinct lineage (clade A) that is resistant to clinically deployed azoles. Strains from this clade are more likely to be cross resistant to agricultural antifungals with unrelated modes of action suggesting they may be adapting rapidly to antifungal challenge. Here we show that this multi-azole resistant lineage is associated with increased mutation rates due to variants in the mismatch repair system. A variant in *msh6* is found near exclusively within clade A, occurs in 88% of multi-azole resistant isolates harbouring the *cyp51A* azole resistance allelic variant TR_{34}/L98H and is globally distributed. Natural isolates with this variant display 4 to 9-times higher mutation rate. Through the pervasive anthropogenic use of azoles, a lineage of *A. fumigatus* has emerged that is not only multi-azole resistant but also displays increased adaptive capability. Compounding this, ipflufenoquin, a novel agricultural antifungal has been approved for use in crop protection and shares the same mechanism of action as olorofim, a next generation clinical antifungal. We show that ipflufenoquin can select for high-level cross-resistance to olorofim. The dual use of these novel classes of antifungal drugs coupled with increased mutation rates in azole resistant isolates increases the probability of the accumulation of multiple independent resistance mechanisms within clade A to both azole and novel antifungal compounds, which ultimately may lead to the evolution of a lineage with pan-drug resistance.

224 **Interspecific hybridisation as a new evolutionary fungicide resistance mechanism in the fungal pathogen *Pyrenophora teres*** Chala Turo[1], Wesley Mair[2], Anke Martin[3], Simon Ellwood[1], Richard Oliver[4], Francisco Lopez-Ruiz[1] [1]MLS, Curtin University, [2]Curtin University, [3]University of Southern Queensland, [4]University of Nottingham

The barley net blotch diseases are caused by two fungal species of the Pyrenophora genus. Specifically, spot form net blotch is caused by *P. teres f. sp. maculata* (Ptm) whereas net form net blotch is caused by *P. teres f. sp. teres* (Ptt). Ptt and Ptm show high genetic diversity in the field due to intraspecific sexual recombination and interspecific hybridisation of the two species, although the latter is considered rare. Here we describe the detection of Ptt × Ptm hybrids with demethylase inhibitor (DMI) fungicide resistance ("HR Ptm") and discuss the implications for barley disease management. The genomes of one putative hybrid, three Ptm, and ten Ptt isolates were sequenced, and recombination analyses performed in the intergenic and whole genome level. Of the 12 chromosomes, 11 showed significant ($P < 0.05$) recombination events in the intergenic regions while variable recombination rate showed significant recombination across all the chromosomes. Further genotyping using Diversity Arrays Technology markers of fourteen Ptt, fifteen Ptm, 48 HR Ptm, and two *P. teres* isolates from barley grass, showed that all HR Ptm isolates were clonal and not clustered with Ptt or Ptm. The Nei's genetic differentiation among the observed clusters accounted for over 99% of the

total variation. Interestingly, lower genetic distance was found between HR Ptm and Ptm isolates (0.189) than HR Ptm and Ptt (0.807) isolates, indicating HR Ptm are more closely related to Ptm. Further locus specific analyses of the DMI target Cyp51A gene showed at least four recombination breakpoints, including the F489L point mutation that is correlated with DMI resistance. The result confirms occurrence of natural recombination between Ptt and Ptm and indicates that the HR Ptm likely acquired DMI resistance through interspecific recombination, followed by clonal expansion of this genotype in barley-growing areas of Western Australia. The use of effective fungicides in integrated disease management tactics will be crucial to minimise and restrict further dissemination of these adaptive HR Ptm isolates.

225 **The proteomic response of Aspergillus fumigatus to Amphotericin B (AmB) reveals the involvement of the RTA-like protein RtaA in AmB resistance** Sophie M. Tröger-Görler[1], Ammar Abou-Kandil[2], Annica Pschibul[1], Thomas Krüger[1], Maira Rosin[1,3], Franziska Schmidt[1,3], Parastoo Akbarimoghaddam[4], Arjun Sakar[4], Zoltán Cseresnyés[4], Yana Shadkchan[2], Thorsten Heinekamp[1], Markus Gräler[5,6,7], Marc T. Figge[3,4], Axel A. Brakhage[1,3], Nir Osherov[2], Olaf Kniemeyer[1] [1]Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), [2]Dept of Clinical Microbiology and Immunology, Sackler School of Medicine, [3]Institute of Microbiology, Friedrich Schiller University (FSU), [4]Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), [5]Dept of Anesthesiology and Intensive Care Medicine, Jena University Hospital, [6]Center for Molecular Biomedicine (CMB), Jena University Hospital, [7]Center for Sepsis Control and Care (CSCC), Jena University Hospital

The opportunistic human pathogen *Aspergillus fumigatus* poses a significant threat by causing mycoses, which can be fatal especially in immunocompromised individuals. Due to the increase of azole resistance in *A. fumigatus*, treatment options are often limited to amphotericin B (AmB), a member of the polyene family of antifungals that has well known side effects. A rising number of resistant isolates against AmB as well as limited knowledge about resistance and compensatory mechanisms give rise to concerns.

To elucidate the effects of AMB on the fungal proteome, we conducted liquid chromatography-tandem mass spectrometry analyses to identify changes in the proteomic profiles of *A. fumigatus* treated with sublethal concentrations of AmB and its liposomal formulation. Selected proteins with significant increase in abundance upon AmB exposure were then characterized.

By comparison of the proteomic response of AmB-treated samples and untreated controls, we found significant increases in the abundance of proteins belonging to secondary metabolite biosynthesis gene clusters, proteins anchored to the membrane, involved in catabolic processes or aromatic acid degradation. One of the proteins with the highest increase in abundance was RtaA, a fungal Rta1-like family protein. While deletion of *rtaA* led to increased sensitivity against AmB, overexpression resulted in a two-fold increase of resistance. Interestingly, only treatment with AmB and nystatin resulted in a rise of *rtaA* transcript levels, which hints towards a specific protection mechanism against polyenes. Deletion of *rtaA* did not significantly change the ergosterol content and intracellular lipid droplets of *A. fumigatus.* While not being crucial for the virulence of *A. fumigatus* itself, RtaA is most likely involved in the resistance against AmB by maintaining lipid homeostasis and membrane stability. These findings reveal a novel polyene resistance mechanism.

226 **High-throughput genetics, essential gene discovery, and fluconazole resistance in *Cryptococcus neoformans*** Joshua Lyon[1,2], Caroline Craig[3], Michael Eickbush[3], SaraH Zanders[3], Blake Billmyre[1,2] [1]Pharmaceutical and Biomedical Sciences, University of Georgia, [2]Infectious Diseases, University of Georgia, [3]Stowers Institute for Medical Research

Cryptococcus neoformans causes nearly 200,000 deaths annually. Treating Cryptococcosis is complicated by the limited set of effective antifungal drugs and by antifungal drug resistance. Development of novel targeted therapies will be facilitated by a better understanding of which genes are required for growth in order to enable prioritization of targeted drug development. We have developed a high-throughput quantitative genetics approach in *Cryptococcus neoformans* using massively parallel insertional mutagenesis coupled with targeted sequencing to identify genes that tolerate (nonessential) or do not tolerate (essential) insertional disruption. The *C. neoformans* genome includes dozens of genes whose orthologs are nonessential in other fungi but required in *C. neoformans* and vice versa. In addition, we have used this same high-throughput approach to map genes that affect susceptibility to fluconazole. The identified hits include genes from pathways with previously identified roles in drug resistance, including the HOG pathway and *PKA1/NRG1*. In addition, we have identified novel contributors to drug susceptibility and resistance, including the *RIM101* pathway and multiple individual genes of unknown function. Transposon inserts in regulatory regions of essential genes generate sensitized strains that enable genome-wide analysis of the contribution of previously intractable essential genes to stress responses including fluconazole resistance. Taken together, this work produces a global understanding of fluconazole resistance and a map of essential genome function to guide future drug development in *C.*

neoformans. We are currently expanding our TN-seq approach in order to establish the *Cryptococcus* genus as a model fungal genus to understand the evolution of virulence-related traits.

227 **Characterization of spatio-temporal dynamics of the constrained network of the filamentous fungus *Podospora anserina* using a geomatics-based approach** Clara Ledoux, Cecilia Bobee, Eva Cabet, Pascal David, Frederic Filaine, Sabrina Hachimi, Christophe Lalanne, Gwenael Ruprich-Robert, Eric Herbert, Florence Chapeland-Leclerc LIED Universite Paris Cite

In their natural environment, fungi are subjected to a wide variety of environmental stresses which they must cope with by constantly adapting the architecture of their growing network. In this work, our objective was to finely characterize the thallus development of the filamentous fungus *Podospora anserina* subjected to different constraints that are simple to implement in vitro and that can be considered as relevant environmental stresses, such as a nutrient-poor environment or non-optimal temperatures. At the Petri dish scale, the observations showed that the fungal thallus is differentially affected according to the stresses but these observations remain qualitative. At the hyphal scale, we showed that the extraction of the usual quantities (i.e. apex, node, length) does not allow to distinguish the different thallus under stress, these quantities being globally affected by the application of a stress in comparison with a thallus having grown under optimal conditions.

Thanks to an original geomatics-based approach based on the use of automatized Geographic Information System (GIS) tools, we were able to produce maps and metrics characterizing the growth dynamics of the networks and then to highlight some very different dynamics of network densification according to the applied stresses. The fungal thallus is then considered as a map and we are no longer interested in the quantity of material (hyphae) produced but in the empty spaces between the hyphae, the intra-thallus surfaces. This study contributes to a better understanding of how filamentous fungi adapt the growth and densification of their network to potentially adverse environmental changes.

228 **Morphological diversity as an adaptation strategy of extremotolerant fungi** Cene Gostincar, Nina Gunde-Cimerman Dept of Biology, University of Ljubljana, Biotechnical Faculty

Fungi that thrive in extreme environments exhibit a diverse repertoire of adaptations that are critical to their survival in conditions hostile to most other species. Many studies have focused on molecular adaptations, but the diverse and often peculiar morphology of extremotolerant and extremophilic fungi is also thought to play a role in their evolutionary success. Two broad strategies of extremotolerance have been described – extremotolerant generalists such as *Cladosporium halotolerans* and *Aureobasidium pullulans* show rapid growth and can often outcompete other microorganisms even under temperate conditions, while specialists such as *Hortaea werneckii* and *Wallemia ichthyophaga* grow slowly and are often restricted to extreme habitats. Asexual reproduction is common, but not the rule in all extremotolerant species, as recent genomic population data show. Asexuality can be explained either as a means of diverting energy into the energetically costly cellular mechanisms of extremotolerance or as a means of maintaining adapted genomic configurations. Dimorphic growth is typical of extremotolerant black yeasts, such as species of the genus *Aureobasidium*, in which both budding hyphae and yeast cells are observed in different strains and under different environmental conditions. But these and other extremotolerant species go beyond this simple yeast-hyphae dichotomy. Sometimes a single isolate forms many morphologies even on a single, relatively homogeneous laboratory medium. One of the survival strategies in extreme environments is meristematic growth, which minimizes the surface-to-volume ratio and thus shields against environmental stressors. The protective role of thickened cell walls enriched with compounds such as melanin or hydrophobins, either complementing meristematic growth or strenghtening yeast cells or hyphae, is evident in diverse and phylogenetically distant extremophilic fungi. In particular, the plasticity of morphologies in extremotolerant fungi is proposed as a form of bet hedging, enhancing adaptability to various environmental challenges. This talk will attempt to shed light on the remarkable morphological adaptations in extremophilic fungi as part of their survival strategies in some of the most hostile places on our planet.

229 **Role of stop-loss editing of *efd4* and *efd7* in fruiting body development and ascospore physiology in *Sordaria macrospora*** Metaxenia Skendrou[1], Jana Grygosch[1], Laura Bleeken[2], Fransziska Krekel[2], Ines Teichert[2] [1]Allgemeine und Molekulare Botanik, Ruhr-University Bochum, [2]Forest Botany and Tree Physiology, University of Göttingen

RNA editing is the selective insertion, deletion, or substitution of nucleotides and is conserved in all domains of life. RNA editing of protein-coding transcripts leads to sequence changes in the transcript as well as the protein that could alternatively be directly encoded in the DNA. In filamentous ascomycetes, adenosine (A) to inosine (I) RNA editing was recently detected to occur in protein-coding transcripts during sexual reproduction. Interestingly, in fungi, amino acid codons, but also stop codons tend to be affected by editing, the latter leading to a change of *TAG* or *TGA* codons to *TGG* tryptophan codons. Why editing occurs during sexual

development, how it is mediated and why the induced protein sequence changes are not directly DNA-encoded, is still under investigation. However, it has been hypothesized that editing is required for ascospore formation and / or ascospore germination and that the diversified proteome provides an advantage for the progeny.

To gain insight into the biological role of editing, we analyzed genes whose transcripts are affected by editing in the ascomycete *Sordaria macrospora*. Deletion of *efd4* and *efd7* encoding for an RNA-binding protein and a sorting nexin indeed revealed a function of these genes during ascospore formation. Complementation studies with mutations of the native stop codon to a *TGG* (always long protein) or a *TAA* (always short protein) revealed possible functions for the editing sites. Further studies on the function of editing during ascospore germination in different physiological conditions are underway, as are studies on the editing mechanism.

230 **Cell and Network Dynamics in Arbuscular Mycorrhizal Fungi** Vasileios Kokkoris A-LIFE Ecology & Evolution, Vrije Universiteit (VU) Amsterdam

Arbuscular mycorrhizal (AM) fungi form one of the most widespread terrestrial symbiosis on earth. Plant roots and AM fungal hyphae form complex interconnected networks because AM fungi can interact with multiple plant species simultaneously and a single plant host can be colonized by multiple AM fungi. As a result, underground mycorrhizal highways, known as common mycorrhizal networks (CMN), spread for hundreds of meters. While the importance and ubiquity of fungal networks has been well established, the underlying genetic system that controls AMF reproduction, and the resulting CMN connectivity is unknown. Here we examine how individual AM fungal networks grow and how they can interact when meeting neighboring, genetically distinct strains.

231 **Cytoskeletal Mechanisms Driving 3D Cellularization of Multinucleated Chytrid Fungi** Edgar M Medina, Lillian Fritz-Laylin Dept of Biology, University of Massachusetts Amherst

Chytrids are the only members of the fungi that have motile flagellated cells without a cell wall—the zoospore. Zoospores are produced by 3D multinuclear cellularization, a specialized form of cytokinesis. in which a single multinucleated mother cell gives rise to multiple uninucleated daughter cells arranged in a packed 3D lattice. Although we understand many molecular mechanisms driving cellularization in one-dimensional rods (fission yeast sporulation) and two-dimensional sheets (Drosophila blastoderm cellularization), how these principles extrapolate to 3D cellularization programs is unclear. To address this problem, we have developed genetic tools for chytrid fungi, a lineage that undergoes synchronous 3D multinuclear cellularization during their development. Here we describe the dynamics and core mechanisms of 3D cellularization in the chytrid fungus Spizellomyces punctatus for the first time. By combining live cell fluorescence imaging, laser ablation, and pharmacological perturbations we address three core questions: how are cleavage furrows initiated, the sources of membrane used for building the cleavage furrows, and the cytoskeletal driving force underlying furrow formation, ingression, and establishment of the 3D cellularization lattice. We show that furrows are initiated by a combination of nuclear cortical migration and interaction between the nuclear microtubule organizing center (MTOC) and the plasma membrane. These furrows extend using membranes sourced from Golgi-derived vesicles. While microtubules improve the accuracy and precision of the cleavage pattern, they do not play a role in furrow initiation, ingression, and formation of the cleavage network. Instead, we find that actomyosin networks provide the driving force of furrow initiation, ingression, and establishment of the cellularization lattice, forming a polymer with viscoelastic properties consistent with a contractile network. Together, this work suggests that the mechanisms underlying 3D cellularization in chytrids use the same machinery as that used for 1D and 2D cellularization in animals and yeast, but deploy them differently. Our findings reiterate the need to study fundamental cellular processes in diverse lineages and cell types rather than assuming the conservation of molecular mechanisms based on parts lists.

232 ***Fusarium graminearum* on barley: Novel encounters between a fungal pathogen and its grain host.** Frances Trail, Rebecca N. Shay Plant Biology, Michigan State Univ

Fusarium graminearum causes a devastating disease of wheat, barley and corn, which leaves grain moldy and contaminated with toxins. We have characterized the formation of biofilms in *F. graminearum* on the plant surface. Plant hosts infected with pathogens generally respond by producing reactive oxygen species as a defense response. Biofilms form a multicellular matrix to protect fungi from oxidative stress. Thus, the formation of biofilms strongly suggests a role in protecting cells from host responses during initiation of plant disease. Infection occurs in association with host trichomes, triggered by an unusual surface sensing mechanism. This work has provided evidence of a finely tuned relationship between host and pathogen, and has revealed new information that dictates approaches to disease control.

233 **Identification of environmental and genetic regulators of apothecium development in *Sclerotinia sclerotiorum*** Jeffrey Rollins[1], Ulla A Benny[2], Chenggang Wang[2] [1]Plant Pathology, Univ Florida, [2]University of Florida
Multicellular fruiting body development by Ascomycota fungi requires canalized genetic pathways that remain responsive to environmental input. Additionally, cooperation between ascogenous and vegetative hyphae is required to produce the final, functional form. The stipitate apothecium of *Sclerotinia sclerotiorum* (Lib.) de Bary is a macroscopic, uni-parental sexual fruiting body that lends itself to manipulation via environmental and genetic experimentation. Both the undifferentiated stipe and the developing apothecial disc are photoresponsive. We have characterized the role of UV-A, red and blue wavebands of light in this developmental process as well as the effects of light deprivation at various phases of development on developmental fate. These studies have revealed a number of photoresponses including positive and negative phototropism, photomorphogenesis controlled by UV-A, de-etiolation of undifferentiated stipes and photo-determinacy. From a genetic perspective, we have employed random mutagenesis as well as targeted gene mutation to identify genes that affect patterns of development as well as tissue determinacy. Through a forward genetic screen, we have determined that a fungal-specific zinc binuclear cluster transcriptional regulator is required for apothecial disc expansion. Rather than developing mature discs, loss of function mutants reiteratively bifurcate at the stipe apex to produce a coralloid form, yet remains fertile. A second mutant identified through reverse genetics of a conserved MAPK-encoding gene produces elongated stipes with incompletely expanded discs indicative of a defect downstream of light perception. Collectively, these experiments utilizing environmental and genetic manipulations provide new insights into pattern development and tissue determinacy in Ascomycota fruiting body development.

234 **Comparative approaches for understanding mushroom development** Laszlo G. Nagy Synthetic and Systems Biology Unit, BRC, HUN-REN Biological Research Center
Mushroom development may be the most complex morphogenetic process in fungi, which includes regulated transition from fractal-like mycelial to 3-dimensional growth, the differentiation of cell types, patterning through space and time, and culminates in the formation of fruiting bodies with genetically encoded shape, size and color. Deciphering the general principles of this process is a century-old challenge in mycology, and we are recently witnessing a revival of interest due to the expansion of industries utilizing fungi. In this talk I provide an overview of efforts to identify conserved genetic circuits that regulate fruiting body development. Comparative approaches proved useful for sourcing genes based on sequence or gene expression conservation for reverse genetics and evo-devo studies, allowing the identification of genes with large developmental effects. These allow tackling some of the fundamental processes associated with multicellular development, such as cell differentiation or the formation of morphological structures, but also fungal-specific processes, such as rapid/expansive growth or sporulation. In the long term, these efforts may contribute to a new genetic synthesis on the development of mushroom fruiting bodies and to better harnessing fungal morphogenetic potentials

235 **RNA Editing of Genomic Neighbors Controls Antiviral Response in *Neurospora crassa*** Shinji Honda[1], Ayumi Yokoyama[1], Nobuhiro Suzuki[2] [1]Faculty of Medical Science, University of Fukui, [2]Institute of Plant Science and Resources, Okayama University
Viruses that weaken pathogenic fungi and reduce their virulence are being considered as biological control agents. However, the mechanism behind viral symptom expression is not fully understood, partly due to the unidentified genes involved. Here, we used *Neurospora crassa*, a newly established model of fungal virology to demonstrate that ribonucleic acid (RNA) editing controls the antiviral response. The player genes comprised two pairs of adjacent genes in the genome, one being an A-to-I RNA editing enzyme, *OTT_1508-like deaminase* (*old*), and the other being its target, *zinc fingers adjacent to old* (*zao*). Mutants lacking both *zao* showed no antiviral transcriptional responses upon viral infection, whereas overexpression of RNA-edited *zao* induced severe symptoms without viral infection. Thus, the RNA editing system controls antiviral responses in *N. crassa*.

236 **Conserved antiviral factors repress pathogenic proliferation of the L-A RNA mycovirus in budding yeast** Jie Gao[1], Sabrina Chau[1], Minyoung Chung[1], Michael Costanzo[1], Annette Diao[2], Brenda Andrews[1], Charles Boone[1], Alan Davidson[1], Marc Meneghini[1] [1]Molecular Genetics, University of Toronto, [2]Biology, Massachusetts Institute of Technology

Studies of the killer phenomenon in the budding yeast *S. cerevisiae* led to the discovery of the double-stranded RNA totivirus L-A over 40 years ago. L-A is required for the replication of a collection of satellite dsRNA species referred to as M, which encode toxins that kill uninfected cells. While L-A and M have historically been regarded as a commensal or even beneficial residents of the cytosol, we have established that redundant host antiviral pathways must collaborate to maintain these viruses at copy numbers tolerable to the host cell. We first observed the pathogenic consequences of L-A and M proliferation through studies of the cytoplasmic exosome-associated Ski complex and the conserved mitochondrial DNA/RNA nuclease Nuc1. In mutants lacking both Nuc1 and Ski complex activity, viral copy number is significantly increased at the detriment of the host cell. During sporulation, accumulation of the M toxin in meiotic progeny leads to spore inviability, and further analysis showed that Nuc1 and the Ski complex must function throughout sporulation to protect spores from M and the L-A virus. During mitotic growth, high L-A copy number in these mutants causes defective respiratory metabolism and lethal heat intolerance. We leveraged the latter discovery

to perform genome-wide synthetic genetic array screening for novel antiviral factors and identified hundreds of functionally diverse candidate host factors that attenuate L-A pathogenesis, many of which are broadly conserved. We confirmed many candidates as bona fide antiviral factors and our further efforts have focused on characterising the mechanisms of Nuc1 and the RNA exonuclease Rex2. Both are conserved across all domains of life and their functions are poorly understood. Our studies reveal that the antiviral functions of Nuc1 and Rex2 require nucleolytic activity but are carried out in the mitochondria and nucleus respectively. Neither appear to contact the site of viral replication in the cytosol, suggesting that these nucleases act through an indirect mechanism that limits the availability of pro-viral host RNA species in a manner evocative of other RNA viruses that co-opt host RNA species for their replication. This insight, along with findings from our studies of other validated antiviral factors, serve to highlight the potential of budding yeast as a model system for the discovery and characterisation of host antiviral factors.

237 **Identification of the Viral Determinant of Hypovirulence and Host Range in Sclerotiniaceae of a Genomovirus Reconstructed from the Plant Metagenome** Shin-Yi Marzano USDA-ARS

The majority of the known mycoviruses are RNA viruses, but few have been found to have single-stranded DNA (ssDNA) genome. Uncharacterized ssDNA viruses have been discovered by metagenomics/metatranscriptomics approaches from various sources without hosts identified. Some of these novel viruses are classified in the newly formed family *Genomoviridae*. Here, we determined the host range of a novel genomovirus, SlaGemV-1, through the transfection of *Sclerotinia sclerotiorum* with infectious clones. Inoculating with the rescued virions, we further transfected *Botrytis cinerea* and *Monilinia fructicola*, two economically important members of the family Sclerotiniaceae, and *Fusarium oxysporum*. SlaGemV-1 causes hypovirulence in *S. sclerotiorum, B. cinerea*, and *M. fructicola*. SlaGemV-1 also replicates in *Spodoptera frugiperda* insect cells but not in *Caenorhabditis elegans* or plants. By expressing viral genes separately through site-specific integration, the replication protein alone was sufficient to cause debilitation. Our study was the first to demonstrate the reconstruction of a metagenomically discovered genomovirus without known hosts with the potential of inducing hypovirulence, and the rescued mycovirus will allow for studying mechanisms of genomovirus-host interactions that are conserved across genera, including fungal-cell entry and assembly.

238 **Characterization of a single-stranded DNA mycovirus infecting the plant pathogenic fungus *Botrytis cinerea*** María A. Ayllón[1,2] [1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA/CSIC), Universidad Politécnica de Madrid, [2]Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM)

Botrytis cinerea is one of the most destructive plant pathogenic fungi worldwide. The fungus causes grey mold disease in over 200 crops, resulting in enormous annual economic losses. Disease control methods usually involve the application of chemical fungicides. However, the development of resistant fungal strains, to more frequently used fungicides, compromises this type of control. Then, new alternatives based in the advance of biological control has promoted the study of mycoviruses which induce a reduction in fungal virulence in plant host.

Our group has made significant progress in the molecular characterization of atypical mycoviruses infecting *B. cinerea* over the last decade, which will be revised in this communication. In particular, it is remarkable the characterization of a segmented mycovirus, belonging to the family *Genomoviridae*, Botrytis cinerea ssDNA virus 1 (BcssDV1). Only three mycoviruses, included in this recently established family, have been entirely characterized, and all of them induce hypovirulence in their fungal hosts. BcssDV1 was initially identified through RNA-Seq screening in pools of Spanish and Italian field isolates obtained from grapevine. A more extended characterization of BcssDV1 revealed that it is a tetrasegmented single-stranded DNA virus which encompasses four genomic segments of approximately 1,7 kb each. These segments, named DNA-A, DNA-B, DNA-C and DNA-D, encode the rolling-circle replication initiation protein, the capsid protein, and two hypothetical proteins, respectively. BcssDV1 variants were subsequently detected in independent field isolates, indicating a high incidence in several Italian and Spanish regions. Analysis of the mycoviral nucleotide and amino acid sequences showed low variability among the different mycoviral variants. DNA-A and DNA-D were the more conserved genomic segments among variants, while DNA-B and DNA-C were the most variable ones. In the absence of an infectious clone of the mycovirus, and to confirm or hypothesize about the function of the proteins coded by each genomic segment, we conducted predictions of the tertiary structures of the four proteins. The results corroborated the function of the proteins encoded by DNA-A and DNA-B, and suggested the involvement of proteins coded by DNA-C and DNA-D in fungal replication cell cycle.

239 **From multi- to single-mycoviral infection in the plant pathogenic fungus *Botrytis cinerea*** Julián Méndez-García[1], Julio L Rodríguez-Romero[1,2], María A Ayllón[1,2] [1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid

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The use of mycoviruses has been explored as an innovative strategy for the biological control of fungal infections, as it was discovered that they can decrease the virulence of the fungi on their hosts. *Botrytis cinerea* is one of the most important plant-pathogenic fungi, as it is responsible of the gray mold disease in more than 200 crops worldwide. In a previous study, an RNA-Seq analysis allowed the detection of the mycovirome of *B. cinerea*, in pools of 248 field isolates, collected from vineyards in different regions of Europe (Italy and Spain).

The RNA-Seq analysis of the mycovirome of three independent field isolates allowed us to demonstrate that multi-mycoviral infection is a common phenomenon in natural populations of *B. cinerea*. This analysis showed the presence of more than 20 mycoviruses infecting one single field isolate.

To study the independent effect of mycoviruses on a fungus, it is necessary to obtain fungal strains infected with a single mycovirus, and, then, analyse the effect of this infection compared to the isogenic line free of this mycovirus infection. In this sense, we combined different mycoviral curing strategies, including single-spore or protoplast isolation, co-culture transmission and hyphal tipping. These strategies were performed consecutively from the three independent field isolates.

The mycovirome of the distinct resulting cured strains was also analyzed in separate steps of the curing pipeline, through RNA-Seq analysis and detection by PCR amplification. These curing steps reduced the mycoviral content to over ten mycoviruses in a single-spore strain, under ten in a strain after co-culture transmission, and one single-mycovirus infection after hyphal-tipping.

In conclusion, the newly obtained strains showed a reduction in the number of infecting mycoviruses. Additionally, this analysis also demonstrates that multimycoviral infection is frequently maintained in *B. cinerea*, pointing out the difficulties in obtaining a singlemycoviral-infected fungal strain. Despite the pointed difficulties of the process, we successfully navigated the way from a multi- to a single-mycoviral infected *B. cinerea* strain. Interestingly, phenotypic and virulence analysis showed differences between isogenic strains that only varies in mycoviral population.

240 **Virome characterization of a collection of *Botrytis cinerea* from Australia** Lorena Rodriguez[1,2], Rosalie Sabburg[3], Donald M Gardiner[3], Kim M Plummer[1], Scott Mattner[1,2,4], Anthony Gendall[1,2] [1]Deparment Animal, Plant and, La Trobe University, [2]Australian Research Council Research Hub for Sustainable Crop Protection, [3]The University of Queensland, [4]Victoria Strawberry Industry Certification Authority

Gray mould, caused by *Botrytis cinerea*, is a highly damaging pathogen impacting a wide range of crops, with prevalent fungicide resistance. Products derived from microorganisms offer an ecofriendly alternative to chemical controls that could be incorporated into biocontrol strategies. Among these biologicals, mycoviruses have emerged as potential biological control agents. Mycoviruses are widespread viruses infecting fungi, including *B. cinerea*, that are being investigated for their role in such approaches. Notably, there are currently no reports of the mycoviral diversity in *B. cinerea* in Australia. In this study, we conducted an exploration of the mycovirome of *B. cinerea* isolates from various hosts across different states in Australia. RNASeq analyses was used to investigate mycovirus diversity in 24 Australian *B. cinerea* isolates from diverse hosts and geographic regions. This lead to the identification of sequence contigs that corresponded to either partial or complete genomes of mycoviruses. Most isolates were infected with more than one mycovirus, and some isolates from different hosts shared identical or near-identical mycoviruses suggesting a recent transmission. To examine the impact of the mycovirus on the host, we documented alterations in the *in vitro* and *in planta* phenotype and growth characteristics of isolates with different mycoviromes. This study has enhanced our understanding of mycoviral diversity, and identified mycoviruses that could serve as active ingredients in biological products for the effective control of this devastating fungus. Furthermore, the identification of fungal viruses is a crucial step in initiating an understanding of the dynamic relationship between mycoviruses and the RNA silencing machinery (RNAi) in fungi. This opens up a new field of study where viruses influence or manipulate host gene expression regulation, with implications for both fundamental mycology and potential applications.

241 **The evolution and distribution of endogenous DNA viruses in early-divergent fungi** Mark Yacoub[1], Rebecca Clemons[2], Timothy Y James[2], Jason E Stajich[1], Evelyn Faust[2], Luis F Toledo[3], Thomas S Jenkinson[4], Tamilie Carvalho[2], Rabern Simmons[2], Erik Kalinka[5], Lillian K Fritz-Laylin[5] [1]Dept of Microbiology and Plant Pathology, University of California, Riverside, [2]Dept of Ecology and Evolutionary Biology, University of Michigan, [3]Departamento de Biologia Animal Instituto de Biologia (IB), Universidade Estadual de Campinas, [4]Dept of Biological Sciences California State University, East Bay, [5]Dept of Biology, University of Massachusetts Amherst

Batrachochytrium dendrobatidis (*Bd*) is a chytrid fungus that causes the global amphibian disease chytridiomycosis. Unlike many pathogens that have a narrow host range, *Bd* is capable of causing skin necrosis across a wide range of amphibians. This has led to the extinction of frog species across six continents, making *Bd* the most significant pathogen contributor to biodiversity decline. *Bd* is divided into distinct clades: Global Panzootic Lineage (GPL), CAPE, BRAZIL, and ASIA. Of these, GPL is globally distributed and responsible for amphibian decline on nearly every continent, while the other clades are considered enzootic. We detected a novel ssDNA virus (BdDV-1) integrated at a single locus in the genome of a *Bd*-BRAZIL strain that is largely absent in GPL strains. The discovery of BdDV-1 provides an opportunity to investigate the molecular interactions between chytrid fungi and endogenous mycoviruses. In this study we leverage DNA and RNA sequencing methods to study the interactions between infected *Bd* strains and BdDV-1. We identified differentially expressed genes associated with the presence of BdDV-1 as well as evidence for co-evolution between fungus and virus. We expand on this discovery by revealing the presence of an additional 249 endogenous DNA viral elements in the genomes of other early-divergent fungi, indicating the scope of fungal history with DNA viruses

242 **Viro-Fungal Tag-Team: Aspergillus dsRNA virus drives fungal fitness and pathogenicity in the mammalian host** Vanda Lerer[1], Marina Rocha[1], John Adeoye[1], Neta Shlezinger[2] [1]The Koret School of Veterinary Medicine, The Hebrew University, [2]The Hebrew University

Fungal pathogens pose a significant threat to global health. As eukaryotes, they share considerable homology with their hosts, requiring the development of innovative, non-cross-reactive therapies. *Aspergillus fumigatus* accounts for approximately 65% of all invasive fungal infections in humans, with mortality rates from aspergillosis reaching nearly 50%. Fungal virulence in plant pathogenic fungi can be modified by mycoviruses, which are viruses that infect fungi. However, their impact on fungal pathogenesis in mammals has remained largely unexplored. Here, utilizing an *A. fumigatus* strain naturally infected with *Aspergillus fumigatus polymycovirus-1 (AfuPmV-1)*, we found that the mycovirus confers a significant survival advantage to the fungus under conditions of oxidative stress, heat stress, and within the murine lung. Thus, *AfuPmV-1* modulates fungal fitness, resulting in increased virulence and the progression of exacerbated fungal disease. Moreover, antiviral treatment reverses the exacerbated *AfuPmV-1M*-Mediated Virulence. Therefore, antiviral drugs that target viral replication represent promising "antipathogenicity" treatments against virus-bearing pathogenic fungi. Taken together, these data suggest that mycoviruses play a significant role as "backseat drivers" in human fungal diseases, presenting critical clinical implications.

243A **Genomic spoilage determinants and evolutionary history of diastatic *Saccharomyces cerevisiae* strains** Jeremy R Smith Food Science, Cornell University

Diastatic yeasts are a subset of *Saccharomyces cerevisiae* brewing strains which have acquired the ability to hydrolyze terminal α-1,4 glycosidic bonds found in poly- and oligosaccharides. The breakdown of complex carbon sources by diastatic yeast and subsequent fermentation of released glucose monomers results in super-attenuated beer (i.e., beer which is fermented well below normal specific gravity values). The super-attenuating process, while desirable for some beer styles, is slow and often occurs post-bottling in cases of unexpected diastatic contamination. Spoilage by diastatic yeast can impart undesirable sensory characteristics to final products and produce dangerous high-pressure systems at the risk of consumer safety. The extracellular glucoamylase responsible for this phenotype is encoded for by *STA1*, *STA2*, and *STA3*, three highly related genes which formed through a gene fusion between *FLO11* (GPI-anchored cell surface glycoprotein) and *SGA1* (intracellular sporulation-specific glucoamylase). While all diastatic brewing strains contain either *STA1*, *STA2*, or *STA3*, identification of gene presence alone is insufficient to accurately predict strain spoilage potential. By cross-referencing genomic and functional assay data, we were able to identify conserved allelic variations which influence transcriptional regulation and enzymatic function of the glucoamylase. Additionally, genomic investigation has yielded evidence for sequential gene fusion and translocation events of *STA* variants and has provided insights towards elucidating the evolutionary history of diastatic strains.

244A **A protein kinase coordinates *Magnaporthe oryzae* metabolism during biotrophy to drive growth in living host rice cells** Nawaraj Dulal, Gang Li, Ziwen Gong, Richard A Wilson Plant Pathology, University of Nebraska-Lincoln

Magnporthe oryzae, a hemibiotroph and an important class of eukaryotic microbial plant pathogen, causes blast, the most devastating disease of cultivated rice. It is also a threat to global wheat production. Following breaching of the rice cuticle using

specialized cells called appressoria, *M. oryzae* elaborates bulbous, branching invasive hyphae (IH) that grow undetected and in intimate contact with living host rice cells for the first 3-4 days of infection before switching from this biotrophic growth phase to necrotrophy, when host cells die and disease symptoms appear. To establish infection and avoid host detection, *M. oryzae* neutralizes the host ROS burst that otherwise triggers plant immunity while deploying effectors into both the apoplastic space between IH and the plant membrane-derived extra invasive hyphal membrane (EIHM), and into the plant membrane-rich biotrophic interfacial complex (BIC), where they are translocated into host cells. However, the molecular decision-making processes that integrate fungal growth and metabolism in living host rice cells with biotrophic interfacial membrane integrity and plant defense suppression are poorly understood. Eukaryotic cellular growth requires activated Target of Rapamycin (TOR) signaling, which inhibits autophagy. Here, using reverse genetics, live-cell imaging, plate growth tests and multiomics approaches, we show how, during biotrophy, the *M. oryzae* serine/threonine protein kinase Rim15 connects autophagy with TOR signaling to drive colonization of the living host rice cell. Specifically, examination of the Δ*rim15* deletion mutant strain revealed that Rim15-dependent cycles of autophagy and glutaminolysis liberate α-ketoglutarate, which reactivates TOR signaling and supports biotrophic growth as a preferred carbon source while conserving glucose for antioxidation-mediated plant defense suppression. Our findings thus shed new light on the metabolic strategies integrating host plant defense suppression with fungal growth under the nutrient-restricted conditions of the host rice cell. We expect these insights will help uncover new targets to combat *M. oryzae* and other recalcitrant fungal pathogens.

245A **Predicting fungal secondary metabolite activity from biosynthetic gene cluster data using machine learning** Olivia Riedling[1], Allison S Walker[2], Antonis Rokas[1] [1]Biological Sciences, Vanderbilt University, [2]Chemistry, Vanderbilt University

Fungal secondary metabolites (SMs) contribute to the diversity of fungal ecological communities, niches, and lifestyles. Many fungal SMs have one or more medically and industrially important activities (e.g., antifungal, antibacterial, and antitumor). The genes necessary for fungal SM biosynthesis are typically located right next to each other in the genome and are known as biosynthetic gene clusters (BGCs). However, whether fungal SM bioactivity can be predicted from specific attributes of genes in BGCs remains an open question. We adapted machine learning models that predicted SM bioactivity from bacterial BGC data with accuracies as high as 80% to fungal BGC data. We trained our models to predict antibacterial, antifungal, and cytotoxic/antitumor bioactivity of fungal SMs on two datasets: 1) fungal BGCs (dataset comprised of 314 BGCs), and 2) fungal (314 BGCs) and bacterial BGCs (1,003 BGCs). We found that models trained on fungal BGCs had balanced accuracies between 51-68%, whereas training on bacterial and fungal BGCs had balanced accuracies between 56-68%. The low prediction accuracy of fungal SM bioactivities likely stems from the small size of the dataset; this lack of data, coupled with our finding that including bacterial BGC data in the training data did not substantially change accuracies, currently limits application of machine learning approaches to fungal SM studies. With >15,000 characterized fungal SMs, millions of putative BGCs in fungal genomes, and increased demand for novel drugs, efforts that systematically link fungal SM bioactivity to BGCs are urgently needed.

246A **Lipid flippase regulation of antifungal drug resistance and virulence in *Cryptococcus neoformans*** Robert Tancer, Siddhi Pawar, Chengjun Cao, yina wang, Chaoyang Xue Rutgers University

Echinocandins show fungicidal activity against common invasive mycoses but are ineffective against cryptococcosis. The underlying mechanism for echinocandin resistance in *Cryptococcus neoformans* remains poorly understood. Our forward genetic screen identified Cdc50, the regulatory subunit of lipid flippase, as a key contributor of caspofungin resistance. Further suppressor screen identified a mechanosensitive channel protein Crm1 (Caspofungin Resistant Mutation 1) that interacts with Cdc50 to regulate intracellular calcium levels ($[Ca^{2+}]c$). Together, our results demonstrate that Cdc50 and Crm1 regulation of the calcineurin pathway and cytoplasmic calcium homeostasis may underlie caspofungin resistance in *C. neoformans*. In addition, we also found that Cdc50 is essential for fungal virulence. The *cdc50*Δ mutant cells are rapidly engulfed by macrophages in vitro and are cleared in the infected lung in a murine model of systemic cryptococcosis. We are currently testing the hypothesis that the accumulation of phosphatidylserine on the outer leaflet of lipid bilayer membrane contributes to both the increased sensitively of *cdc50*Δ cells against caspofungin and the loss of fungal virulence. We propose that fungal lipid flippase is an excellent drug target and are developing peptide-based lipid flippase inhibitors to block Cdc50-ATPase interaction as potential antifungal agents. Our preliminary study has yielded a stable peptide inhibitor that shows synergetic effect with several existing antifungal drugs.

247A **An improved CRISPR-Cas12a editing system uncovers the role of horizontally-transferred metabolic pathways in mitochondria of the oomycete *Phytophthora infestans*** Carl S Mendoza, Howard S Judelson Microbiology & Plant Pathology, University of California, Riverside

CRISPR-Cas gene editing systems have proved to be powerful tools for biological research and bioengineering, but their effectiveness varies depending on the organism. This hinders research progress in many important filamentous fungi and oomycetes, among them *Phytophthora*, which includes some of the world's most destructive plant pathogens. A Cas12a-mediated editing system recently developed for the potato and tomato pathogen *Phytophthora infestans* was improved by using a mutant LbCas12a combined with elevated post-transformation incubation temperatures, increasing the rate of editing by three- to ten-fold. We also tested whether the efficiency of editing via homology-directed repair (HDR), which carries broader applications that include gene replacement and protein tagging, could be improved by disabling a component of the non-homologous end-joining (NHEJ) pathway in *P. infestans*, Ku70. We found that disrupting Ku70 increased the rate of editing via HDR by about four-fold, while not impacting growth, development, or pathogenesis. The editing system is now being used to test the function of a novel feature of glycolysis unique to stramenopiles (which includes oomycetes) in which enzymes for the ATP-generating pay-off phase occur in both the cytosol and mitochondria. We hypothesize that the mitochondrial glycolytic pathway is functionally linked to serine biosynthesis in that organelle through 3-phosphoglycerate. Knocking out the mitochondrial enzymes involved in glycolysis and serine biosynthesis caused slower growth with more frequent hyphal branching. Metabolomic analyses to understand how *P. infestans* adapts to disruptions in the pathways are underway. In summary, we have developed improvements to the CRISPR-Cas system which should be useful for many species, and demonstrated its utility by testing the function of a unique feature of oomycete metabolism which sheds light on the evolution of metabolic pathways and metabolic compartmentalization in eukaryotes.

248A **The hypoxia regulator Sre1 controls cryptococcal response to nickel, a micronutrient for fungi** Amber R Matha, Xiaofeng Xie, Robert J Maier, Xiaorong Lin Microbiology, University of Georgia

Nickel is an abundant element on Earth. While humans do not produce enzymes that require nickel as a cofactor, many microbes, including various pathogenic microbes, use nickel for various enzymatic activities. There are nine nickel requiring proteins known in microbes, and only one is known in the fungal kingdom. Nickel-requiring urease is one of the first established virulence factors in the opportunistic human fungal pathogen *Cryptococcus neoformans*. Unlike our knowledge of other metals, the way *C. neoformans* responds to Ni is unknown despite the abundance of Ni in soil, the environmental niche of this organism. Here, we found that the transcription factor Sre1 is required for growth on media supplemented with nickel and that Ni causes alterations in ergosterol and lipid biosynthesis. Overexpression of *ERG25*, a known iron binding protein in the ergosterol biosynthesis pathway, increases resistance to Ni in the wildtype and also rescues the growth defect of *sre1*Δ. Mutation of two histidine residues in Erg25 that are predicted to interact with a cation abolishes its ability to rescue *sre1*Δ on Ni. Collectively, we show that ergosterol biosynthesis pathway is impacted by the presence of Ni and that Sre1 and Erg25 play a vital role in responding to this metal.

249A **A role for melanin and perylene quinones for abiotic and biotic stress tolerance** Jia Gao, Reinhard Fischer Dept of Microbiology, Institute of Applied Biosciences

The black mold *Alternaria alternata* is one of the most widespread contaminants of food and feed, and a weak plant pathogen. It produces a large diversity of secondary metabolites with alternariol and its derivatives as prominent examples. Other important phyto- and mycotoxins are perylene quinones (PQs), some of which exhibiting anticancer activity. We discovered recently that the PQ altertoxin (ATX) biosynthesis shares most enzymes with the DHN-melanin pathway[1]. However, melanin is formed in aerial hyphae and spores, but ATXs are synthesized in substrate hyphae. Furthermore, we proved that 1,8-DHN is the last common intermediate required for DHN-melanin and ATXs formation. The enzyme dimerizing 1,8-DHN to ATXs remained unknown. To identify the dimerization enzyme encoding gene, we performed genome-wide expression analyses with different mutant strains producing much more or much less ATXs as compared to the wild type. A small gene cluster was discovered where the expression in the different mutants correlated well with the amount of ATXs formed. The cluster contains six genes, namely the transcription factor encoding gene *atbA*, the dehydrogenase encoding gene *atbB*, the anthrone oxygenase encoding gene *atbC*, the oxidoreductase encoding gene *atbD*, the cytochrome P450 encoding gene *atbE* and the major facilitator superfamily transporter encoding gene *atbF*. Deletion of *atbA and C-E* resulted in strains unable to produce ATXs, whereas the absence of AtbB caused the accumulation of ATXs, suggesting a key role of this gene cluster in ATXs biosynthesis. HRMS and MS/HRMS analyses to reveal how 1,8-DHN is dimerized to form ATXs are on the way. Furthermore, co-incubation of *A. alternata* strains with *Aspergillus nidulans*, *Trichoderma guizhouense*and *Penicillium species*, proved that ATXs help *A. alternata* to compete with other fungi. In

summary, this study revealed that *A. alternata* uses two gene clusters plus several genes scattered in the genome to produce DHN-melanin and PQs and that both products are important to cope with the environmental stress UV light and competing fungi.

Gao, J., Wenderoth, M., Doppler, M., Schuhmacher, R., Marko, D., & Fischer, R. (2022). Fungal melanin biosynthesis pathway as source for fungal toxins. *mBio*, **13**: e00219-00222.

250A **Tailor-made biosurfactant production in the corn smut *Ustilago maydis*** Jessica Tiefenbacher[1], Fabienne Becker[2], Johannes Freitag[1], Uwe Linne[3,3], Björn Sandrock[1] [1]Genetics, Philipps-University Marburg, [2]Biochemie, Justus-Liebig-University Giessen, [3]Chemistry, Philipps-University Marburg

Under nutrient limiting conditions fungi produce a large variety of secondary metabolites with diverse molecular architecture and biological activities including antibiotics and toxins. The smut fungus *Ustilago maydis* and related basidiomycetes synthesize huge amounts of glycolipids in response to nitrogen limitation. Two types of glycolipids from *U. maydis* were discovered in the middle of the last century: ustilagic acids (UAs) and mannosylerythritol lipids (MELs) (1). UAs kill other fungi and bacteria and are discussed as biocontrol agents. MELs are surface active substances that have antibacterial properties and may become a valuable future resource for pharmaceutical and biotechnological industry, e.g. they can be used as biodegradable surfactants. Here we present our results to generate tailor-made MELs in *U. maydis* (2) using the different characteristics of the acyltransferases Mac1 and Mac2 from 11 different MEL producing fungi. MEL variants are analyzed with regard to a variety of properties like surface tension, hemolysis, biocontrol and emulsification.

251A **Mechanism of circadian clock control of rhythmic translation in *Neurospora crassa*** Ebimobowei Preh, Deborah Bell-Pedersen Biology, Texas A&M University

The circadian clock in *Neurospora crassa* regulates daily rhythms in the phosphorylation and daytime inactivation of the conserved translation initiation factor eIF2α. Clock control of eIF2α activity is responsible for the rhythmic translation of ~15% of mRNAs. Cycling phosphorylated eIF2α levels require rhythmic activation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). However, how the clock controls the activity of CPC-3 is not known, and this information is critical to determine the mechanisms underlying rhythmic protein synthesis. To be activated, CPC-3 forms a complex with GCN1, which helps to bring uncharged tRNAs to the tRNA binding domain on CPC-3. In *Saccharomyces cerevisiae*, activation of GCN2 under stress conditions requires direct interaction of GCN1 and GCN2 with ribosomes. Furthermore, CPC-3 and GCN1 levels are clock-controlled in *N. crassa*. Based on these data, I hypothesized that *N. crassa* GCN1 and CPC-3 rhythmically interact with the ribosome, and that this interaction is necessary for rhythmic CPC-3 activity and eIF2α-controlled translation initiation. To test this hypothesis, the interaction of CPC-3 and GCN1 with ribosomes was examined. Ribosomes were pelleted from cultures grown in constant dark (DD) and harvested every 4 hours in a circadian time course. While CPC-3 and GCN1 were found to co-sediment with monosomes and polysomes, the interaction of CPC-3 and GCN1 with ribosomes was arrhythmic in DD. Data will be presented showing that CPC-3 interaction with the ribosome is necessary for acute stress induction of eIF2α phosphorylation, but not for circadian clock regulation of eIF2α phosphorylation. Taken together, these data suggest that CPC-3 and GCN1 ribosome interaction is not necessary for rhythmic CPC-3 activity. In addition, work is in progress to investigate the alternative hypothesis that the clock regulates the interaction between uncharged tRNA and GCN1, leading to rhythmic activity of the kinase CPC-3, and ultimately driving rhythms in the translation of select mRNAs.

252A **Aspartyl peptidase May1 induces host inflammatory response by altering cell wall composition in the fungal pathogen *Cryptococcus neoformans*** yeqi li[1], Benjamin Chadwick[2], Tuyetnhu Pham[2], Xiaofeng Xie[1], Xiaorong Lin[1,2] [1]Microbiology, University of Georgia, [2]Plant Biology, University of Georgia

The opportunistic fungal pathogen *Cryptococcus neoformans* causes cryptococcal meningoencephalitis, a disease that kills more than 180,000 out of 225,000 infected people annually. Contributing to its success as a pathogen is its cell wall surrounded by a polysaccharide capsule. However, when cryptococcal cell wall is compromised, exposed pathogen-associated molecular pattern molecules (PAMPs) could trigger host recognition and initiate attack against this fungus. Thus, cell wall composition and structure are tightly regulated. Cryptococcal cell wall is unusual in that chitosan, the acetylated form of chitin, is predominant over chitin and is essential for cryptococcal virulence. Recently, it was shown that acidic pH causes weakened cell wall and increased exposure of PAMPs partly due to decreased chitosan levels. However, the molecular mechanism responsible for the cell wall remodeling in acidic pH is unknown. In this study, by screening for genes involved in cryptococcal tolerance to high levels of CO_2, we serendipitously discovered that the aspartyl peptidase May1 contributes to cryptococcal sensitivity to acidic pH in unbuffered media due to high levels of CO_2. Overexpression of *MAY1* increases cryptococcal cell size and elevates PAMP exposure, causing a hyper-inflammatory response in the host while the deletion of the *MAY1* gene does the opposite. We found that May1 weakens

the cell wall and reduces the chitosan level, partly due to its interference on the subcellular localization of Chs3, the sole chitin synthase that supplies chitin to be converted to chitosan. Consistently, overexpression of *CHS3* largely rescued the phenotype of *MAY1*^{oe} in acidic media. Collectively, we demonstrate that May1 remodels *C. neoformans* cell wall in acidic pH by reducing chitosan levels through its influence on Chs3.

253A **Constitutive excretion of melanin induced by nonsense mutation in *Exophiala* species** Quin Barton[1], Erin C Carr[1], Steven D Harris[2], Wayne R Riekhof[1] [1]School of Biological Sciences, University of Nebraska-Lincoln, [2]Plant Pathology, Entomology, and Microbiology, Iowa State University

Polyextremotolerant fungi are characterized by melanin in their cell wall, which allows them to tolerate a range of extreme environments. Melanin is metabolically expensive, but provides a great deal of protection against abiotic factors such as UV exposure, desiccation, osmotic pressure, metal toxicity, reactive oxygen species, and more. *Exophiala viscosa* is a novel species of polyextromotolerant fungus which is capable not only of melanin production, but also excretion of melanin into its environment. A recent spontaneous mutant of *E. viscosa*, referred to as Creamy, is defined by its ability to excrete a great deal more melanin than the wild type. There are 113,175 single nucleotide polymorphisms in Creamy compared to the wild type *E. viscosa*, with one mutation creating a nonsense mutation in the gene *yel1* (yellow-green enzyme, aka: *ayg1*). This is the second enzyme in the conical melanin production pathway, which is believed to be the source of this fungus' intense over-excretion of secondary metabolites. The excreted substance mimics melanin in its UV-Vis spectrum, however the mutation within *yel1* entails alternate possibilities such as increased production of flaviolin or a combination of flaviolin and melanin overproduction. Finally, unlike *E. viscosa* wild type, Creamy's "melanin" is constitutively excreted on all media types tested. This note of intrigue prompts further insight into genetic regulation of secondary metabolites via characterization of *E. viscosa* and its mutants.

254A **RNAseq and targeted metabolomics analyses implicate G protein signaling in regulation of arginine and ornithine metabolism and compartmentation in Neurospora crassa** Monique A Quinn[1], Alexander Carrillo[2], Lida Halilovic[2], Katherine Borkovich[1] [1]Microbiology & Plant Pathology, University of California, Riverside, [2]University of California, Riverside

Resistance to Inhibitors of Cholinesterase 8 (RIC8) functions as a non-receptor guanine nucleotide exchange factor (GEF) and protein chaperone for heterotrimeric Gα subunits. In *Neurospora crassa*, RIC8 acts as a GEF for the Gα subunits GNA-1 and GNA-3 in vitro and affects the stability of all three Gα subunits in vivo. The wild type, Δ*gna-1*, Δ*gna-3*, and Δ*ric8* strains represent a continuum of growth and asexual development, with wild type and Δ*gna-1* strains producing hyphae in submerged cultures, while Δ*gna-3* and Δ*ric8* mutants develop conidiophores, particularly in the Δ*ric8* mutant. Previous work in our laboratory showed that supplementation with peptone leads to a partial correction of the macroconidiation defect in Δ*gna-3* and Δ*ric8* mutants. This suggested a role for RIC8 in metabolism through G-protein signaling. Our laboratory performed RNAseq and targeted LC-MS on submerged cultures to analyze the Δ*gna-1*, Δ*gna-3*, and Δ*ric8* mutants for possible effects on metabolism in *N. crassa*. We observed that several metabolites involved in the arginine biosynthetic pathway were present at different levels in the Δ*ric8* and wild type strains. RNAseq analysis revealed that the genes encoding enzymes involved in arginine biosynthesis were not differentially expressed. This result suggested that the enzymes may be regulated at the post-transcriptional level. However, results from enzymatic assays for several pathway enzymes did not fully account for the differences between Δ*ric8* and wild type strains. Previous work in *N. crassa* has shown that some metabolites involved in arginine metabolism are present at lower levels in conidia compared to hyphae, and that the majority of the cellular arginine and certain other pathway metabolite pools are contained in the vacuole. We identified 11 putative vacuolar arginine transporters in *N. crassa* based on homology to known yeast genes. Five of these transporters were mis-regulated at the transcriptional level in the Δ*ric8* mutant. Utilizing ion exchange chromatography, we measured the arginine and ornithine pools in submerged cultures from *N. crassa* knockout mutants lacking these vacuolar amino acid transporters. Analysis of these pools allowed us to pinpoint decreased retention of arginine and ornithine in the vacuole as the most likely mechanism leading to the low levels of arginine and ornithine in macroconidia.

255A **The *Aspergillus nidulans sarB* gene encodes a putative N-acetylglucosamine transporter involved in amino acid utilization** Heather D Forster, Joel T Steyer, Sara M Hopkins, Richard B Todd Plant Pathology, Kansas State University

Nutrient acquisition is an essential and controlled process. In *Aspergillus nidulans*, the transcription factor AreA regulates nitrogen utilization genes. The *areA102* pleiotropic altered function mutation confers increased growth on histidine as a nitrogen source. The suppressor of *areA102* mutants *sarA* and *sarB* suppress this strong growth on histidine. The *sarA* gene (AN2350) is characterized and encodes an L-amino acid oxidase (LAAO), but *sarB* has remained unidentified. *sarB* was mapped to chromosome VII, 0.26 cM from *xprG*. As 1 cM usually represents ~5-10 kbp in *A. nidulans, sarB* was expected to lie within 2.6 kbp of *xprG*. This project aims to identify the *sarB* gene and understand its role in nitrogen acquisition. Nine candidate *sarB* genes adjacent to *xprG* failed to complement the *sarB7* phenotype in transformation experiments, suggesting that *sarB* is physically further away from *xprG* than predicted. We therefore adopted a Whole Genome Sequencing approach. The genomes of the *sarB7* mutant and its parent were sequenced and compared with the reference genome. Three SNPs on chromosome VII were unique to

the *sarB7* mutant. The closest SNP to *xprG* was ~118 kbp away in the uncharacterized gene AN8875. AN8875 and *xprG* are close to, but separated by, the centromere, consistent with the larger physical distance than expected. The SNP introduces a stop codon about one-third of the way through the gene, producing a truncated protein. To investigate if AN8875 is *sarB*, the wild-type AN8875 gene was transformed into the *areA102 sarB7* strain and transformants were obtained by direct selection for growth on histidine, indicating that AN8875 complemented the *sarB7* phenotype. To determine the phenotype of complete loss of AN8875, the gene was deleted. In a wild-type background, the AN8875Δ mutation showed no phenotype on histidine, but in an *areA102* background, it phenocopied the *sarB7* phenotype of weak growth on histidine. These experiments provide strong evidence that AN8875 is *sarB*. AN8875 encodes a putative N-acetylglucosamine (GlcNAc) transporter conserved in fungi. Its orthologs in *Saccharomyces cerevisiae* and *Hansenula polymorpha* are involved in cell wall chitin biosynthesis, and the *Kluyveromyces lactis* ortholog is involved in N-glycosylation. Because mutations in *sarA* and *sarB* produce the same phenotype, we hypothesize SarB is necessary for SarA LAAO function.

256A **Transcription factor Adr1 and its role in citrate utilization and gluconeogenesis in *C. albicans*** Amelia White, Aaron Mitchell Microbiology, University of Georgia

Opportunistic fungal pathogen *Candida albicans* often encounters host niches where it must survive on alternative carbon sources alone. In *Saccharomyces cerevisiae,* transcription factor Adr1 activates genes required for acetate and glycerol utilization. However, previous researchers found that Ca*ADR1* deletion mutants have robust growth on acetate and glycerol. We have found that Adr1 is required for growth on citrate and malate, two intermediates of the TCA cycle. This phenotype is manifested among multiple strains isolated from all known host niches. RNA sequencing and Nanostring analyses of *adr1Δ/Δ* and wild-type strains show that Adr1 is required for expression of genes within the TCA cycle and gluconeogenesis. Major targets include enzymes Mdh1 and Pck1. Both *mdh1*Δ/Δ and *pck1*Δ/Δ mutants are defective for growth on citrate similar to *adr1Δ/Δ* mutants, suggesting they are downstream of and activated via the Adr1 transcription factor. However, unlike *adr1Δ/Δ* and *pck1Δ/Δ* mutants, *mdh1*Δ/Δ mutants are able to grow on malate, suggesting that there may be a compensatory mechanism. Further investigation of CaAdr1 will allow for a better understanding of alternative carbon metabolism and its regulation within *C. albicans*

257A **Investigating the activation model of a wheat tandem kinase upon effector recognition from *Magnaporthe oryzae* pathotype *Triticum*** Yi-Chang Sung[1], Yinghui Li[1,2,3], Suji Baik[1], Tzion Fahima[4], Gitta Coaker[5] [1]University of California, Davis, [2]Triticeae Research Institute, Sichuan Agricultural University, [3]University of Haifa, [4]Institute of Evolution, University of Haifa, [5]Plant Pathology, University of California, Davis

Pathogens deliver effector proteins into plants to enhance their virulence and fitness. Some effector proteins are recognized by corresponding plant resistance proteins triggering immune responses. Here, we explored the relationship between the wheat tandem kinase RWT4 and the effector PWT4 from *Magnaporthe oryzae* pathotype *Tricitum* (MoT). RWT4 belongs to a newly identified resistance protein family with unique features of two fused kinase domains called Tandem Kinase Proteins (TKPs). Although TKPs are common across the plant kingdom, TKPs conferring disease resistance have only been identified in monocots and their activation mechanisms remain unclear. In this study, we developed a rice protoplast expression system to investigate TKP activation. In rice protoplasts, RWT4 specifically recognizes the AvrPWT4 effector, leading to immune responses including programmed cell death and activation of defense marker genes. The interaction between RWT4 and AvrPWT4 was validated through yeast two-hybrid and a recombinant protein-based pull-down assay. Furthermore, purified RWT4 is an active kinase and kinase activity is required for defense signaling. RWT4 can trans-phosphorylate AvrPWT4 but not unrecognized PWT4 alleles. Using Alphafold multimer modeling, we identified specific regions of RWT4 required for effector interaction and recognition. Collectively, these data demonstrate TKPs are a novel class of resistance proteins and the RWT4 TKP can directly recognize the AvrPWT4 effector to activate plant defense.

258A **Alternative ergosterol biosynthetic pathways in *Mucor lusitanicus* and their connection with antifungal resistance** Gabor Nagy[1,2], Bence Rafael[1,2], Kitti Bauer[1,2], Sandor Kiss-Vetrab[1,2], Bernadett Vago[1,2], Monika Varga[1], Csaba Vagvolgyi[1], Tamas Papp[1,3] [1]Dept of Microbiology, University of Szeged, [2]HUN-REN-SZTE Patomechanism of Fungal Infections Research Group, University of Szeged, [3]HUN-REN-SZTE Patomecahnism of Fungal Infections Research Group, University of Szeged

Several species of the order Mucorales can cause often fatal, systemic infections in immunosuppressed patients, known as mucormycosis. Infections are associated with high mortality rates and rapid clinical progression. Lipid formulations of amphotericin B are used for the first-choice therapy, while certain azoles (posaconazole, isavuconazole) are recommended as salvage therapy. Ergosterol, an important component of the fungal cell membrane, and its biosynthesis pathway are the most important antifungal drug targets.

The main goal of this study is to investigate the role of the *erg3* and *erg6* genes in the main and alternative ergosterol biosynthesis and antifungal resistance in *Mucor lusitanicus*.

The genome of *M. lusitanicus* encodes three sterol C-24 methyltransferase genes (*erg6*a, *erg6b,* and *erg6c*), and one sterol C-5 desaturase gene (*erg3*). Erg6 plays a role in growth at high temperature and virulence in *Cryptococcus neoformans* and it plays a role in the alternative ergosterol biosynthesis pathway in yeast. Mutation of Erg3 can influence the virulence and azole resistance in *Candia albicans*. We have created *erg3* and *erg6* knockout mutants using a CRISPR-Cas9 system. Growth ability, sporulation capacity, sterol content, virulence, and sensitivity to azoles and amphotericin B of the mutants were examined. Lack of *erg6b* resulted in decreased ergosterol and eburicol levels, while lanosterol, zymosterol, and 7-dehydrodesmosterol were significantly increased in the mutant strain. Deletion of *erg3* resulted in increased sensitivity to lower temperature and osmotic- and cell wall stressors. Ergosterol content significantly decreased in Δ*erg3* mutants as well. In *Galleria mellonella* larvae, virulence of the created mutants was tested. Lack of *erg6b* and *erg3* resulted in significantly decreased virulence of the tested mutants.

Sterol composition of *erg6* and *erg3* knockout mutants significantly altered compared to the control strains and revealed the presence of at least four alternative sterol biosynthesis pathways. Furthermore, *in silico* analysis of amphotericin B docking to ergosterol and ergosterol biosynthesis intermediates showed that amphotericin B can bind to the intermediates with the same affinity and energy as to the ergosterol.

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259A **A novel glycosyltransferase organizes glyogen and cell wall glucans in *Cryptococcus neoformans*** Liza Loza, Tamara Doering Washington University in St. Louis

The basidiomycete yeast *Cryptococcus neoformans* (Cn) is a devastating opportunistic pathogen, accounting for 15% of AIDS-associated mortality. In immunocompromised people, Cn can proliferate in the lungs and disseminate to cause meningoencephalitis.

Carbohydrate storage molecules are a conserved strategy to address carbon limitation, which Cn encounters in the environment and host. Glycogen is a glucose storage molecule comprised of a glycogenin protein core surrounded by α-1,4-linked glucose chains with α-1,6-linked branchpoints. A predicted glycogenin, Glg1, is required for normal pathogenesis. Deletion of *GLG1* or perturbation of its catalytic residue causes a glycogen defect, while its expression in a *S. cerevisiae* (Sc) strain lacking glycogenins rescues glycogen synthesis; together, these results confirm its identity.

A hypothetical glycosyltranferase that we call Glucan organizing enzyme (Goe1) has 35% amino acid similarity to Glg1. However, our studies show that it is not a second glycogenin and in fact has novel cellular roles. Deletion of *GOE1* results in reduced glycogen and mislocalization of glycogen rosettes. Interestingly, *goe1Δ* also displays aberrant cell wall morphology, likely due to the loss of alkali-insoluble β-1,3-glucan. Experiments are underway to investigate the biochemical activity of Goe1 and its possible interactions with other enzymes. Altogether, this work illuminates an unexplored connection between glycogen and cell wall synthesis.

260B **Rhythmic interaction between ZUOTIN and ribosomes may promote daily rhythms in protein folding and activity** Madhusree Gangopadhyay, Teresa Lamb, Deborah Bell-Pedersen Texas A&M University

Several ribosome-associated chaperones bind nascent polypeptide chains to mediate co-translational folding. Zuotin (ZUO) is a part of the ribosome-associated complex (RAC) that functions as a ribosome co-chaperone. In yeast cells, ~30% of ribosomes associate with the RAC, suggesting some level of specificity. Quantitative mass spectrometry across circadian time in *Neurospora crassa* revealed that the interaction between ZUO and ribosomes is clock-controlled. Thus, we hypothesized that the rhythmic ZUO/ribosome interaction may lead to rhythms in the folding of specific protein targets. Protein aggregation assays confirmed that ZUO promotes proper protein folding in *N. crassa*. Identification of misfolded proteins by mass spectrometry revealed that ZUO-dependent folding targets included copper transporters, and proteins involved in snRNA processing and mRNA catabolic process. Protein aggregates are more likely to form when the ZUO-ribosome interaction is low during the day under control of the clock, supporting that protein folding of select targets is clock-controlled and dependent on ZUO. Interestingly, while the interaction of

ZUO with ribosomes is clock-controlled, ZUO protein levels do not cycle. Thus, the daily rhythm in folding of specific protein targets, potentially including ZUO itself, likely contributes to protein activity rhythms in the absence of rhythmic abundance.

261B **Circadian clock regulation of translation fidelity through the methionyl-tRNA synthetase in *Neurospora crassa*** Griffin Best Texas A&M University

About half of the proteins synthesized in eukaryotic cells under control of the circadian clock arise from arrhythmic mRNAs, supporting a role for clock control of posttranscriptional mechanisms. In *Neurospora crassa*, the circadian clock controls rhythmic mRNA translation, in part, through regulation of the eIF2α kinase CPC-3 (the homolog of yeast and mammalian GCN2). CPC-3 phosphorylates and inactivates the translation initiation factor eIF2α during the subjective day, leading to rhythmic translation initiation of select mRNAs. We discovered that clock control of CPC-3 activity requires the rhythmic charging of $tRNA^{val}$ and rhythmic levels of the valyl-tRNA synthetase (ValRS). These data supported that circadian rhythms in tRNA synthetases (RS) lead to rhythms in the ratio of charged versus uncharged tRNAs that drive rhythmic CPC-3 activity, P-eIF2α levels, and translation. To determine if rhythmic CPC-3 regulation is specific to ValRS, we examined five other RS's: MetRS, PheRS, GlnRS, LeuRS and AspRS, and found that they are clock-controlled and peak in the subjective night. We were particularly interested in MetRS, because MetRS is phosphorylated by clock-controlled MAP kinases, ERK1 and ERK2, when mammalian cells are exposed to oxidative stress. When MetRS is phosphorylated, it's specificity for $tRNA^{met}$ is reduced, and this leads to methionylation of non-cognate tRNAs (Met-misacylation) and Met misincorporation into polypeptides. We hypothesized that rhythmic phosphorylation of MetRS by ERK1/2 drives daily rhythms in Met misincorporation. Consistent with this hypothesis, we show that Met misincorporation into an mCherry reporter is clock-controlled. Met can sequester reactive oxygen species (ROS) that accumulates during oxidative stress. We are testing the intriguing idea that clock control of Met misincorporation in polypeptides not only produces protein variants beyond what is encoded in the genome, but also protects the organism from circadian oscillations in ROS levels.

262B **Elucidating cryptococcal capsule synthesis through proximity labeling methods** Daphne Ko, Tamara L Doering Molecular Microbiology, Washington University in St. Louis

Cryptococcus neoformans is an opportunistic fungal pathogen that causes over 150,000 cases of cryptococcal meningitis annually. *C. neoformans* is unique from other fungal pathogens in being surrounded by a polysaccharide capsule that is essential for virulence. The capsule is composed of two large polysaccharides: glucuronoxylomannan and glucuronoxylomannogalactan. While the structures of these two polysaccharides are well-known, their synthetic pathways are largely uncharacterized. Glycosyltransferases (GTs) are enzymes that transfer glycan moieties from nucleotide sugar donors to glycosyl acceptor molecules and are key for capsule synthesis. To date, only one GT has been shown to act in capsule synthesis: Cryptococcal xylosyltransferase1 (Cxt1). Other GTs predicted to be involved in capsule synthesis have been difficult to enzymatically characterize. We hypothesize that Cxt1 works in concert with other GTs to form capsule. To identify these potential interactors, and possibly identify novel glycoactive enzymes, we are using proximity-dependent biotinylation. Fusing a promiscuous biotin ligase (TurboID) to Cxt1 should cause biotinylation of nearby proteins under appropriate conditions. We have generated a *C. neoformans* strain expressing Cxt1-TurboID and performed streptavidin pulldowns of biotinylated proteins followed by mass spectrometry to identify Cxt1-interacting proteins. Initial results yielded broad classes of proteins localized to varied cellular compartments. Currently, we are refining our protocol to enrich for membrane-associated proteins to increase the likelihood of detecting Golgi-localized glycoactive enzymes. We will then prioritize putative interactors based on function and degree of enrichment for further study. We hope to discover novel glycosyltransferases or other glycoactive enzymes to further elucidate the mechanism of capsule synthesis.

263B **The heat shock transcription factor HsfA plays a role in membrane lipids biosynthesis connecting thermotolerance and unsaturated fatty acid metabolism in *Aspergillus fumigatus*** Jonatas Erick Maimoni Campanella[1], Joao Henrique Tadini Marilhano Fabri[1], Caroline Mota Fernandes[2], Marina Campos Rocha[1], Gilberto Yanes[1], Anderson Ferreira da Cunha[1], Maurizio Del Poeta[2], Iran Malavazi[1] [1]Genetics and Evolution, Federal University of Sao Carlos, [2]Microbiology and Immunology, Stony Brook University

Thermotolerance is a remarkable virulence attribute of *Aspergillus fumigatus,* but the consequences of heat shock (HS) to the cell membrane of the fungal cells are unknown, although this structure is one of the first to detect changes in ambient temperature. In general, HS imposes an adaptative response to the fungal cell *via* the activation of heat shock transcription factors and chaperones to protect the cell against heat damage. Concomitantly, the membrane adapts to heat and maintains the physical-chemical properties, such as the balance between saturated/unsaturated fatty acids and fluidity. In yeast, a lower accumulation of phospholipids with unsaturated fatty acid (FA) chains occurs in response to HS, directly affecting plasma membrane composition

and physiological responses against stress conditions. The synthesis of unsaturated fatty acids is catalyzed by Δ9-fatty acid desaturases, whose expression is temperature-modulated, highlighting a connection between HS response and plasma membrane lipids composition. Here, we aimed to understand the relationship between HS and saturated/unsaturated FA balance in membrane lipids in the thermophile *A. fumigatus*. To achieve this goal, we combined biochemical and genetic approaches, such as lipid quantification by mass spectrometry, protein co-immunoprecipitation and Western blot, RT-qPCR, and confocal fluorescence microscopy. Here, we show that HsfA responds to plasma membrane stress and has a role in the biosynthesis of unsaturated sphingolipids and phospholipids. Besides, we reported that the *A. fumigatus* Δ9-fatty acid desaturase *sdeA is* an essential gene required for unsaturated FA biosynthesis, although it did not directly affect the total levels of phospholipids and sphingolipids. We also demonstrate that the *hsfA* controls *sdeA* expression while SdeA is an Hsp90 client protein. Also, we show that the SdeA::GFP is an endoplasmic reticulum protein that significantly changed its localization in response to antifungals. Additionally, *sdeA* depletion significantly sensitizes mature *A. fumigatus* biofilms to caspofungin. HsfA is required for the adaptation of the fungal plasma membrane to HS and points out a close relationship between thermotolerance and FA metabolism in *A. fumigatus*. We argue that the SdeA/Hsp90 regulatory circuitry interference can be further exploited as a promising antifungal target.

264B **Exploring effect of Ethyl 3-methylbutanoate on fumonisin production and *FUM* gene expression in *Fusarium verticillioides*** Antonia Susca[1], Alessandra Villani[2], Laurie Josselin[3], Vincenzo Lippolis[2], Salvatore Cervellieri[4], Thomas Netti[5], Daria Carella[1], Robert H Proctor[6], Antonio Moretti[1] [1]Institute of Sciences of Food Production (ISPA), National Research Council of Italy, [2]National Research Council of Italy, [3]Gembloux Agro-Bio Tech, Liege University, [4]Institute of Sciences of Food Production (ISPA, National Research Council of Italy, [5]Institute of Sciences of Food Production, National Research Council of Italy, [6]United States Dept of Agriculture, Agriculture Research Service, National Center for Agricultural Utilization Research
Volatile organic compounds (VOCs) are secondary metabolites emitted by organisms and play a role during interactions with other organisms. In the context of fungi, VOCs can serve various purposes, including intraspecies communication, attraction or repulsion of other organisms, and regulation of growth and development. *Fusarium verticillioides* is a major fungal pathogen of maize and produces fumonisins, mycotoxins of worldwide concern to food and feed safety. Therefore, developing innovative control strategies is essential to reduce the negative impacts of fumonisin contamination in maize. Recently, we found that the VOC ethyl-3-methylbutanoate (E3MB) is emitted by fumonisin-nonproducing mutants of *F. verticillioides* but not by their wild-type progenitor strain. To study the potential of E3MB as a fumonisin inhibitor and understand its mode of action, fumonisins and *FUM* gene expression were monitored in cultures of the mutant and wild type following exposure to E3MB. Two application modes were investigated: a *contact* condition, where E3MB was introduced into the substrate, and a *non-contact* condition, where E3MB was only introduced into headspace of cultures. Although E3MB inhibited fumonisin production in the wild type under both conditions, its effects on *FUM* gene expression varied significantly depending on the application mode. In the *contact* condition, *FUM* genes were overexpressed, while in the *non-contact* condition, most *FUM* genes were down-regulated. These results suggest that E3MB inhibits fumonisin production by different mechanisms in the different application modes. These findings provide insights for potential biocontrol strategies against fumonisin contamination caused by *F. verticillioides*. However, additional investigations, including epigenetic analysis, are needed to fully understand the mechanism by which E3MB inhibits fumonisin production.

265B **FuNTAP: A *Fusarium graminearum* Protein Interaction Network of Trichothecene Biosynthesis Pathways** Gopal Subramaniam[1], Armand Mirmiran[2], Nilesh Kumar[3], Chris Blackman[1,2], Margaret Balcerzak[1], Amanda Sproule[1], Tom Witte[1], Maryam Nourimand[1], David Overy[1], Chris Rampitsch[1], Shahid Mukhtar[3], Darrell Desveaux[2] [1]Agriculture Canada, [2]University of Toronto, [3]Univesity of Alabama

The activation of biosynthetic gene clusters (BGCs) in fungi requires coordination and integration of many environmental cues. As an example, *Fusarium graminearum*, the main causal agent of the Fusarium head blight disease (FHB) in wheat produces an array of secondary metabolites including the mycotoxin deoxynivalenol (DON) that are regulated by carbon and nitrogen sources and the physiological status of the cell. To understand the contribution of many input sources that regulate BGC in *F. graminearum*, we undertook a protein interaction network analysis in yeast of ~ 300 *F. graminearum* proteins that are involved directly or indirectly in the regulation of DON. The small-scale network identified protein hubs not only involved in DON regulation but also in other biosynthetic pathways. Genetic analysis, combined with metabolomic profiling provided corroborative evidence. Finally, the expression of one of the major hubs tagged with TurboID in the fungus validated our supposition that major hubs exert their control by interacting with various proteins, temporally and spatially.

266B **Modulating lipid composition in the filamentous fungus *Ashbya gossypii* alters assembly of the septin cytoskeleton** Brandy N Curtis[1], Amy S Gladfelter[2] [1]Cell Biology, Duke University, [2]Duke University

The properties of the plasma membrane (PM) are carefully modulated during cellular processes and cell stress by adjusting thousands of types of lipid molecules. Lipids with defined chemical properties are used to localize and organize many membrane proteins involved in cell signaling. Septins are a class of membrane-binding cytoskeletal proteins often at regions of membrane curvature where cells undergo changes in shape, e.g. during cytokinesis or polarized growth. Septins assemble into distinct arrangements of septin filaments to scaffold cell signaling factors that facilitate cell shape changes in both space and time. While negatively charged lipids are required to recruit septins, septin assemblies are likely anchored to the membrane through an amphipathic helix (AH) domain. The AH is also required to localize septins to convex membrane curvature, presumably by embedding into lipid "packing defects". Lipid packing defects are transient pockets where the hydrophobic core of the membrane is exposed. Defects arise when the membrane bends but are also induced by lipid composition. By changing the fatty acid tail saturation in simple membrane systems in vitro, we can control the size and number of packing defects and measure septin binding. When there are few packing defects, i.e. the membrane is "tightly" packed, septin binding is occluded on flat lipid bilayers, whereas when the membrane is "loosely" packed (many larger defects) septins bind robustly. Interestingly, tightly packed lipid compositions reduce, but do not fully prevent, septin binding on convexly curved membranes, in-line with their ability to sense curvature. To assess whether the property of lipid packing impacts septin assembly in cells, we moved into the filamentous fungus *Ashbya gossypii* (Ag). Ag has a common pool of septins that is separated into distinct assemblies both at curved and flat regions of the PM. By treating Ag with a fatty acid synthase inhibitor and adding saturated or unsaturated fatty acids to growth media, we can change the lipid packing of the PM and observe septin assemblies. After acute treatments with saturated fatty acids, septins are lost at flat regions of the PM but are preserved on curved regions, in direct alignment with in vitro experiments. This work indicates that septins are sensitive to lipid packing both in vitro and in cells, and that modulating lipid composition could be a mechanism to localize septins in vivo.

267B **Galactose growth in budding yeast species lacking the canonical *GAL* pathway** Emily J Ubbelohde[1], Marie-Claire Harrison[2], Antonis Rokas[2], Chris T Hittinger[1,3] [1]Laboratory of Genetics, University of Wisconsin - Madison, [2]Dept of Biological Sciences, Vanderbilt University, [3]Wisconsin Energy Institute, University of Wisconsin - Madison

Galactose catabolism via the *GAL* (Leloir) pathway in the yeast species *Saccharomyces cerevisiae* is a classic model used to explore gene regulation, metabolic control, and pathway evolution driven by environmental adaptation. The *GAL* pathway is widely recognized as the only galactose catabolism pathway in *S. cerevisiae* and other budding yeast species. With the help of machine learning, we have discovered several yeast species that do not possess any of the *GAL* genes, yet have been functionally confirmed to metabolize galactose. This discovery led us to ask several questions: what genes are responsible for regulating and encoding the proteins involved in galactose catabolism in these yeast species, how did this novel pathway evolve, or alternatively, has this pathway been lost in species possessing the *GAL* pathway? The identification of core genes involved in this pathway will lay the foundation to answer these evolutionary questions. Here, I explore the hypothesis that galactose catabolism proceeds via an oxidoreductive pathway.

A decrease in the concentration of extracellular galactose was observed and confirmed using HPLC for nine species. In three of those species, I performed enzymatic assays to investigate whether NADPH-dependent enzymatic activity was occurring in cells grown on galactose. When galactose was added as the sole substrate, a decrease in NADPH was observed over time, indicating that NADPH was being oxidized to NADP+. This observation further supports the hypothesis that galactose catabolism is proceeding via an oxidoreductive pathway. Because the genes and enzymes responsible for this pathway's proteins and regulation remain unknown, I am also gathering transcriptomic data for these three species in three growth conditions to identify candidate genes for future functional experiments.

This putative NADPH-dependent enzyme may be the first step in a galactose metabolism pathway like the oxidoreductive galactose pathway found in some filamentous fungi. Alternatively, this enzyme could be involved in a novel pathway which has yet to be described. Regardless, yeasts possessing this newly discovered pathway provide us with a unique opportunity to further explore potential candidates for metabolic engineering for rare sugar production and possible convergent evolution events not previously described.

268B **Feedback regulation of secondary metabolite production via a G-protein coupled receptor adjusting several metabolic pathways** Wolfgang Hinterdobler[1,2], Miriam Schalamun[2], Sabrina Beier[2], Jana Krautloher[2], Hoda Bazafkan[2], Monika Schmoll[2,3] [1]MyPilz GmbH, [2]Bioresources, AIT Austrian Institute of Technology GmbH, [3]Dept of Microbiology and Ecosystem Science, University of Vienna

Fungi live in highly complex habitats where they have to acquire nutrients, efficiently reproduce and fend off foes to compete and survive. Balancing resource distribution among these tasks requires delicate adjustment of diverse metabolic pathways to the given environment, which is sensed by G-protein coupled receptors (GPCRs).

We previously showed concentration-gated glucose and plant sensing as well as regulation of posttranscriptional cellulase expression by the GPCR CSG1 in the ascomycete *T. reesei*. Deletion of *csg1* abolishes transcription of another GPCR-encoding gene. Hence lack of glucose sensing causes shifts in environmental sensing. This GPCR, GPR16 is required for cellulase production in darkness and for proper sexual development. Transcriptome analyses revealed extensive alterations in secondary metabolism and carbohydrate metabolism under both conditions. Additionally, we detected considerable sorbicillin overproduction and self-inhibition with a deletion mutant of *gpr16*. Testing extracts from this mutant, we found that inhibition is dependent on the presence of the biosynthetic genes of the SOR cluster, which is responsible for sorbicillin production. The compound sensed by GPR16 is hence required for limiting metabolite production once a certain level is reached, which hints at feedback via GPR16-mediated sensing and is reminiscent of quorum sensing.

We conclude that GPR16 is required for balancing secondary metabolism with enzyme production and development for optimal resource distribution adjusted to the environment.

269B **Are the type strains of Aspergillus oryzae and A. sojae truly domesticated?** Jens C Frisvad[1], Jos Houbraken[2], Giancarlo Perrone[3], Massimo Ferrara[4], Kristian Barrett[5], Jakob B Nielsen[5], Thomas O Larsen[5], Lene Lange[6] [1]DTU-Bioengineering, Technical University of Danmark, [2]Food and Indoor Mycology, Westerdijk Fungal Diversity Institute, [3]Institute of Sciences of Food Production, [4]massimo.ferrara@, Institute of Sciences of Food Production, [5]DTU-Bioengineering, [6]LL-Bioeconomy

Aspergillus oryzae is one of the most important species used in biotechnology, in R&D as transformation host and for industrial enzymes as production host; and in addition, as a production organism in use for production of soy sauce, miso, shoyu and many other foods and beverages. *A. oryzae* is regarded as a domesticated form of the producer of the mycotoxins aflatoxins, cyclopiazonic acid and 3-nitropropionic acid, *A. flavus*. Domesticated forms in the major genera *Aspergillus* and *Penicillium* have rarely been accepted as species, the few examples being *A. oryzae*, *A. sojae*, *Penicillium camemberti* and *Penicillium caseifulvum*. *A. oryzae* has often been separated from *A. flavus* by production of aflatoxins in the latter, and inability to produce aflatoxin in the former. Paradoxically, the ex-type culture of *A. flavus* does not produce aflatoxins, while the ex-type culture of *A. oryzae* can produce aflatoxins. The presently selected ex-neotype of *A. oryzae* produces sclerotia, indicating that the strain originates from nature and that it is not domesticated. A new ex-neotype of *A. oryzae* should thus be selected, one that is genuinely domesticated. We have compared cultures identified as *A. oryzae*, *A. flavus* and *A. aflatoxiformans*, regarding production of specialized metabolites and CAZymes, to find distinguishing characters between those three important taxa. The first genome sequenced strain of *A. oryzae*, RIB40 and an "industrial strain" IFO 4177 are also producing sclerotia, similar to *A. oryzae* as now neo-typified, while real domesticated *A. oryzae* strains do not produce sclerotia, aspergillic acid and aflatoxins. Nearly all strains of *A. flavus sensu stricto* and *A. aflatoxiformans* produce aspergillic acid, and often aflatoxins, while *A. flavus sensu stricto* produces the species-specific compound flavimin. Recently, a similar behavior has been observed also in the ex-type culture of *A. sojae* (CBS 100928) which resulted in detection of aflatoxin B1, also this domesticated species needs further insight and a possible retyping of the original ex type strain.

270B **Unraveling *Cryptococcus neoformans* Metabolic Adaptations: Implications for Therapeutic Targets** Arohi Singhal Genetics and Biochemistry, Clemson University

Cryptococcus neoformans, the leading cause of fungal meningitis, claims over 200,000 lives annually despite treatments like amphotericin-B and 5-fluorocytosine. As an opportunistic pathogen found in the soil and in decaying material, it enters the human body via lung inhalation, adapting to nutrient shifts and utilizing alternative carbon sources like acetate. L-carnitine is required for the transport of activated acetyl/acyl units into mitochondria. Carnitine biosynthesis in *Cryptococcus* involves a series of four enzymatic reactions. Notably, deletion mutants of genes encoding the first, third, and fourth steps exhibit defects in melanin production—a pigment crucial for countering oxidative stress encountered during infection. However, the genes encoding hydroxytrimethyllysine aldolase (HTMLA), the enzyme involved at the second step, are currently unknown. CNAG_02851 is a

promising candidate gene because (1) it encodes threonine aldolase, which catalyzes pyridoxal phosphate-dependent condensation between amino acids (principally glycine) and aldehydes such as acetaldehyde, and (2) it is homologically identical to the gene, *Orf19.6305*, which encodes HTMLA in *Candida albicans*. To address this hypothesis, we have generated CNAG_02851 knockout using the TRACE (Transient CRISPR-Cas9 Coupled with Electroporation) method. We are currently studying the effects of this knockout on cell growth with various carbon sources, virulence factors, like melanin production and capsule formation.

Metabolic pathways in *C. neoformans* are not only vital for assimilating nutrition and growth but also play a crucial role in resisting host immune attacks. An intriguing aspect of the fungus's metabolic flexibility lies in its ability to synthesize arginine de novo. Arginine, a crucial amino acid involved in various cellular processes, is synthesized through a functional arginine biosynthesis pathway. The involvement of glutamate N-acetyltransferase (Arg7, CNAG_01238), acetylornithine transaminase (Arg8, CNAG_05134), and arginosuccinate synthase (Arg1, CNAG_00930) in arginine biosynthesis were investigated. Even though *ARG7* is essential for growth on minimal media lacking arginine, *arg8Δ* and *arg1Δ* mutants were able to thrive. *Arg7Δ* mutants have virulence factor deficiencies that can be corrected by supplementing with arginine.

Understanding arginine and carnitine pathways unveils promising therapeutic targets, disrupting fungal adaptability and laying the foundation for targeted antifungal drug development. This exploration enhances strategies against cryptococcal infections, contributing to improved patient outcomes.

271B **Oryzapsin, orthologs of yeast yapsin in *Aspergillus oryzae*, are involved in ergosterol biosynthesis** Natsuno Shimizu[1], Tamaki Katagiri[1], Akira Matsumoto[1], Yoshihiko Matsuda[2], Hiroshi Arai[1], Nobumitsu Sasaki[1], Keietsu Abe[2], Toru Katase[3], Hiroki Ishida[4], Ken-Ichi Kusumoto[5], Michio Takeuchi[1], Youhei Yamagata[1] [1]Tokyo University of A & T, [2]Tohoku University, [3]Amano Enzyme Inc., [4]Gekkeikan Sake Co., Ltd., [5]Osaka University

The oryzapsin genes *opsA* and *opsB* in *Aspergillus oryzae* encoding glycosylphosphatidylinositol (GPI)-anchored aspartic endopeptidase are homologs of *Saccharomyces cerevisiae* yapsins. However, the profiles and roles of the proteins encoded by these genes have not yet been clarified. First, we produced *opsA* and *opsB*-overexpression strains and performed enzymatic analyses, revealing that OpsA and OpsB can attack sites other than the carboxyl-terminal peptide bonds of basic amino acids. Second, *opsA* and *opsB* single-deletion and double-deletion strains (ΔopsA, ΔopsB, and ΔopsAΔopsB) were constructed to explore the expected roles of oryzapsins in cell wall synthesis, similar to the role of yapsins. The transcription level of *mpkA* in the cell wall integrity pathway was increased in ΔopsB and ΔopsAΔopsB strains, suggesting that OpsB might be involved in processing cell wall synthesis-related proteins. Treatment with an ergosterol biosynthesis inhibitor reduced the growth of the ΔopsAΔopsB strain. Moreover, the mRNA levels of A*oerg1*, A*oerg3-1*, A*oerg3-2*, A*oerg7b*, A*oerg11*, and A*ohmg1,2* showed a decreasing tendency in the ΔopsAΔopsB strain, and the ergosterol content in the membrane was reduced in the ΔopsAΔopsB strain. These results suggest that oryzapsins play roles in the formation of cell walls and cell membranes, especially ergosterol biosynthesis.

272B **The CakA kinase links the cell cycle with secondary metabolism in *Aspergillus nidulans*** Zhiqiang Dong[1], Agnieszka Gacek-Matthews[2], Franz Zehetbauer[2], Niranjan Shirgaonkar[1], Kaeling Tan[1], Chris Koon Ho Wong[1], David Cánovas[3], Joseph Strauss[4] [1]Faculty of Health Sciences, University of Macau, [2]Institute of Microbial Genetics, University of Natural Resources and Life Sciences, [3]Dept of Genetics, University of Sevilla, [4]Dept of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU)

Cell cycle control is indispensable for the growth and development of all organisms, governing many crucial cellular functions including cell proliferation, differentiation, and cell homeostasis. In *Saccharomyces cerevisiae*, the mitotic and meiotic cell cycles are regulated by Cdc28, the catalytic subunit of the main cyclin-dependent kinase (CDK1). CDK1 is activated by cyclins and the Cyclin-dependent kinase-activating kinase (Cak1). The *CAK1* gene is conserved among fungi and is essential for *S. cerevisiae* (Espinoza *et al*., 1998). Interestingly, the *A. nidulans CAK1* ortholog (*cakA*) is not essential, suggesting the existence of redundant kinase(s) or a different function for CakA. Here, we study the global role(s) of CakA using a proteomic and functional genomics approach. BioID analysis (proximity-dependent biotin identification) showed that CakA interacts with the Cdc7 orthologue (AN3450), which is another essential kinase involved in the regulation of the cell cycle (De Souza *et al*., 2014). Consistent with this observation, transcription profiling analysis of the *cakA*Δ mutant showed that CakA affects expression of genes involving in growth, development, cell cycle and response to stresses. In addition, our result also revealed a role of CakA in regulation of secondary metabolite biosynthesis gene clusters (BGCs). We have found several downstream transcription factors of secondary metabolism upregulated in the *cakA*Δ mutant. To test whether interference with cell cycle progression generally affects secondary metabolism, we inhibited the cell cycle using a genetic approach with the temperature-sensitive mutant alleles of the cell cycle regulators *nimX*^{cdc28} and *bimE*^{apc1} and a pharmacological approach with hydroxyurea or torin 1. Transcriptional and metabolic HPLC

MS/MS analysis of cell cycle mutants and HU/Torin1-treated wild type cells revealed drastic changes in SM profiles and associated BGC transcription. Moreover, cyclins are differentially expressed between BGC-silencing and BGC activating conditions and this transcriptional program changes in some tested kinase mutants. Therefore, our work demonstrates that the cell cycle can influence secondary metabolism in *A. nidulans*.

273B **Sulfur metabolism-mediated fungal glutathione biosynthesis is essential for oxidative stress resistance and pathogenicity in the plant pathogenic fungus *Fusarium graminearum*** Jiyeun Park[1], Jae Woo Han[2], Nahyun Lee[3], Sieun Kim[1], Soyoung Choi[1], Hyun-Hee Lee[4], Jung-Eun Kim[1], Young-Su Seo[4], Gyung Ja Choi[2], Yin-Won Lee[1], Hun Kim[2], Hokyoung Son[1] [1]Dept of Agricultural Biotechnology, Seoul National University, [2]Korea Research Institute of Chemical Technology, [3]Seoul National University, [4]Pusan National University

The oxidative stress response is required for plant pathogens to endure host-derived oxidative stress during infection. Previously, we identified the eight transcription factors (TFs) involved in the oxidative stress response in the plant pathogenic fungus *Fusarium graminearum* and found that of these TFs, the deletion of *FgbZIP007* caused hypersensitivity to oxidative stress. However, the underlying mechanisms of Fgbzip007 are not fully understood. Based on chromatin immunoprecipitation followed by sequencing (ChIP-seq) analysis, we found the regulons of Fgbzip007, and further genetic studies demonstrated that Fgbzip007 is a key regulator for sulfur assimilation. The deletion strains of *FgbZIP007* and its regulons exhibited low level of glutathione biosynthesis, which led to characterize glutathione biosynthesis. Fgbzip007-mediated sulfur assimilation is required for glutathione biosynthesis, which is essential for oxidative stress resistance and pathogenicity in *F. graminearum*. Although the reduced resistance of glutathione-deficient mutants against oxidative stress was restored by overexpression of *FCA7*, encoding a core peroxidase, but not on pathogenicity, suggesting that glutathione in pathogenesis is independent of antioxidant properties. This study characterized the function of genes of glutathione biosynthesis, provides specific insight into how Fgbzip007 regulates pathogenesis in *F. graminearum*, and establishes a genetic framework for the molecular dissection of a TF Fgbzip007 with the integration of pathogen responses to oxidative stress.

274B **Molecular Insights into Glycerol Transport in *Neurospora crassa*** Basant Ibrahim Abdelaziz Elsayed Nada, Elisabeth Tamayo, J. Philipp Benz Fungal Biotechnology in Wood Science, Technical University of Munich

Glycerol transport in fungi is fundamental to cellular physiology, serving as a critical component in both metabolic processes and stress adaptation. Fungi, encountering diverse environmental conditions, leverage glycerol as a versatile molecule essential for osmoregulation. Glycerol uptake across cellular membranes is mediated by specific proteins, including aquaporins and glycerol transporters, which thereby contribute significantly to the maintenance of cellular homeostasis. Distinctively, the filamentous fungus *Neurospora crassa* possesses only a single predicted protein from within the aquaporin family, NCU08052. To elucidate the mechanisms of glycerol transport in *N. crassa*, our study focused on this putative aquaporin and the glycerol:H^+ symporter NCU02591. Our results signified a marked reduction in glycerol uptake in single mutant deletion strains of those genes under optimal gene induction conditions, as compared to the wild type. This reduction was verified by observed growth defects on glycerol as a sole carbon source. Furthermore, we explored the transport capabilities of these proteins for other sugar alcohols to ascertain their substrate specificity. A significantly reduced growth of the single mutant Δ*NCU08052* compared to the wild type indicated that NCU08052 could be also responsible for the transport of some other polyols such as xylitol, arabitol and myoinositol. To evaluate the relationship between glycerol transport and osmoregulation, Δ*NCU08052* and Δ*NCU02591* knockout strains were subjected to high salt concentrations using solid and liquid growth assays. Both knockout strains displayed a growth defect under salt stress compared to the wild type, especially in liquid cultures, emphasizing the intricate connection between glycerol transport and fungal response to osmotic stress.

275B
An essential telomere binding protein regulating the transition from primary to secondary metabolism in *Aspergillus nidulans* Shuhui Guo, Xiaofeng Liu, Lakhansing Pardeshi, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau

Fungi can produce a wide range of diverse secondary metabolites (SMs). These SMs are synthesized by specialized enzymes encoded by genes that are often organized into clusters located near the telomeres of chromosomes. The sub-telomeric arrangement is believed to facilitate gene silencing, preventing secondary metabolism during the active growth stage when the cell primarily focuses on primary metabolism for energy and macromolecule biosynthesis. Under nutrient limitation or during the stationary growth phase, the cell undergoes a transition from primary to secondary metabolism, producing SMs that are crucial for fungal survival in specific environmental niches. Although various global regulators of secondary metabolism and SM cluster-specific transcription factors have been identified, the molecular mechanism underlying the coordination between primary and

secondary metabolisms remains unclear. In this study, we have identified a hitherto uncharacterized protein that binds to telomeres as well as numerous secondary metabolism genes of different SM biosynthetic gene clusters in *Aspergillus nidulans*. Transcription profiling analysis reveals the protein's function as a transcriptional activator for the secondary metabolism genes. Interestingly, this protein also negatively regulates gene functions that are essential for the active growth stage, such as primary carbon and nitrogen metabolism, energy production, and nucleotide metabolism. Based on these findings, we propose that the telomere binding protein acts as a molecular switch, regulating the transition from primary to secondary metabolism.

276C **Characterization of annularins in the filamentous fungus *Podospora anserina* by an interdisciplinary study** Xiaoyue Peng[1], Thomas Gaslonde[2], Eva Cabet[1], Xavier Cachet[2], Florence Chapeland-Leclerc[1], Gwenael Ruprich-Robert[1] [1]LIED Universite Paris Cite, [2]Citcom Universite Paris Cite

Podospora anserina is an efficient laboratory model ascomycete with a large reservoir of secondary metabolites (SMs) encoded by at least 43 biosynthetic gene clusters (BGCs). Numerous in-depth studies have been carried out on development, senescence and hyphal interference. On the other hand, chemical studies have been much less numerous, leaving the potential for chemical diversity unexploited and the contribution of SMs during the life cycle of P. anserina uncertain. With the whole genome sequenced and annotated, the transformation process known, the *PaStcA*, *PaVvd* and *PaNsdD* genes have been characterized and their implications in the sterigmagtocystin biosynthetic pathway elucidated. During these studies, a small molecule, annularin F, was isolated. This molecule, possessing an alpha-pyrone core, belongs to the family of polyketide molecules. Using an interdisciplinary approach between molecular genetic biology and natural product chemistry, the annularin BGC was searched. The presence of methoxy substituents on the molecule enabled us to narrow down the number of candidates to 4 BGCs with putative methyltransferases. The 4 targeted PKS gene inactivation mutants were then constructed. LC-MS analyses of extracts from these mutants were compared with those from the wild-type strain and revealed that there was no longer any identifiable annularin F from the culture of one deleted mutant. This BGC was therefore a good candidate for annularin biosynthesis in *P. anserina*. This was confirmed by the restoration of compound production in the complemented strain. Furthermore, a significant increase in annularin F was observed in a strain overexpressing the gene encoding a putative BGC transcription factor, by placing it under the control of the strong promoter of the *AS4* gene. This confirmed the role of this transcription factor in BGC. In addition, annularin analogues accumulated progressively while annularin F decreased in this overexpression strain as incubation time lengthened, indicating a biodegradation or biotransformation process during the life cycle of this strain. On the other hand, to elucidate the annularin F biological pathway, we attempted to annotate the functions of the adapting enzymes by constructing deletion mutants of the other BGC genes. We thus characterized the gene encoding cytochrome P450 leading to the accumulation of the annularin D precursor and providing additional elements to decipher the annularin biological pathway.

277C **On the role of natural products as virulence factors in fungi with a predatory lifestyle** Maria C. Stroe[1], Xi Yu[2], Xiaodi C. Hu[1], Reinhard C. Fischer[1] [1]Institute for Applied Microbiology, Karlsruhe Institute for Technology, [2]Karlsruhe Institute for Technology

An intriguing and fascinating inter-kingdom interaction occurs between nematodes and fungi that trap and prey on them. Nematode-trapping fungi are soil microbes which can switch from a saprotrophic lifestyle to a predatory behavior in the presence of nematodes[1,2]. Among them, *Arthrobotrys flagrans* is a typical member of the soil microbiome and is able to form adhesive, three-dimensional trap networks to catch nematodes. Using *Caenorhabditis elegans* as model, we previously showed that secondary metabolites produced by *A. flagrans* are crucial for attracting the nematodes into fungal colonies and traps and for controlling trap formation, together with nematode-derived ascarosides[3]. Bioinformatic analysis has revealed that *A. flagrans* only encodes 3 PKSs, 3 NRPSs and 3 NRPS-like biosynthetic gene clusters, which is a relatively small collection of clusters compared to other fungi. Therefore, the *A. flagrans* - *C. elegans* interaction offers the perfect system and opportunity to elucidate the entire secondary metabolism of *A. flagrans,* as well as the importance of the produced compounds for nematode predation. Here we focus on the two uncharacterized PKS-encoding genes of *A. flagrans*. We show that the two genes are expressed exclusively in the fungal traps and in trophic hyphae inside the nematode. Chemical analysis revealed that the two PKSs work in a concerted fashion and biosynthesize volatile natural products with synergistic effects, increasing the attraction of nematodes into the hyphal traps. The molecular nematode targets are currently under investigation.

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278C **Accessory chromosomes are reservoirs of unique secondary metabolite biodiversity in *Fusarium poae*** Thomas Witte[1,2], Linda J. Harris[2], Jason Ma[1], Anne Hermans[2], Amanda Sproule[2], Anne Johnston[2], Michael Darnowski[1], Whynn Bosnich[3], Danielle Schneiderman[3], Hai D.T. Nguyen[3], Xiben Wang[4], Christopher N. Boddy[1], David P. Overy[2] [1]Chemistry and Biomolecular Sciences, University of Ottawa, [2]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, [3]ORDC, Agriculture and Agri-Food Canada, [4]Morden Research and Development Centre, Agriculture and Agri-Food Canada

Fusarium poae is of concern to plant pathologists as it is globally distributed, frequently detected in surveys of *Fusarium* head blight-symptomatic crops, and threatens consumers by the production of harmful mycotoxins. Recently, genomic and metabolomic profiling of *F. poae* strains revealed the presence of small chromosomes with structural features and genetic content suggestive of rapidly evolving 'accessory' genomes. Termed 'supernumerary' or 'accessory' chromosomes, these small chromosomes harbour strain-specific genetic content including transcriptionally active secondary metabolite biosynthetic gene clusters (BGCs), high numbers of transposable elements and frequent gene duplications or disruptions. The presence of accessory chromosomes with active BGCs provides a promising area for metabolomic screening projects to discover new and rapidly evolving fungal chemistry within a population.

Recent whole-genome sequencing and untargeted metabolomics profiling of Canadian *F. poae* populations by Agriculture and Agri-Food Canada researchers will be presented. Telomere-to-telomere genome assemblies revealed the presence of highly polymorphic accessory chromosomes affecting mycotoxin profiles detected *in vitro* and *in planta*. Specifically, approximately ten percent of profiled strains have an accessory chromosome expressing the BGC for the acutely toxic cyclic peptide apicidin, not previously associated with *F. poae.* Additionally, we have characterized a rare BGC on an accessory chromosome, which produces and incorporates 2,3-diaminopropionic acid into a novel small peptide secondary metabolite – a first for the fungal kingdom. Finally, the implications of rapidly mutating accessory chromosomes in a fungal species with no characterized sexual cycle will be briefly discussed.

279C **The transcriptomic landscape of lignocellulose degradation by anaerobic fungi** Jessica L Matthews[1], Matt J Blow[2], Igor V Grigoriev[2], Kurt Labutti[2], Vivian Ng[2], Stephen C Fry[3], Jolanda van Munster[1] [1]SRUC, [2]JGI, [3]University of Edinburgh

Anaerobic fungi are powerful degraders of lignocellulose, but to fully exploit this in renewable-based technologies, we must understand their degradative mechanisms. Here, we propose to use time-course RNA-SEQ and untargeted proteomics to identify gene expression and enzyme production of two species of anaerobic fungi with distinct characteristics, during the colonisation of wheat straw – a major biofuel feedstock.

Following fungal responses from inoculation, mid-log, and stationary growth, will allow us to assess if anaerobic fungi have constitutive degradation activity, or if the changing composition of wheat straw elicits temporal changes in their activity. The observed responses will also allow a comparison of different strategies that both fungal species may use for lignocellulose degradation.

To investigate enzyme expression in further detail, the transcriptomic responses of both species will also be assessed to individual plant components of potential industrial interest, e.g., cellulose, arabinoxylan, and galactomannan. As some of the polysaccharides are expected to induce enzyme expression but cannot support anaerobic fungi growth, we investigated how enzyme induction was affected by a combination of these polysaccharides together with a carbohydrate that can support fungal growth, (e.g., glucose, cellobiose, and cellulose).

Together, these studies enable the identification of enzymes that two different types of anaerobic fungi employ as part of their degradative mechanisms and elucidate how enzyme production is affected by lignocellulose-derived inducers. This knowledge will be exploited to create combinations of anaerobic fungi and lignocellulose with increased potential in selective lignocellulose pre-treatments for bioprocessing.

280C **Acquired amphotericin B resistance and fitness trade-off compensation in *Candida auris*** Hans Carolus[1], Dimitrios Sofras[1], Giorgio Boccarella[1], Poppy Septhon-Clark[2], Celia Lobo Romero[1], Rudy Vergauwen[1], Saleh Yazdani[1], Siebe Pierson[1], Stef Jacobs[1], Paul Vandecruys[1], Stefanie Wijnants[1], Jacques F Meis[3], Pieter van den Berg[1], Jeffrey M Rybak[4], Christina Cuomo[2], Patrick Van Dijck[1] [1]KU Leuven, [2]Broad Institute, [3]University of Cologne, [4]St. Jude Children's Research Hospital

Candida auris is an emergent human fungal pathogen of growing concern due to common drug resistance to all major antifungal drug classes. Although resistance to amphotericin B (AMB) has been detected in 30 to 60% of clinical isolates of *C. auris*, mechanisms of AMB resistance remain poorly characterized. Here we present a large-scale investigation of how AMB resistance can be acquired through genetic adaptation. We typed 441 *in vitro* and *in vivo* evolved *C. auris* lineages from four AMB-susceptible clinical strains of different clades. We show a great diversity of acquired resistance responses with resistance magnitude- and strain-dependent fitness trade-offs. Genotyping and membrane sterol analyses of selected lineages show four major types of membrane sterol alterations. Using a novel, plasmid-based CRISPR-Cas9 allele editing method and Cas9-RNP meditated gene deletions, we show that AMB resistance can be acquired through variation in several sterol biosynthesis regulators including *ERG6, NCP1, ERG11, ERG3, HMG1, ERG10* and *ERG12*. Additionally, we show how aneuploidies in chromosomes 4 and 6 emerge during AMB resistance evolution. By leveraging fitness trade-off phenotyping and mathematical modelling of the *in vivo* environment during treatment, we evaluated the potential of different mechanisms to establish resistant infections and discovered a potential mechanism of fitness trade-off compensation. Variation in *CDC25* substantially enhanced the capacity to establish a resistant infection and may have played a role in facilitating the sole documented clinical case of acquired AMB resistance during treatment in *C. auris*. In summary, our findings show that fitness trade-off compensation along with several sterol modulating mechanisms of acquired AMB resistance represent a potential risk for AMB treatment failure in the clinic.

281C **Inter-fungal warfare in the maize kernel: mechanism of pyrrocidine-induced fumonisin elimination** Lily W Lofton[1,2], Maria Doppler[3], Christoph Bueschl[4], David Ruso[3], Timothy R Satterlee[2], Anthony E Glenn[2], Rainer Schuhmacher[4], Scott E Gold[2] [1]Dept of Plant Pathology, University of Georgia, [2]USDA, ARS, US National Poultry Research Center, Toxicology & Mycotoxin Research Unit, [3]Dept of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna (BOKU), Core Facility Bioactive Molecules: Screening and Analysis, [4]Dept of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna (BOKU)
Fusarium verticillioides, a mycotoxigenic fungus, coinhabits maize kernels with the protective endophyte *Sarocladium zeae*. *S. zeae* produces secondary metabolites, pyrrocidines A and B, that inhibit *F. verticillioides* fumonisin biosynthesis. Transcriptomics previously identified *FvZBD1* (FVEG_00314), a gene adjacent to the fumonisin biosynthetic gene cluster. Pyrrocidines induced *FvZBD1* gene expression 4,000-fold. *FvZBD1* deletion resulted in dramatic increases (FB_1 > 30-fold, FB_2 > 40-fold) in fumonisin production, suggesting its role as a genetic repressor of fumonisin biosynthesis. Fungal biology, transcriptomics, proteomics, and metabolomics will elucidate the molecular mechanisms by which pyrrocidine, through *FvZBD1*, abolishes fumonisin biosynthesis in *F. verticillioides*. Recent pyrrocidine dose-response studies demonstrate (1) the discovery of synergy between pyrrocidines A and B, and (2) that *FvZBD1* confers partial tolerance to pyrrocidine. In collaboration with the Institute of Bioanalytics and Agro-Metabolomics (BOKU, Austria), untargeted metabolomics was carried out to elucidate how pyrrocidine and *FvZBD1* induce changes to the *F. verticillioides* metabolic profile, revealing the biochemical pathways – and efficacy – of pyrrocidine-induced fumonisin elimination in *F. verticillioides*. Representing the first report of an untargeted metabolomics experiment conducted on *F. verticillioides*, this study sheds new light broadly on its mycotoxin production potential, and uniquely provides evidence that pyrrocidine functions through *FvZBD1* to eliminate fumonisin biosynthesis. The thorough analysis and implications of this metabolic data will be discussed. This study advances our knowledge of the role of secondary metabolites in fungal communication and mycotoxin control, and will inform the development of a biological control strain of *S. zeae*, optimized to eliminate fumonisin contamination in maize across conventional, organic, and subsistence agriculture.

282C **Unsilencing the cryptic isocyanides and secondary metabolites of *Penicillium expansum*** Justin L Eagan[1], Mira Syahfriena Amir Rawa[1], Christina M Hull[1,2], Nancy P Keller[1,3] [1]Medical Microbiology and Immunology, University of Wisconsin - Madison, [2]Dept of Biomolecular Chemistry, University of Wisconsin - Madison, [3]Dept of Plant Pathology, University of Wisconsin - Madison

Fungal secondary metabolites are an important source of novel chemistry, but the majority remain silent or "cryptic" in standard laboratory conditions, which challenges research with large rates of known compound rediscovery. For example, the pome fruit pathogen *Penicillium expansum* contains over 70 biosynthetic gene clusters (BGCs) of which only 6 are linked to known metabolites. To characterize cryptic SMs in *P. expansum,* we are taking two approaches to activate silent BGCs. The first approach focuses on activating isocyanide synthase (ICS) BGCs, which we hypothesize are regulated by trace metals based on previous studies from our lab (Lim *et al.* 2018, Won *et al.* 2022). One intriguing ICS BGC is predicted to contain two isocyanide synthases, an MFS-domain transporter and a DJ-1/PfpI-like protease. In parallel with promoter replacements of each cluster member in a single background to create overexpression stains, we are also cloning the entire cluster into an AMA1 plasmid to heterologously express in *Aspergillus nidulans*. Our second approach is to create a baseline *P. expansum* strain (*Pexp*ΔSM) that is inactivated for all known secondary metabolites, which can allow characterization of otherwise masked metabolites. LC-MS/MS data show unique peaks produced in the *Pexp*ΔSM strain, which we speculate could represent cryptic metabolites expressed through alterations in

precursor flux. Our approaches so far show promise in activating cryptic BGCs, and gene expression data will be utilized to link any novel compounds to putative biosynthetic genes.

283C **Deciphering the Genotype-Phenotype Connection: Environmental Influence on Secondary Metabolite Production in *Basidiobolus*** Kimberly C. Syring[1], Gisele Rodriguez[2], George Neuhaus[2], Lluvia Vargas-Gastélum[1], Kerry McPhail[2], Joseph Spatafora[1] [1]Botany and Plant Pathology, Oregon State University, [2]College of Pharmacy, Oregon State University

Basidiobolus is a genus of fungi with a complex life cycle characterized by unique life history stages that have been ecologically adapted to survive in numerous environmental niches. In addition to spending a portion of its life cycle living freely on detritus as a filamentous saprobe, it is also found within the digestive systems of reptiles and amphibians in a yeast-like palmella stage. Furthermore, the exploration of *Basidiobolus* genomes has identified a strong signal of bioactive specialized metabolites such as cyclic peptides. To elucidate variation in secondary metabolism (SM) under contrasting environmental conditions and gain insight into the ecological function of SM within the genus, we tested and compared different strains of *Basidiobolus* by growing them on multiple media types under aerobic and anaerobic conditions. Methanol extractions of fungal biomass were subsequently analyzed via LC-MS/MS to determine feature masses present in each sample. PCA analysis revealed that *Basidiobolous* exhibits variation in SM production across different strains, medias, and anaerobic and aerobic conditions. Aerobic growth conditions resulted in greater variation of SM within and across species when compared to paired anaerobic conditions, suggesting that the exposure to anaerobic environments may result in a more conserved production of secondary metabolites that may function within the gut environment. LCMS feature tables were used to create feature based molecular networks to predict if molecular families were conserved or differentially produced across species and conditions. Of particular interest is the recognition of an eight-residue cyclic peptide across multiple species and treatments. Upon evaluation of *Basidiobolus* genomes, only a single biosynthetic gene cluster encoding an eight-module NRPS was predicted, establishing a putative genotype-phenotype connection for additional analyses. We will present data and analyses that explore the differential production of SM as a function of environmental variables (aerobic vs. anaerobic) relevant to the life history of *Basidiobolus* and the predicted biosynthetic gene clusters responsible for their biosynthesis.

284C **Exploring biosynthetic gene clusters in *Aspergillus fischeri*** Karin Steffen[1], David Rinker[1], Thomas Sauters[1], Adiyantara Gumilang[1], Manuel Rangel-Grimaldo[2], Huzefa Raja[2], Nicholas Oberlies[2], Gustavo H Goldman[3], Antonis Rokas[1] [1]Vanderbilt University, [2]University of North Carolina Greensboro, [3]University of Saõ Paulo

Biosynthetic gene clusters (BGCs) are groups of genes in close physical proximity that produce specialized metabolites (SMs) and other natural products. Many BGCs and their SMs are considered virulence factors in pathogens. Therefore, BGCs are typically studied in pathogens such as *Aspergillus fumigatus*, the causal agent of aspergillosis. However, in addition to this pivotal role in pathogens BGCs are also found in non-pathogens indicating BGC and SM functions beyond virulence in nature. Our aim was to gain a better understanding of the true diversity of BGCs and SMs, their presence and prevalence in other *Aspergillus* species. To that end, we studied *Aspergillus fischeri*, a close relative of *A. fumigatus* investigating BGC content and SM repertoire across 16 strains. We mined whole genome assemblies with antiSMASH and initially detected 37 known and 35 unknown potential BGCs. This corroborates that the non-pathogen has a higher number of BGCs detected by antiSMASH than its pathogenic relative. We combined the genomics approach with metabolomics to verify predictions via the detections of corresponding SMs when possible. However, we emphasize the importance of manual curation as we rejected the prediction of several (false positive) BGCs while also recovering additional BGCs (false negatives). This richness in BGCs in *A. fischeri* indicates the versatility and wide range of BGC and SM functions in the ubiquitous non-pathogenic soil fungus. Beyond this overall assessment of known BGCs some of the hypothetical new BGCs may represent new avenues for future natural product discovery.

285C **Using TN-seq to identify molecular targets of fungal spore germination inhibitors** Jackie Spieles[1], R. Blake Billmyre[2], Christina Hull[1] [1]University of Wisconsin-Madison, [2]The University of Georgia

Cryptococcus is an environmental fungus that produces spores as a fundamental survival mechanism. Germination of these spores is required for subsequent vegetative growth in new environments, including mammalian hosts. Spore germination has therefore been identified as a potential reservoir for new molecular targets for antifungal drug development. Previously, our lab identified 191 small molecule inhibitors of *Cryptococcus* spore germination. Within this pool, eight prevalent substructure groups were identified, many of which also inhibit yeast growth and exhibit low cytotoxicity to mammalian fibroblasts. As an initial approach to identify targets of these novel antifungal molecules, we are employing genome-wide transposon mutagenesis with high-throughput sequencing (TN-seq). With this approach, we use a library of transposon insertion mutants in which each cell has a single transposon insertion, and the population is saturated to include mutations of every gene in the genome (an average one insertion per 13 bp genome-wide). When exposed to selective conditions (i.e., germination and growth inhibitors), cells with

transposon-mediated mutations in genes required for survival become under-represented in the population. By sequencing just outside the transposon sequence in the selected population, we can identify genes differentially influenced by inhibitors during vegetative growth and spore germination. This method has been previously employed in *Cryptococcus* to verify targets of the well-known antifungal drug fluconazole. Thus, with TN-seq we anticipate identifying affected pathways that will implicate molecular targets of these novel inhibitors of *Cryptococcus* spore germination.

286C **New Regulators of Gliotoxin Synthesis, HsfA and RogA, Identified through the Systems Biology Network GRAsp** Hye-won Seo[1], Natalia Wassano[2], Nancy Keller[3] [1]Microbiology and Immunology, UW-Madison, [2]Biochemistry and Tissue Biology, University of Campinas (UNICAMP), [3]UW-Madison

Aspergillus fumigatus, a pathogenic fungus, is a significant medical threat and causal agent of invasive aspergillosis. The fungus employs toxic secondary metabolites, notably gliotoxin, as key virulence factors to attack the host's immune cells. Gliotoxin also exhibits antifungal properties and *A. fumigatus* has evolved self-protection strategies against gliotoxin including GliT affecting disulfide bridge closure in the gliotoxin molecule and GtmA generating the less toxic bisdethiobis(methylthio)gliotoxin. Although the regulation of the *gli* biosynthetic genes is well known and orchestrated by the transcription factor GliZ, GliZ does not regulate *gliT* or *gtmA.* Here we utilized our recently developed gene regulatory network named GRAsp (**G**ene **R**egulation of ***Asp****ergillus fumigatus*) to explore the regulatory mechanisms governing gliotoxin self-protection in *A. fumigatus* . GRAsp analysis pinpointed 2 genes, AFUA_5G01900 and AFUA_3G11990, potentially involved in gliotoxin regulation. AFUA5G01900 encodes the heat shock protein HsfA and AFUA3G11990 encodes a C6 transcription factor we termed RogA (Regulator of Gliotoxin). GRAsp predicted that RogA regulated all of the biosynthetic gene cluster (BGC) genes responsible for gliotoxin synthesis as well as both *gliT* and *gtmA*. HsfA also was predicted to regulate *gliT* and *gtmA.* Gene expression data showed that RogA and HsfA negatively regulated all *gli* biosynthetic genes as well as both *gliT* and *gtmA.* Overexpression of both genes increased gliotoxin synthesis simultaneously with *gliT* and *gtmA* expression. By creation of single and double RogA and HsfA mutants, we present a model where HsfA regulates *gli* and *gtmA* expression through induction of RogA expression. Our work highlights the use of computational modeling to provide insight into previously unknown regulatory systems in self-protection mechanisms against endogenous toxins.

287C **Sugar, sugar: Development of the trehalose biosynthesis enzymes as antifungal drug targets** Erica J Washington[1], Ye Zhou[2], Jiuyu Liu[3], Joe Heitman[1], John Perfect[4], Richard Lee[3], Mario Borgnia[5], Alberto Bartesaghi[6], Richard Brennan[2] [1]Molecular Genetics and Microbiology, Duke University, [2]Biochemistry Dept, Duke University, [3]Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, [4]Infectious Disease, Duke University, [5]Genomic Integrity and Structural Biology, NIEHS, [6]Computer Science, Duke University

Invasive fungal diseases are a major threat to human health, resulting in more than 1.5 million deaths worldwide each year. Yet the arsenal of antifungal therapeutics is limited. Trehalose is a non-reducing disaccharide composed of two molecules of glucose that is required for pathogenic fungi, such as *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*, to survive in their human hosts. Trehalose biosynthesis is a two-step process. Trehalose-6-phosphate synthase (Tps1) converts UDP-glucose and glucose-6-phosphate to trehalose-6-phosphate (T6P). Subsequently, trehalose-6-phosphate phosphatase (Tps2) converts T6P to trehalose. Interestingly, the trehalose biosynthesis pathway has been identified as a top candidate for novel antifungal development. However, there are currently no known antifungal agents that target the trehalose biosynthesis pathway. We demonstrated that inhibition of protein-protein interactions among trehalose biosynthesis proteins results in the loss of the ability of fungi to produce disease in mice. Therefore, the long-range goal of this project is to develop a broad-spectrum antifungal drug that disrupts complex formation among trehalose biosynthesis proteins. This shall require an atomic view of the homo- and hetero-oligomers of the trehalose biosynthesis proteins. Using cryo-electron microscopy (cryo-EM) we solved the structure of *apo C. neoformans* Tps1 (CnTps1), which is the first visualization of a ligand/substrate-free Tps1 homo-tetramer from a human fungal pathogen. Here, we also report the cryo-EM structure of CnTps1 bound to uridine diphosphate (UDP) and glucose-6-phosphate (G6P). Both *apo* and ligand-bound forms of CnTps1 form homo-tetrameric complexes. There is significant movement in the N-terminus of each CnTps1 protomer upon ligand binding. Additionally, detailed analysis of substrate-binding residues reveals several mutations that result in a loss of enzymatic activity *in vitro*. These data demonstrate that trehalose biosynthesis proteins form tetrameric complexes. Future work shall include determining the structures of hetero-complexes of trehalose biosynthesis proteins. In conclusion, these studies expand our knowledge of the role of complex formation among trehalose biosynthesis proteins and highlight the potential of developing antifungal therapeutics that disrupt protein-protein interactions among the complexes of proteins required to synthesize trehalose.

289C **Powdery mildew breaks down host chloroplasts for nutrient acquisition** Hang Xue[1], Mary C. Wildermuth[2], Krishna K.. Niyogi[2], Johan C. Jaenisch[1] [1]University of California, Berkeley, [2]PMB, University of California, Berkeley

Chloroplasts are important hubs connecting photosynthesis and plant immunity. Many plant pathogens target host chloroplasts to subvert host physiology for the benefits of the pathogens. The obligate biotrophic powdery mildew fungus, *Golovinomyces orontii*, grows on the model organism *Arabidopsis thaliana* and relies entirely on host nutrients to complete its life cycle. We found *G.orontii* infection induces the localized breakdown of host chloroplast thylakoids concurrent with its asexual reproduction when lipid demand is high. In addition, host lipid bodies were observed in the cytosol, adjacent to and inside chloroplasts in cells underlying the infection site. Furthermore, transmission electron and confocal microscopy showed an abundance of lipid bodies in the fungal haustorium. Genetic and molecular analyses show *Arabidopsis* chloroplast-localized DIACYLGLYCEROL ACYLTRANSFERASE 3 (DGAT3) to be largely responsible for powdery mildew-induced chloroplast TAGs and to promote fungal asexual reproduction. DGAT3 is known to prefer thylakoid membrane-associated FAs and we see increased triacylglycerols containing these FAs with infection. Therefore, we hypothesize that fungal haustorium lipid bodies derive from host chloroplast lipid bodies containing TAGs synthesized by DGAT3. In summary, *G. orontii* infection induces a source/sink transition promoting chloroplast photosynthetic membrane breakdown to facilitate acquisition of energy-dense lipids by *G. orontii* to support its asexual reproduction.

290C **Nonsense-mediated decay (NMD) in *Cryptococcus neoformans* utilizes human-like SMG downstream effectors with non-redundant functions** Sean R Duffy, John C Panepinto Microbiology and Immunology, SUNY at Buffalo Jacobs School of Medicine and Biomedical Sciences

Cryptococcus neoformans is an opportunistic fungal pathogen that primarily causes disease in individuals with compromised immunity. The current standard of treatment for cryptococcal infections is a dual therapy of amphotericin B and flucytosine. However, resource-limited regions still rely on fluconazole monotherapy which is less efficacious due to the fungistatic affect of fluconazole on *C. neoformans*. Identifying pathways that influence fluconazole tolerance may reveal strategies to augment the efficacy of fluconazole as an antifungal. Nonsense-mediated decay (NMD) is a conserved eukaryotic RNA decay pathway that degrades mRNAs with premature termination codons (PTCs). This pathway is regulated by Upf (1-3) proteins which identify PTC-containing mRNAs and recruit Smg (5-7) proteins that then facilitate mRNA decay. Deletion of any one of the Upf proteins causes fluconazole sensitivity in *C. neoformans*. However, the overall mechanism of NMD and its downstream effectors (i.e. Smg proteins) as well as its physiological roles within *C. neoformans* are unknown. In this work, we identify a role of NMD in regulating mitochondrial function in *C. neoformans*. We also identify homologs of Smg6 and Smg7 within *C. neoformans*. Phenotypically, *smg6*Δ and *smg7*Δ mutants possess subsets of phenotypes observed in the *upf1*Δ mutant, suggesting a bifurcation of the branches of NMD in *C. neoformans* in which Smg6 and Smg7 exert non-overlapping functions. This contrasts with what is observed in humans, as the Smg6- and Smg7-mediated branches have been shown to be functionally redundant. Overall this work will elucidate the role of NMD in *C. neoformans* and identify new NMD components that could be targeted to boost fluconazole efficacy in treating cryptococcal infection.

291C **Manipulation of plant host cell cycle and lipid metabolism to fuel powdery mildew spore production** Mary C. Wildermuth, Johan Jaenisch, Hang Xue Plant & Microbial Biology, University of California Berkeley

Powdery mildews are obligate biotrophic fungi that manipulate plant responses to facilitate their colonization, growth, and reproduction. Site-specific transcriptomics led us to investigate potential alteration of the plant cell cycle in response to powdery mildew infection. We found the powdery mildew *Golovinomyces orontii* induces endoreduplication in mesophyll cells underlying the epidermal cell containing the fungal feeding structure, concurrent with fungal asexual reproduction. Furthermore, the number of asexual reproductive structures was found to be highly correlated with the ploidy index of these mesophyll cells. Analysis of transcriptome data for plant development and biotrophic-plant interactions in which induced endoreduplication is associated with enhanced metabolic capacity showed specific metabolic pathways exhibit a non-additive increase in expression across systems. This led us to identify powdery mildew-induced use of the plant pyruvate dehydrogenase complex (PDHc) bypass to fuel increased acetyl-CoA and lipid metabolism for fungal asexual reproduction in which new conidia filled with lipid bodies are formed. Labeling studies find this acetyl-CoA is incorporated into both leaf and spore storage lipids, triacylglycerols (TAGs) with a 3.5-fold increase in TAGs of powdery mildew-infected. Genetic analyses found leaf TAGS that promote powdery mildew conidia formation are not synthesized using the canonical ER pathway, but instead use *DIACYLGLYCEROL ACYLTRANSFERASE 3 (DGAT3)*, localized to the chloroplast. Thylakoid membranes are disassembled and leaf galactolipids and associated FAs decrease. Leaf and spore TAGs are dominated by thylakoid-membrane derived fatty acids, such as 18:3, and DGAT3 preferentially incorporates 18:3 into TAGs. Furthermore, silencing powdery mildew genes that break down lipids via spray-induced gene silencing (SIGS) limits powdery

mildew asexual reproduction. Product development and field testing of SIGS to control powdery mildew of grapevine shows this approach to be a promising, more sustainable alternative to increasingly restricted chemical fungicides.

292A **Developing Innovative Antifungal Drug Delivery Systems to Fight *Fusarium oxysporum* Infection in Humans** Siyuan Wu[1,2], Harun Cerkezi[2], Eunji Hong[3,4], Himani Patel[2], Rachel Griffiths[2], Muhammad Aamir Hassan[5], Vincent Rotello[5], Siyuan Rao[3,4], Li-Jun Ma[2] [1]Molecular and Cellular Biology Graduate Program, University of Massachusetts Amherst, [2]Dept of Biochemistry and Molecular Biology, University of Massachusetts Amherst, [3]Dept of Biomedical Engineering, University of Massachusetts Amherst, [4]Dept of Biomedical Engineering, Binghamton University, [5]Dept of Chemistry, University of Massachusetts Amherst
Fusarium oxysporum species complex has plant pathogens that cause vascular wilts on over 100 plant hosts and was listed among the top five most important plant pathogens. It also includes human pathogens that cause both disseminated fusariosis in immunocompromised patients and *fusarium* keratitis in immunocompetent individuals, presenting a serious public health concern and listed among high-priority fungi in the first fungal priority pathogens list published by the World Health Organization. It is difficult to treat *Fusarium* infections, as this group of fungi exhibit intrinsic multidrug resistance. Therefore, the development of anti-*Fusarium* agents characterized by both efficacy and safety is crucial. Take advantage of the versatility of nanoparticle, this study employed two different nanoparticle constructs, Amphotericin B (AmpB) liposomes and Carvacrol-gelatin nanoemulsions (C-GNE), for site-specific, target-oriented drug deliveries to treat *Fusarium* infections. We tested the drug efficiency using a tomato pathogen F. oxysporum f. sp. lycospersici strain Fo4287 and a human pathogenic strain Fo32931. Multiple human cell lines were used to test toxicity of these nanoparticles. Detailed results will be presented and potential mechanisms of these novel nanoparticles and novel delivery systems will be discussed.

293A **A novel isomaltose sensor/transporter identification involved in the activation of the transcription factor AmyR in *Aspergillus oryzae* and *A. nidulans*** Da Min Jeong, Jikian Tokashiki, Tomoko Shintani, Takahiro Shintani, Katsuya Gomi The graduate school of agriculture, Tohoku University

Aspergillus oryzae, or koji mold, is known for its ability to produce high levels of hydrolytic enzymes including amylolytic and proteolytic enzymes, which are used in various industrial applications such as Japanese fermented foods and beverages. In *A. oryzae*, the regulation of amylolytic enzyme gene expression is controlled by the fungal-specific transcription factor AmyR, which regulates the expression of α-amylase, glucoamylase, and α-glucosidase genes. The induction of AmyR activity is triggered by isomaltose most effectively compared to glucose or maltose. However, isomaltose was found to be barely incorporated into the fungal cells both in *A. oryzae* and *Aspergillus nidulans*, suggesting the existence of an unidentified isomaltose sensor or transporter on the plasma membrane. In this study, to identify the isomaltose sensor/transporter, *A. nidulans* mutant strains showing good growth on isomaltose agar were isolated from the strain that showed a defective growth due to overexpression of *brlA*, a transcription factor required for conidiation, with the strong α-amylase gene promoter. Genomic DNA of the most promising candidates were used for whole genome sequencing to compare genetic polymorphisms between mutant strains. SNP analysis revealed that mutation in the putative coding sequences (CDS) was included in the major facilitator superfamily (MFS) transport proteins. Interestingly, different kinds of polymorphisms were identified in this CDS in common. This MFS transporter is considered to be an isomaltose sensor/transporter of interest in this study, because deletion of the gene restored growth of the *A. nidulans* strain harboring the *brlA* overexpression cassette on isomaltose agar medium. Furthermore, the relative expression level of amylolytic genes as well as *amyR* decrease significantly in the disruption mutant, demonstrating this CDS affects on the AmyR induction pathway and its regulation of amylolytic gene expression.

294A **Control of *Penicillium camemberti* morphology to influence conidia production in an industrial environment** Aymeric Paradis[1,2], Valérie Gautier[1], Nicolas Hardy[2], Philippe Silar[1] [1]Université Paris Cité, [2]International Flavors and Fragrances
Background: The white mold *Penicillium camemberti* is used in the ripening of soft cheeses such as Camembert or Brie. To be sold as a ferment, its spores are produced on an industrial scale using the asexual reproduction cycle, which can develop of in two forms: a vegetative one called mycelium, or dormant asexual spores called conidia. The last bioprocess used by industry today is submerged fermentation in stirred bioreactors, which enables easy control of culture parameters to influence factors impacting the cell cycle. However, the main difficulty with this liquid fermentation process is controlling the morphological phenotype of the mycelium during bioreactor growth.
Objective: As we know no works clearly establish a correlation between macromorphology and conidia production. The aim of this work is to gain new fundamental knowledge of the link between fungal growth/morphology and conidia formation.
Method: To study the cell cycle, which takes place on a large industrial scale (cubic meters) bioreactors, the chosen method was to scale-down this submerged fermentation to laboratory scale (millimeters), to increase experimental throughput.
Results: Differences in morphological phenotypes depend on the experimental conditions tested, reproduction of dispersed morphology is possible on a laboratory scale, and the link between morphology and conidiation is clearly established. Many carbon

sources were tested and leads to a dispersed morphology but in the presence of sucrose, the culture is more homogeneous with less viscosity, and the biomass concentration is higher. In addition, conidiation is reduced in presence of sucrose compared to other sugars. Finally, the addition of hydrogen peroxide fluidizes morphology, changing from hybrid to fully dispersed, and this effect seems to be correlated with a dose-effect on conidia production. Conclusion: Using this laboratory model, the link between morphology and conidiation was clearly demonstrated, and several critical parameters influencing this cycle were identified. Four different and reproducible nutrient conditions were therefore set up to obtain different growths and conidiations levels, and total RNA was extracted for sequencing and gene expression studies.

295A **Roles for heterotrimeric G-proteins and adenylyl cyclase in differential regulation of cellulase gene expression and cellulase activity in *Neurospora crassa*** Yagna Oza, Logan A Collier, Katherine A Borkovich Microbiology and Plant Pathology, University of California, Riverside

Transcriptional regulators of genes encoding plant cell wall degrading enzymes have been characterized in several filamentous fungi, but the upstream signal transduction pathways have yet to be fully understood. We previously demonstrated that two heterotrimeric G protein Gα genes (*gna-1* and *gna-3*) and adenylyl cyclase (*cr-1*) are required for normal cellulase activity in *Neurospora crassa*. To further our understanding of this regulation, we have compared the transcriptomes of wild type and these three mutants after pre-growth on glucose and then transfer to glucose or cellulose as a carbon source. Functional categorization of differentially expressed genes in the mutants vs. wild-type during growth on glucose yielded the greatest number of up- and down-regulated genes in the *gna-3* mutant for all major categories. In contrast, the *cr-1* mutant possessed the highest number of up- and down-regulated genes across all categories during growth on cellulose. The results also showed that multiple cellulases have greatly reduced expression in *gna-1, gna-3,* and *cr-1* mutants compared to the wild-type strain during growth on cellulose. Specific assays were performed on these mutants for endoglucanase, cellobiohydrolase, and beta-glucosidase activity, with the results consistent with the reduction/loss of enzyme activity resulting from transcriptional regulation. Furthermore, the findings demonstrated that *cr-1* is essential for cellobiohydrolase activity. Transcript levels of the transcription factors *clr-1* and *clr-2*, known to regulate cellulase gene expression in *N. crassa*, are reduced in *gna-1, gna-3,* and *cr-1* mutants. *gna-3* impacts both *clr-1* and *clr-2*, whereas *gna-1* primarily regulates *clr-2* gene expression. We are currently testing for an epistatic relationship between both Gα subunits and CLR-2 through over-expression of the *clr-2* gene in the G-protein mutant background. We have also performed cellulase activity assays on mutants lacking multiple Gα genes, with the results showing that *gna-1* and *gna-3* are required for endoglucanase activity, whereas *gna-2* and *gna-3* work together to regulate cellobiohydrolase activity.

Summarizing, our results reveal the transcriptional regulation of cellulase enzyme families by G-proteins and adenylyl cyclase, the crucial role of *cr-1* and multiple Gα genes in regulation of specific enzyme activities, and the relationship between G-protein subunits and the transcription factors regulating cellulase gene expression.

296A **Functional Characterization of the *pgs* Gene provides insights into the molecular basis of pathogenicity in an important pine pathogen.** Alida van Dijk[1], Andi Wilson[1], Bianke Marx[2], Bianca Hough[1], Benedicta Swalarsk-Parry[2], Lieschen De Vos[1], Michael Wingfield[1], Brenda Wingfield[1], Emma Steenkamp[1] [1]BGM, University of Pretoria, [2]University of Pretoria

Fusarium circinatum, the causal agent of pine pitch canker disease, poses a significant threat to the health of *Pinus* species worldwide. The pathogen, native to Mexico and the Caribbean, has spread globally causing damage in nurseries, plantations as well as in natural pine forests. *F. circinatum* can cause root and collar rot in seedlings and resinous cankers on mature trees, resulting in substantial losses. To combat the impact of this pathogen, a detailed understanding of the molecular interplay between pine and *F. circinatum* is needed. Despite extensive research on the pathogen, knowledge of its underlying mechanisms of pathogenicity remains limited. A recent genome-wide association study identified the potential role of a putative pathogenicity protein in virulence. To functionally characterize the putative pathogenicity gene, a CRISPR-Cas9 genome-editing system was established for the first time in *F. circinatum*. Functional characterization was conducted by comparing knockout isolates to their corresponding wild type and transformant control, in various phenotypic traits including a pathogenicity trial, growth in culture and conidial production. Three knockout and transformant control isolates were generated from three wild type isolates. Successful knockout of the *pgs* gene was confirmed using PCR and Southern Blotting, demonstrating the precision and efficacy of the genome-editing technology. The knockout isolates exhibited significantly smaller lesions compared to their wild-type and transformant control counterparts in pathogenicity trials conducted on *Pinus patula,* confirming a role in pathogenicity for this gene. The mutant isolates also grew significantly slower and produced significantly fewer conidia than the corresponding wild type isolates, suggesting that the *pgs* gene plays a role in growth as well as conidial production. Given these results, we suggest the gene be termed *pgs*, as it plays a role in **p**athogenicity, **g**rowth, and **s**porulation of this pathogen. Identification and characterization of pathogenicity genes will allow for the development of effective strategies to manage and mitigate pitch canker

disease. Furthermore, understanding the molecular basis of resistance and pathogenicity paves the way to produce resilient planting stock and targeted control measures. Notably, the establishment of CRISPR-Cas9 genome editing technology expands the molecular toolbox available for this pathogen, allowing many more insights into *F. circinatum.*

297A **Exploring the role of alpha-1,3-glucan synthases on fungal cell wall integrity in Aspergillus niger** Katharina J. Ost[1], Mark Arentshorst[2], Arthur F.J. Ram[2], Bruno M. Moerschbacher[3], Mareike E. Dirks-Hofmeister[1] [1]Food Biotechnology, Osnabrück University of Applied Sciences, [2]Biology, Leiden University, [3]Plant Biology and Biotechnology (IBBP), University of Münster

Alpha-glucan synthases (ags) play a key role in the synthesis of alpha-1,3-glucan, a crucial component of the fungal cell wall that is (i) contributing to its structural integrity and is (ii) involved in cross-linking of different polymers, so that it influences the composition of both: the outer shell and inner membrane of fungal cells. In the filamentous fungi Aspergillus niger five ags genes are annotated, of which agsA and agsE were shown to be the most highly expressed genes during different stages of development. In addition, an agsE deletion mutant caused smaller micro-colonies as well as a shift in the secretome composition of A. niger, without affecting biomass production. Concurrently, intracellular cross-linking between chitin and beta-glucan is primarily mediated by the seven-membered cell wall-related transglycosylase gene family (crh). Although an impact on cell wall integrity has been expected when deleting the entire crh gene cluster in A. niger, significant alterations in cell wall integrity became only evident when the crh gene cluster deletion was combined with the reduction of alpha-glucan and galactomannan by deleting respective agsE or ugmA. With this background, we aimed to further explore the impact of ags gene deletions – both individually and as an entire family – on fungal cell wall integrity of A. niger. Using targeted CRISPR/Cas9 technology, we engineered various ags-deficient strains, including the deletion of the entire gene family (ΔagsA-E) in a mutant strain lacking all chitin-glucan cross-linking enzymes (Δcrh, TLF39). Subsequent morphological and biochemical characterization of these mutants pinpoint the importance of agsE for maintaining cell wall stability and suggesting its potential influence on protein production and/or secretion. These findings therefor provide not only new insights into fungal biology but also potential targets for biotechnological applications.

298A **Development of *Trichoderma reesei* capable of producing products at higher production temperature without compromising productivity** Cherry Lin[1], Damian Long[1], Steve EauClaire[2], Jon Palmer[1], Mikhail Karymov[1], Bette Bodie[1] [1]IFF, IFF, [2]IFF, Former employee at IFF

The ascomycete fungus *Trichoderma reesei* (teleomorph *Hypocrea jecorina*) is widely used for industrial cellulase production and a wide variety of other products. While the Strains such as QM6a, RUT-C30 and RL-P37 grow faster at 34°C, the optimum temperature for protein production is 25-28C. During large scale industrial fed-batch fermentation, reducing temperature from growth at 34C to 25-28C for protein production requires cooling equipment that could incur significant capital and operating costs. Using evolution and screening we identified a mutation in the SPT5 gene that enables *T. reesei* to secrete proteins at 29C without loss of productivity. Isolation of the mutant strain and characterization of the SPT5 mutation will be described.

299A **Sub-genomic RNAi-assisted strain evolution of filamentous fungi for enhanced protein production** xianhua Sun[1], xiaoyun Su[2] [1]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, [2]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Genetic engineering at the genomic scale provides a rapid means to evolve microbes for desirable traits. However, in many filamentous fungi, such trials are daunted by low transformation efficiency. Differentially expressed genes under certain conditions may contain important regulatory factors. Accordingly, while manipulating these subsets of genes only can largely reduce the time and labor, engineering at such a sub-genomic level may also be able to improve the microbial performance. Herein, first using the industrially important cellulase-producing filamentous fungus *Trichoderma reesei* as a model organism, we constructed suppression subtractive hybridization (SSH) libraries enriched with differentially expressed genes under cellulase induction (MM-Avicel) and cellulase repression conditions (MM-Glucose). The libraries, in combination with RNA interference, enabled sub-genomic engineering of *T. reesei* for enhanced cellulase production. The ability of *T. reesei* to produce endoglucanase was improved by 2.8~3.3-fold. In addition, novel regulatory genes (*tre49304*, *tre120391*, *tre123541* and *tre21982*) were identified to affect cellulase expression in *T. reesei*. Iterative manipulation using the same strategy further increased the yield of endoglucanase activity to 75.6 U/ml, which was 7.0 times as high as that of the wild-type (10.8 U/ml). Moreover, using *Humicola insolens* as an example, such a sub-genomic RNAi-assisted strain evolution proved to be also useful in other industrially important filamentous fungi. By combining SSH and RNAi, a strain of *H. insolens* producing 28,500±288 U/ml of catalase was obtained, which was 1.9 times as high as that of the parent strain. The same strategy may also be expanded to engineering other microorganisms for enhanced production of proteins, organic acids, and secondary metabolites.

300A **Deciphering the Regulatory Mechanisms Governing Recombinant Protein Secretion in Filamentous Fungi** Everton Paschoal Antoniel, Jaqueline Gerhardt, Natália Wassano, Fernanda Lopes de Figueiredo, André Damasio University of Campinas

Filamentous fungi represent promising hosts for the production of industrially important enzymes, owing to their robust secretory machinery and capacity to yield complex proteins at high levels. These attributes are particularly significant in producing biofuels and various other bioproducts. However, despite their exceptional capacity for protein secretion, there is still room for improvement in recombinant protein production. Thus, comprehending the intricate regulatory mechanisms that underlie the secretion of these proteins is paramount for enhancing its biotechnological applications. In this context, we propose a systems biology approach, involving the analysis of high-throughput data from various fungi, such as A. nidulans, A. oryzae, and A. niger, all of which overexpressing recombinant enzymes. Our systematic approach entailed the filtration of up- and down-regulated genes, followed by the selection of their respective promoter regions for thorough motif analysis. Subsequently, these promoter regions were analyzed to identify specific binding motifs, from which the top ten candidate motifs for each fungal species were selected. To gain further insights, we compared these identified motifs with a collection of known motifs from yeast, facilitating the identification of corresponding transcription factors in S. cerevisiae. Finally, we obtained the transcription factors from the analysis in S. cerevisiae and conducted alignments against the A. nidulans database to search for potential orthologs. Through this intricate process, we successfully identified five common transcription factors in the three Aspergillus species, showcasing significant potential to influence recombinant protein production positively. Subsequent genetic manipulation of these transcription factors resulted in a substantial 2.8-fold and 2.6-fold increase in the secretion of recombinant enzymes from two distinct strains of A. nidulans. Through these investigations, we aim to enhance our understanding of the mechanisms governing protein secretion in filamentous fungi and develop novel strategies to improve the efficiency and yield of recombinant protein secretion in filamentous fungi.

301A **Glycosylation studies of industrial *Trichoderma reesei* strains** Lori Maggio-Hall, Yang-Xiang Li, Carol McCutchen, Suzanne Singles, Raymond Hong, Sergio Nanita, Timothy Caspar, Sharief Barends Health & Biosciences, International Flavors & Fragrances

Trichoderma reesei is a well-known producer of both native and heterologous secreted proteins. Proteins are typically glycosylated as they transit the secretory pathway, with asparagine-linked glycan addition potentially playing a role in protein folding and quality control functions in the endoplasmic reticulum (ER). Here we show the effects of genetic modifications that alter N-glycan processing and retention on production of native and heterologous proteins in industrial strain lineages by using mass spectrometry to track N-glycan occupancy and composition. Elimination of an endogenous endo-N-acetylglucosaminidase gene led to significant retention of N-glycans on proteins after secretion into culture media. Rut-C30 and related strains had previously been found to contain a frameshift in alpha-glucosidase II (Geysens *et al* 2005), an enzyme predicted to control how much time nascent and misfolded proteins spend in contact with ER chaperones (Zhang *et al* 2021). We demonstrated that restoration of the frameshift increased glycan trimming on secreted proteins and in one case led to a 50% increase in protein production. Some lineages of *Trichoderma* produce charged N-glycans, with phosphomannose extensions added to the original core high mannose structure (Stals *et al* 2004). We found that these could be detected on native cellulase enzymes in Rut-C30, in agreement with the literature, but were harder to detect in higher-producing isolates obtained after mutagenesis. Development of a genetic toolbox for multiple glycosylation phenotypes has allowed us to optimize expression, manufacturing and storage stability for a broad number of enzyme products.

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Zhang, J., J. Wu, L. Liu and J. Li (2021) "*The crucial role of demannosylating asparagine-linked glycans in ERADicating misfolded glycoproteins in the endoplasmic reticulum*" Front. Plant Sci. 11: 625033

Stals, I., K. Sandra, B. Devreese, J. Van Beeumen, and M. Claeyssens. (2004) "*Factors influencing glycosylation of* Trichoderma reesei *cellulases. II: N-glycsoylation of Cel7A core protein isolated from different strains*" Glycobiol. 14(8): 725-737.

302B **Expanding the use of targeted liposomes from an antifungal treatment to a fungal glycan capture tool** Quanita Choudhury[1], Suresh Ambati[2], Collin Link[1], Xiaorong Lin[1], Zachary Lewis[1], Richard Meagher[2] [1]Microbiology, University of Georgia, [2]Genetics, University of Georgia

Invasive fungal infections cause over one million deaths globally each year. Issues with existing antifungal treatments include drug toxicity and rising levels of drug resistance. Amphotericin B (AmB) is a broad-spectrum antifungal that is used as a primary treatment of limited duration due to its toxicity. Our research group designed DectiSomes – AmB-loaded liposomes that are coated with immune receptors such as Dectin-1 – in order to target AmB specifically to fungal pathogens. DectiSomes exhibit improved targeting efficacy in vitro against *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans,* and *Rhizopus delemar,* the common causative agents of aspergillosis, candidiasis, cryptococcosis, and mucormycosis, respectively. To see if we could expand our options for DectiSome immune receptors, we constructed a Dectin-3-targeted DectiSome and evaluated its efficacy against *C. albicans, C. neoformans,* and *R. delemar*. Dectin-3 has previously been reported to interact with *C. albicans* and *C. neoformans*, but no interactions with *R. delemar* had been reported. Our data show that Dectin-3-targeted DectiSomes bind to *C. albicans*, *C. neoformans*, and *R. delemar*, and also inhibit *C. albicans* and *R. delemar* in vitro. Our research group's overarching goal is to optimize DectiSomes as a pan-fungal treatment against invasive infections.

Despite their importance in immune functions, the precise extracellular carbohydrate content of most pathogens and the carbohydrate specificity of most Dectins are poorly understood. Therefore, we are also evaluating whether Dectin-targeted liposomes can serve as a glycan capture tool. We propose that Dectin-targeted liposomes will be powerful tools to investigate the ligand specificity of Dectins because the liposomal membrane association of Dectins mimics Dectin behavior on the cell surface. This allows Dectins to efficiently adopt the homo- or heterodimeric conformations that are required to bind ligands. We have generated a biotinylated Dectin-1-targeted liposome that binds to streptavidin beads, and this tool effectively pulls down *R. delemar* germlings in liquid culture compared to biotinylated untargeted liposomes. Given that Dectin-3 is the least characterized of the Dectin receptors, our ultimate goal is to construct a biotinylated Dectin-3-targeted liposome, isolate bound ligands from fungal cultures, and analyze these ligands via mass spectrometry. This will ultimately further our understanding of Dectin interactions with fungal pathogens.

303B **Fungi Unleashed – Rapid Ionomic Profiling with Laser-Induced Breakdown Spectroscopy** Tomas A Rush[1], Ann M Wymore[1], Miguel A Rodriguez[2], Sara A Jawdy[1], Rytas J Vilgalys[3], Madhavi Z Martin[1], Hunter B Andrews[4] [1]Bioscience Division, Oak Ridge National Laboratory, [2]BioEnergy Science Centre, Center for Bioenergy Innovation and Biosciences Division Oak Ridge National Laboratory, [3]Biology Dept, Duke University, [4]Radioisotope Science and Technology Division, Oak Ridge National Laboratory

Nutrient acquisition, delivery, and availability dictates microbes' phenotype, lifestyle, and survival. One of the best microbes to study the influence of substrate availability is fungi, because they are heterotrophic, meaning they cannot produce their food from environmental nutrients. Tapping into this biological process by manipulating the substrate available has led to discoveries in identifying how fungi interact with a host or environment or biological products like alternative sources of protein and natural products. Despite the scientific interest and multifaceted roles that nutrient acquisition plays in controlling fungal behavior and producing biological products, the elements that are obtained and how they differentiate across fungal species have remained a largely unexplored area of research. To address this knowledge gap, we used laser-induced breakdown spectroscopy (LIBS) to identify the fungal ionomic profiles of two genetically different fungal species, *Hyaloscypha finlandica* and *Mucor hiemalis*, grown on defined and undefined substrate media. Through Pearson correlation coefficients, we had identified strong positive correlations with the emissions from carbon, zinc, phosphorus, manganese, and magnesium. The positive correlations seen with these elements in both species indicates their vital role in fungi propagation and survival. When the Pearson correlation coefficients of each fungi species are compared to one another a few noticeable differences are seen. Firstly, *H. finlandica* exhibits strong positive correlations between sodium, hydrogen, and the essential element group. This indicates *H. finlandica* has a reliance on sodium that *M. hiemalis* does not exhibit. A similar behavior is seen with potassium in *H. finlandica*, but generally a medium positive correlation exists between the essential elements and potassium. Interestingly, *M. hiemalis* shows a strong negative correlation between potassium and the essential elements. *M. hiemalis* shows stronger positive correlations between silicon, iron, and the essential elements; although, *H. finlandica* shows a positive correlation between silicon and calcium that *M. hiemalis* does not. Taken together, we provide data for the building blocks of what elements are needed for fungal growth and sustainability and how they differ across genetically diverse fungi.

304B **Biological control of Pythium pathogens in hydroponic greenhouses.** Carren B Burkey[1], Sunday B osamika[1], Jigar B Patel[2], Paul F Morris[1,3] [1]Biological Sciences, Bowling Green State University, [2]LLC, LLC BG BIOLOGICS, [3]LLC BG BIOLOGICS

Major investments in hydroponic greenhouse and vertical farming operations which use LED lighting and controlled environment conditions have enabled the local production of tasty leafy green produce on a year-round basis. These capital-intensive systems are more efficient in their use of land, water and fertilizer than conventional operations, and crop rotations are much faster because of extended photoperiods and elevated CO2. However, once Pythium pathogens get introduced into this artificial

environment, the hyphae produce zoospores that enable rapid dispersal throughout the system. Surface biofilms then provide a continuing inoculum source for new seedlings. Depending on the strain and environmental conditions, root infections can cause yellowing of leaves and complete crop losses, or be limited to impairment of nutrient absorption due to loss of root mass and slower growth rates. The reduced integrity of plant roots may enable pathogenic bacteria in the water to migrate via the plant vascular system into the leaves and potentially cause disease. Our surveys found that pythium strains were present on plant roots of plants from every commercial facility that we have screened to date. To address this problem, we used a high throughput screening program to identify several *Pseudomonas fluorescens* isolates from Lake Erie, that exhibit contact-dependent killing of pythium isolates collected from commercial operations across the country. Several genes that contribute to the killing phenotype in one strain has been identified by sequencing and targeted gene knockouts. We will describe a comparative genomics approach and a high throughput gene knockout strategy to identify other key molecular determinants of these biocontrol agents.

305B **Efficient genetic modifications via CRISPR/Cas9 genome editing in *Aspergillus sojae* and comparative analysis of strain-specific characteristics in soy sauce brewing** Takayuki Igarashi, Takuya Katayama, Jun-ichi Maruyama Dept of Biotechnology, The University of Tokyo

Aspergillus sojae has traditionally been used in soy sauce brewing. Genetic modification techniques have been established in *A. sojae*, but it is difficult to apply them to various industrial strains. In this study, we developed the CRISPR/Cas9 system as an efficient genetic modification technique for *A. sojae*[1].

We constructed autonomous replicable genome-editing plasmids bearing the Cas9 nuclease and sgRNA expression cassettes. When the genome-editing plasmids were introduced into three *A. sojae* industrial strains, mutations in the target sequences were detected in 83.3-100% of transformants. In addition, gene deletion and gene integration by introducing the genome editing plasmids together with donor plasmids were also successfully performed with 38.2-75.0% efficiencies. The results indicate that the genome editing technique allows efficient genetic modifications in *A. sojae*.

Using this technique, we constructed strains overexpressing *prtT*, known to be involved in induction of protease genes, in the three *A. sojae* industrial strains. In all the strains, *prtT* overexpression (*prtT*-OE) resulted in enhanced alkaline protease activity. Furthermore, we performed the deletion of the 250 kb large chromosomal region, which was previously reported to reduce the apparent viscosity of soy sauce mash, *moromi*. The large chromosomal deletion (Δ250-kb) did not reduce apparent viscosity in one of the three strains. Finally, strains with *prtT*-OE and Δ250-kb were obtained using the forced recycling technique of the genome editing plasmid, which had been developed in *Aspergillus oryzae*. Unexpectedly, the reduced apparent viscosity caused by Δ250-kb was suppressed by *prtT*-OE, and their suppression levels varied among the tested strains. These results indicate that the effects of *prtT*-OE and Δ250-kb on the apparent viscosity of *moromi* differ among *A. sojae* industrial strains.

Our technique using the CRISPR/Cas9 system is a powerful tool for genetic modification in *A. sojae* and will allow comparative genetic analyses related to soy sauce brewing.

[1]Igarashi *et al*., *Biosci. Biotechnol. Biochem.*, 87:1236-1248 (2023)

306B **Making Anti-Fungal Peptides (AFPs) More Potent Through Target-Specific Activation** David J Larwood, William F DeGrado Pharmacy / Chemistry and Chemical Biology, University of California, San Francisco

Of some 300 medically important fungi, a few species cause millions of infections annually. Only about 20 out of > 300 *Aspergillus* species cause human disease, dominated by *A. fumigatus*. The profile for *Candida* species is similar with *C. albicans* responsible for more than half the human deaths. Human health care costs of a *Candida* and *Aspergillus* infections each account for about 25% of the US total spend, with the balance caused by other fungal infections. Drug resistance to fluconazole and increasing resistance to echinocandins makes it critical to develop new antifungal drugs.

Antimicrobial peptides (AMPs) found in nature can be very effective at killing microbes by permeabilizing their membranes. Various AMPs significantly inhibit bacteria, viruses or fungi, with some compounds active against multiple classes. Attempts to develop AMPs as drugs have been frustrated due to significant off-target activity. For the better available AMPs, the ratio of safe dose to therapeutic dose (therapeutic index, TI) is barely above 1, acceptable for topical but not for systemic dosing. Our aim is to improve safety by using pro-drug AMPs that remain inactive until near their target site.

We report on progress to design, construct and study a range of pro-drug antifungal peptides (AFPs). An active AFP is aligned with a protective peptide sequence (PPS) that masks and limits activity of the AFP. The PPS and AFP are linked by a proteolytically sensitive sequence designed to be cleaved by a protease active at or near the target site, ensuring AFP release and activation only in the vicinity of the target cell or tissue. We proved cleavable activation in a related system. Work is continuing on antibacterial AMPs.

A first fungal target is using a mellitin derivative as the AFP and serine protease for activation. Additional AFP candidates include several reported novel AFPs, and we are developing more. We are looking at protease candidates as well. These de-novo peptides are designed in significant part with modern generative AI tools. We are making five constructs to confirm primary antifungal design criteria, from which we plan to make and test up to 20 more constructs by March.

307B **Characterization of acid phosphatases in *Aspergillus oryzae* strain with reduced "umami" degradation activity** Kanae Sakai[1], Tadahiro Suzuki[2], Yuichiro Horii[3], Yutaka Wagu[4], Ken-Ichi Kusumoto[5] [1]engineering, Osaka University, [2]National Agriculture and Food Research Organization, NARO, [3]Food Research Center, Niigata Agricultural Research Institute, [4]Bio'c Co., Ltd., [5]Osaka University

Miso, fermented soy bean paste, is a traditional Japanese seasoning. It is made from soybeans, salt, water, and *koji* (solid-state culture of *Aspergillus oryzae* on rice, soybean, or barley). To fit the demand of modern busy lifestyle, production of dashi (broth) containing miso has been increasing in recent decades. In the manufacturing process of dashi containing miso, heat treatment of miso is needed before adding dashi. Since acid phosphatase secreted by *A. oryzae* degrades one of the dashi component ribonucleotides yielding no taste ribonucleosides and phosphoric acid, acid phosphatase should be inactivated by heat treatment. However, heat treatment requires energy and special equipment and it reduces the quality of miso. In this study, we attempted to obtain a strain with low acid phosphatase activity to avoid heat treatment, and analyzed the characteristics of acid phosphatase for breeding purpose.

Through the screening of 503 practical *A. oryzae* strains stocked in Bio'c Co., we found a strain with greatly reduced acid phosphatase activity while maintaining protease and amylase activities sufficient for miso fermentation and named KBN-p [Food Sci. Technol. Res. (2012) 83-90]. Among 13 putative extracellular acid phosphatase genes (*aphA-M*) in *A. oryzae* genome, AphC was considered to be one of the main causes of low acid phosphatase activity in KBN-p strain based on the results of transcriptional analysis and activity test. When AphC amino acid sequence of KBN-p was compared to RIB40 and practical strain for miso koji (No.6020), 5 and 1 amino acid substitutions were found, respectively. So, we analyzed the properties of AphC with three different amino acid sequences. At first, 3 kinds of *aphC* genes were expressed under the *tef1* promoter in each *A. oryzae* strain and found that AphC (KBN-p) have some problem in secretion. However, it was difficult to determine the reason whether the secretion defect of AphC (KBN-p) in KBN-p host lies in the AphC sequence or the host strain itself. Three kinds of AphC were expressed in a unified *aphC* null mutant host and tested the properties of AphC activity in response to heat and NaCl which thought to be related to dashi containing miso making process. As a result, it was found that AphC (KBN-p) was less stable than the other AphCs (RIB40, No.6020). This property is thought to be advantageous for producing dashi containing miso without heat treatment.

308B **Engineering of *Aspergillus niger* for efficient production of xylitol from arabinose** Marcel Rüllke, Veronika Schönrock, J. Philipp Benz Fungal Biotechnology in Wood Science, Technical University of Munich

The ascomycete *A. niger* harbors multiple pathways to metabolize various complex carbon sources such as lignocellulosic biomass. Genetic remodeling of this organism has been shown to create strains with high capacities to convert cheap substrates into valuable products. The industrial sweetener xylitol - an intermediated of the pentose catabolism in *A. niger* - is an interesting bioproduct due to its health-benefits compared to sucrose. Industrial xylitol production relies on energy-intensive and environmental-unfriendly processes. As efficient producer of hemicellulases, *A. niger* is an optimal candidate for the production of xylitol from arabinan-rich agricultural waste streams like sugar beet pulp. In the wild type, arabinan is hydrolyzed and the free L-arabinose further metabolized via the pentose catalytic pathway. To generate a strain that can accumulate D-xylitol, three knock-outs were established in genes of enzymes involved in the degradation of D-xylitol: i) the xylitol dehydrogenase (XdhA) converting xylitol into D-xylulose, ii) the sorbitol dehydrogenase (SdhA) with xylitol-degrading site-activity, and iii) the D-xylulose kinase (XkiA), that phosphorylates D-xylulose to feed it into the pentose phosphate pathway. To minimize xylitol degradation and enable its secretion into the medium, a passive xylitol exporter from *Saccharomyces cerevisiae* was investigated for this approach. In *S. cerevisiae,* the aquaglyceroporin FPS1 was shown to be involved in glycerol transport upon osmotic stress but seems to be also able to transport xylitol. Therefore, a constitutively opened version of this transporter was expressed in *A. niger* under control of multiple constitutive promoters with different expression strengths. The triple KO strain expressing the transporter with lowest

expression levels showed the best results, yielding up to 35 % of D-xylitol from L-arabinose and depicting the lowest side effects during osmotic stress.

Taken together, these results present a promising starting point for environmentally friendly and cheap generation of xylitol as alternative sweetener from arabinan-rich agricultural waste streams.

309B **High-throughput screening of filamentous fungi using droplet digital microfluidic system.** Chiara Leal Alves[1], Mari Valkonen[2], Adiphol Dilokpimol[2], Ulrike Abendroth[2], Steve Shih[1] [1]Center of Structural and Functional Genomics, Concordia University, [2]VTT Technical Research Centre of Finland Ltd

Filamentous fungi represent a diverse group of microorganisms with remarkable physiological and metabolic capabilities, playing key roles in biotechnological processes, environmental remediation, and the production of various bioactive compounds. They are one of the main organisms in industrial biotechnology and bioprocessing, due to their natural ability to secrete a wide array of proteins. For example, the industrial strains of *Trichoderma reesei* may secrete native hydrolytic enzymes in levels of over 100 g/l to the culture broth. However, yields of non-native proteins are often less impressive, which is an issue generally occurring with all fungal hosts. Hence, methods improving protein secretion in filamentous fungal hosts are extremely desirable.

Traditional methods for improving fungal strains and assessing their protein secretion profiles involve time-consuming and labor-intensive procedures. The emergence of microfluidic technologies has revolutionized the screening process by allowing precise control of microscale droplets, enabling high-throughput experimentation in different organisms. However, microfluidics is typically ill-suited for filamentous fungi since, unlike bacteria and yeast, their hyphal growth poses a notable challenge for the microfluidic droplet integrity. Long incubation times are required to screen secreted proteins during which, hyphae usually pierce through the microfluidic droplets. Additionally, optimizing the incubation time is a challenge, as it requires fine-tuning to find the right balance between allowing sufficient time for protein secretion and preventing prolonged incubation that may lead to overgrowth or depletion of nutrients. To address these challenges, we have used colloidal chitin, a naturally occurring biopolymer, as a solid support to control the growth of filamentous fungi within 3nL water in oil droplets. This approach allows longer incubation times, and subsequent sorting of the strains by droplet high-throughput screening. Here, we present an efficient but affordable droplet microfluidic-based high-throughput platform for screening of industrial filamentous fungi *Trichoderma reesei* and *Aspergillus oryzae* with enhanced secretion capabilities. The platform is based on direct screening of fluorescent fusion proteins or enzymatic activity using fluorescent substrates. This novel platform will advance the screening of modified fungal host strains for more efficient production of industrial enzymes.

310B **Role of non-programmed cell death inducing effectors in the *Parastagonospora nodorum*-wheat necrotrophic interaction** Gayan K Kariyawasam[1], Nathan A Wyatt[2], Zhaohui Liu[1], Timothy L Friesen[3] [1]Plant Pathology, North Dakota State University, [2]Sugarbeet and Potato Research Unit, Edward T. Schafer Agricultural Research Center, USDA-ARS, [3]Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, USDA-ARS

Effectors are small, secreted cysteine rich proteins that are involved in host-pathogen interactions. *Parastagonospora nodorum*, which causes septoria nodorum blotch of wheat, has been known to produce multiple necrotrophic effectors that induce programmed cell death (PCD) in wheat genotypes that carrying the corresponding susceptibility genes. To date, five necrotrophic effectors including *SnToxA*, *SnTox1*,*SnTox267*, *SnTox3* and *SnTox5* have been cloned and functionally characterized. However, little to nothing is known about the role of non-PCD inducing effectors during infection. Therefore, in this study the proteome of *P. nodorum* strain Sn2000 was screened using signalP v5.0 and effectorP v3.0 and RNA-Seq data were generated for samples collected at 0, 4, 12, 24, 48, 72, and 96 hours post inoculation of SnTox5 producing isolate Sn2000 on the wheat differential line LP29, which carries the host susceptibility gene *Snn5*. *In silico* analysis predicted a total of 563 effectors whereas RNA-Seq analysis supported *in planta* expression of 435 of those predicted effectors at least once across all the time points. Out of 435, 250 effectors showed at least 100 reads across RNA-Seq libraries and were therefore used in downstream analysis. Differential expression analysis showed 146 effector genes were differentially expressed whereas 104 were constitutively expressed in time points relative to the expression at 0 hpi. InterProScan screening of these effectors revealed, 50 of the predicted effectors are cell wall degrading enzymes, three are chitin binding proteins, seven are proteases, four are necrotrophic effectors, eight provide protection from reactive oxygen species, five provide protection from other host defense mechanisms, and 24 are involved in nutrient break-down. These results, together with temporal gene expression analysis, suggest that these non-PCD inducing effectors not only play a critical role in adhesion, penetration, and colonization, but also provide protection to the fungus in adverse conditions created during PCD. In addition, no function was predicted for 121 effectors and currently we are in the process of functionally characterizing them to understand their role in the *P. nodorum* infection process.

311B **All Hands on Dect: Treating Cryptococcosis with DectiSomes** Nhu Pham, Ran Shi, Suresh Ambati, Richard Meagher, Xiaorong Lin University of Georgia

Cryptococcosis is a deadly disease that affects both immunocompetent and immunocompromised communities. This fungal infection is responsible for 19% of all the AIDS-related deaths. As it stands, systemic cryptococcosis which leads to cryptococcal meningitis is often fatal even with antifungal treatment. The WHO recommended therapy includes an induction therapy with a single high dose of AmB-loaded liposomes (AmB-LLs, AmBisome®) at 10 mg/kg, followed by maintenance therapy with an azole drug. So far, AmB is the most effective but also most toxic drug for treating cryptococcosis. This limits its use to short-term therapy, which affects the treatment outcome and potentially increases relapses in patients. Therefore, there is an urgent need to increase its efficacy without increasing its toxicity. Our group previously developed a targeted antifungal drug delivery system where host-pathogen receptor dectins were used to decorate AmB-LLs (DectiSomes). Dectins are host C-type lectin receptors that are mostly expressed on dendritic cells. These host receptors recognize the oligoglycans decorated on the fungal cell surface or extracellular matrix and initiate an immune response. Due to our DectiSomes having dectins embellished around the liposomes, there is a tighter specificity for the drug to target the fungus. Here, we showed that in both primary pulmonary cryptococcosis and systemic cryptococcosis models, a single dose of Dectin-2 coated liposomal amphotericin B (Dec2-AmB-LLs), relative to the control AmB-LLs, reduced fungal burden and prolonged animal survival. Our results demonstrate the promise of DectiSomes in improving the outcome of cryptococcosis therapy.

312C **Mycelia in the Mix: Unraveling the Impact of Additives on *Ganoderma sessile* and *Trametes versicolor* in 3D Printed Biocomposites** Caleb O Bedsole[1], Al Mazedur Rahman[2], Yeasir Mohammad Akib[2], Jillian Hamilton[2], Lucia Gonzalez Rodriguez[2], Zhijian Pei[2], Brian d Shaw[2] [1]Plant Pathology & Microbiology, Texas A&M University, [2]Texas A&M University

Biocomposites formed by fungi growing within organic substrates from agricultural waste hold promise for sustainable manufacturing. Utilizing these waste products not only aids in reducing the accumulation of materials in landfills but also contributes to the mitigation of carbon emissions by utilizing on-site materials for construction, thereby minimizing the need for transportation. This study utilizes various substrates, including sawdust and wheat straw colonized by *Ganoderma sessile* and *Trametes versicolor* mycelia, through a 3D printing platform first developed by the authors. Here, the impact of additives that enhance the printing process on the growth of the fungus is investigated. Specifically, sodium alginate and calcium chloride were introduced as additives to explore their influence on fungal growth. Sodium alginate is commonly used for 3D printing biological products as it aids in controlling viscosity and as a supportive matrix when submerged in calcium chloride. Both sodium alginate and calcium chloride were found to impede fungal growth at tested concentrations in the 3D printable mixtures. These findings provide valuable insights into the roles of these additives in *Ganoderma sessile* and *Trametes versicolor* 3D printing applications, contributing to our understanding of optimal working concentrations for future applications and optimizing the growth parameters for the preparation of the biocomposites for 3D printing.

313C **From Fungi To High-Tech Cheese: How To Use Precision Fermentation To Rescue Our Food System** Beatrice Bernardi, Bastian Jöhnk Formo Bio GmbH

Our current food system is increasingly discussed as a root cause of climate change and human health (Poore & Nemecek, 2018). The global dairy industry alone is responsible for 4% of global greenhouse gas emission. In addition, livestock production leads to severe losses in biodiversity and fertile farming land each year. Consumer awareness and increased availability of animal-free products on the market is promoting the rise of flexitarian, vegetarian and vegan diets. A recent study on global consumer acceptance of animal-free dairy products, found strong enthusiasm in trying and regularly consuming animal-free dairy products (Zollman & Bryant, 2021). Hence, it is crucial that the food industry responds by enlarging and improving the animal-free dairy market. Plant-based products are a sustainable alternative to animal-based, but plant ingredients can be challenging for the food science industry, especially when emulating taste and texture of traditional dairy products.

The use of microorganisms as food ingredient is usually referred to as processed biomass or to protein purified from it (Graham & Ledesma-Amaro, 2023). For many years microorganisms have been used for drugs and food additives production thanks to the development of precision fermentation technology. Precision fermentation is a refined form of brewing or winemaking, where microbes are fermented to produce a specific molecule, which can be purified from the fermentation broth. This technology can be applied to produce the products of choice at industrial scale, including macronutrients for animal-free replacement products.

At Formo, we are focusing on reimagining dairy products using precision-fermented single protein ingredients. In particular, we are expressing recombinant milk proteins in a variety of expression hosts. Filamentous fungi, due to their ability for high-yield

recombinant protein secretion and their capability of post translational modifications, are a promising platform for milk protein production. However, fungi also pose some inherent challenges like limited genetic accessibility, high proteolytic activity or secretion inhibiting pellet-forming morphology. Therefore, we decided to fix these issues by a series of genetic modifications that led to a strain, which demonstrates high efficiency for targeted genomic modifications, low proteolytic activity and a completely dispersed growth phenotype. This modified base-strain showed superior recombinant protein production compared to the WT.

Using fungi as cell-factories to produce food ingredients through precision fermentation technology is a valuable answer for bringing sustainable, fair, and tasty products into our future food system.

314C **Modernizing high-throughput mycology with robotics and artificial intelligence** Johan V Christiansen, Søren D Petersen, Vilhelm K Møller, David Llorente, Parvathy Krishnan, Steen S Brewer, Katharina Steinert, Alexander R Brems, Sabrina M Pittroff, Mathilde Nordgaard, Vincent Wiebach, Niels Bjerg, Lars Jelsbak, Ling Ding, Jakob B Hoof, Jens C Frisvad, Rasmus J N Frandsen Bioengineering, Technical University of Denmark

Filamentous fungi and their secondary metabolites have been proposed as solutions for many global crises, including as biocontrol agents of agricultural pests. However, high throughput functional screening of filamentous fungi is currently challenging due to the large morphological and physiological diversity, and as most mycological experimental methods have not been developed with a high throughput in mind. Therefore, our project has taken on the task of modernizing mycological methods to create a high throughput screening platform through robotic workflows, automatic data processing and machine learning. The goal is to be able to screen the extensive IBT fungal collection with 38,000+ isolates. To achieve this, we are transferring single-vial strain spore stocks to 96-well plates compatible with robotic workflows, such as cultivation on various defined and complex growth media, metabolite extraction, bioactivity assays and taxonomic profiling using genetic barcodes. We are systematically gathering high resolution mass spectrometry metabolomics data for all isolates to allow for artificial intelligence-based data mining focused on identifying industrial relevant metabolites and predicting which metabolites and species carry bioactivity. For unbiased, fast, and automatic metabolomics data processing and analysis, we have developed a pipeline using state of the art tools wrapped in a Python framework. Additionally, we automated image analysis is used to score the outcome of fungal-fungal interactions and capture basic growth characteristics of the strains. The automated analysis workflow has proven extremely important as our current robotics workflow supports the analysis of 800 strains every two-to-three weeks.

We are currently using this platform to screen IBT isolates for their ability to in vitro inhibit growth of the plant pathogens *Fusarium graminearum* and *Zymoseptoria tritici*. The metabolomics database and taxonomic genotyping data allows for deselection of fungi that produce mycotoxins or are classified as pathogens. The best in vitro performing isolates are continuously tested in planta (green house and field trials) by our commercial partner, FMC. The combined dataset aims to identify fungal isolates that can be used as control agents in agriculture.

316C **Genetically manipulating anaerobic, lignin-degrading microbial communities** Vikram Mubayi[1], Sarah Seagrave[2], Amanda Alker[3], Jaymin Patel[3], Brady F Cress[3,4], Benjamin E Rubin[3,4], Michelle O'Malley[1,2,4] [1]Chemical Engineering, University of California Santa Barbara, [2]Bioengineering, University of California Santa Barbara, [3]University of California Berkeley, [4]Joint BioEnergy Institute

Establishing a circular carbon economy is essential to address climate change by reducing the impact of anthropogenic CO2. Our proposed solution to mitigate this rapid build-up of plastic waste involves improving the biodegradation and conversion of lignocellulose to higher-value chemicals. The recalcitrance of lignin has prevented the efficient utilization of lignocellulose, but increasing numbers of microbes and their natural communities are emerging as specialists in the biodegradation of lignin. Our collaborators through the Joint Bioenergy Institute (JBEI) provided us with a tool to study these anaerobic, lignin-degrading communities. Rubin et al. (2022) developed DNA-editing all-in-one RNA-guided CRISPR–Cas transposase (DART), which allowed our collaborators to modify specific sites within specific bacterial species within a complex microbial community, while keeping the community completely intact.

My research will adapt DART to determine lignin degradation pathways in anaerobic communities and reveal the enzymes relevant for these processes. Initially, as a proof-of-concept, I have been studying the anaerobic bacterium *Prevotella bryantii*, which was previously isolated from the gut of a herbivore and identified as an organism with lignin degrading capabilities. I aim to use DART to genetically modify *P. bryantii* as an isolate, then see if I can perform similar modifications to *P. bryantii* while it is in a complex anaerobic community. I hope to use DART to gain deeper insight into these lignin degrading communities, to study the relevant enzymes and degradative mechanism that anaerobic bacteria and fungi employ to breakdown these recalcitrant materials. Given

the structural similarities between lignin and certain aromatic plastics, I will also apply the knowledge of lignin breakdown to study how these microbial communities respond to plastics.

317C **A two-step method to generate marker-less mutants in *Coccidioides*** M. Alejandra Mandel[1], Mana Ohkura[2], Lisa F Shubitz[1], Hien Trinh[1], Christine Butkiewicz[1], John N. Galgiani[1], Marc J Orbach[3] [1]Valley Fever Center for Excellence, University of Arizona, [2]Oregon State University, [3]School of Plant Sciences, Univ Arizona

Coccidioides species are the causative agents of the mammalian disease Valley fever, or coccidioidomycosis, endemic to the SW US and the Americas. Deletion of the *C. posadasii CPS1* gene resulted in a mutant strain, *Δcps1*, that shows efficacy and safety as a live-attenuated vaccine to prevent coccidioidomycosis and is being developed for commercial use as a canine vaccine. The original Δ*cps1* mutant (Narra et al 2016) was made by replacing the six kb *CPS1* gene with the hygromycin phosphotransferase drug resistance marker (*hphB*), allowing selection for the transformed line. For development of a human vaccine candidate, it would be desirable to have a *CPS1* deletion strain that doesn't contain a foreign gene insertion. To create such a strain, we have developed a novel system using a two-step process to eliminate our gene of interest by incorporating a "suicide gene" vector system, thus creating a marker-less *CPS1* deletion mutant. The Herpes simplex virus gene that encodes for thymidine kinase (HSV-tk) was used as a suicide gene and 5-fluoro-2'-deoxyuridine (F2dU) as counter-selection. First a *Δcps1* strain with *CPS1* replaced by an Hph/HSV-tk cassette was created (Δ*cps1*::hph^R/tk+) using selection for hygromycin resistance. This strain was then transformed with a construct that contains the fused 5' and 3' flanking sequences of the *C. posadasii CPS1* gene using selection on F2dU. This compound is lethal for strains that express the HSV-tk gene. Strains that grew on F2dU were screened for deletion of the Hph/HSV-tk sequences via homologous recombination with the fused *CPS1* flanking sequences. The resulting strain has a *CPS1* deletion containing the fused flanking sequences and no foreign sequences.

This two-step process is key for two reasons: One is the ability to create marker-less mutant strains, which allows marker recycling for creation of strains with multiple mutations. Second, the marker-less *CPS1* deletion mutant may be critical for development of a live-attenuated Δ*cps1* human vaccine.

Mouse studies indicate that the marker-less Δ*cps1* strains appears to be identical to the original Δ*cps1* strain both in virulence and in vaccine protection experiments.

318C **FACS-based method streamlines pooled transformations in *Aspergillus oryzae*** Sarah McFarland[1], Jonathan Pham[2], Sandeep Sharma Khatiwada[1], Eric Carter[3], Ceanne Brunton[1] [1]MSE, Novozymes Inc, [2]MDS, Novozymes Inc, [3]MAA, Novozymes Inc

Through precision fermentation, we use genetically engineered microbes including *Aspergillus oryzae* to produce enzymes, proteins, or other compounds in a controlled, sustainable, and animal-free manner. If we can make more protein/enzymes production from our production organisms for the same amount of input costs, we can make our biosolutions increasing cost competitive. Therefore, we often look for ways to improve the productivity of our production strains. Signal peptides are an important contributor to the secretion potential of a candidate protein and a possible route to increasing production strain performance. Here we discuss the how we tested a library of 100+ signal peptides and developed a high throughput method for pooled transformation, FACS (Fluorescence-activated cell sorting), and automated screening to rank *Aspergillus oryzae* strains based on productivity.

319C **Exploring soil bacteria for aerobic detoxification of deoxynivalenol** Natalia Martínez Reyes[1], Rocío Montes-Ruiz[1], Susan P McCormick[2], Robert H Proctor[2], Rafael Balaña-Fouce[3], Estela Melcón-Fernández[3], Rosa E Cardoza[1], Pedro A Casquero[1], Santiago Gutierrez[1] [1]University Group for Research in Engineering and Sustainable Agriculture (GUIIAS), Area of Microbiology, Universidad de León, [2]Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, Agriculture Research Service, U.S. Dept of Agriculture, [3]Dept of Biomedical Sciences, Faculty of Veterinary Medicine, Universidad de León

Trichothecenes, a group of mycotoxins produced by pathogenic fungi such as *Fusarium*, pose a significant threat to crops and human and animal health due to their inherent toxicity. The use of fungicides can reduce pathogenic fungi and mycotoxin levels. Other strategies include selection of fungi-resistant plants and the use of post-contamination remedies such as thermal treatments, activated carbon, and biological agents like probiotics. Nevertheless, these strategies provide limited effectiveness and can compromise the grain quality. The alternative enzymatic detoxification for contaminated grain selectively deactivates toxins, presenting a significant advantage over the other methods. Thus, this study focuses on identifying and characterizing

aerobic soil bacteria able to detoxify trichothecenes, focusing on the widely prevalent deoxynivalenol (DON) produced by *Fusarium*, offering a promising avenue for mitigating mycotoxin contamination.

More than 80 soil samples from different cultivars of the region were collected. To evaluate DON degradation activity, the screening method included a cultivation approach in a mineral medium using DON as the sole carbon source. After incubation, cultures were analyzed by HPLC to assess DON content.

Significant reductions in DON content were observed across many samples, as evidenced by the chromatographic data. While the majority exhibited a new peak whose amounts correlated with the observed reduction in the level of DON. Some samples displayed unique patterns, including complete DON reduction without any new peaks. Since our objectives extend beyond DON degradation to toxicity reduction, toxicological analyses were performed to assess the cytotoxicity of metabolites produced during bacterial degradation. Preliminary toxicological analyses suggest a correlation between the chromatographic profile and cell toxicity. Interestingly, the consistent peak appearing after DON metabolization is shown to possess a toxicity level similar to DON itself.

Further investigations and collaborations are underway to isolate the bacteria responsible of DON degradation, and to identify newly formed compounds detected by HPLC. This research provides insights into potential soil bacteria for this environmentally friendly approach to mitigate mycotoxin-associated risks in agriculture and food and feed industry.

320C **Understanding the inner workings of the basidiomycete Fomes fomentarius for materials applications** Carsten Pohl[1], Bertram Schmidt[1], Ulla Simon[2], Tamara Nunez-Guitar[1], Carsten Lühr[3], Justus Zillessen[1], Fangxing Zhang[4], Heiko Briesen[4], Hans-Jörg Gusovius[3], Vera Meyer[1] [1]Applied and Molecular Microbiology, Technische Universität Berlin, [2]Chair of Advanced Ceramic Materials, Technische Universität Berlin, [3]Dept Systems Process Engineering, Leibniz-Institute for Agricultural Engineering and Bioeconomy (ATB), [4]Chair of Process Systems Engineering, Technical University of Munich

To achieve climate neutrality, fundamentally new concepts of circularity need to be implemented by the building sector, a significant contributor of anthropogenic CO2 emissions. Recently, we have shown that the polypore Fomes fomentarius feeds well on renewable lignocellulosic biomass and produces composite materials that could potentially replace fossil fuel-based expanded polystyrene as insulation material.

In follow up work, we explored the mechanical and physical properties of F. fomentarius-based composite materials in more detail and determined key performance parameters that are important to evaluate the usability of F. fomentarius-based composite materials in the construction sector. These parameters were determined according to European standards and included compressive strength, modulus of elasticity, thermal conductivity, water vapour permeability, and flammability of uncompressed composites as well as flexural strength, transverse tensile strength, and water absorption capacity of heat-pressed composites, among others.

We furthermore show that heat-pressing can be used to reliably generate stiff and firm particleboards that have the potential to replace current wood-based particleboards that contain synthetic additives. X-ray microcomputed tomography finally visualized for the first time the growth of hyphae of F. fomentarius on and into the hemp shive substrates and generated high-resolution images of the microstructure of F. fomentarius-based composites.

Lastly, we used transcriptomics to characterize the expression of cell wall genes under various growth conditions and attempt to link the impact of the cell wall composition to the observed material properties.

321C **The Dark Side of Anaerobic Digestion: Carbohydrate-Active Enzymes from Uncultured Rumen Fungi** Katharine L Dickson[1], Itai Brand-Thomas[1], Claire Shaw[1], Charles G Brooke[1], Markus DeRaad[2], Robert Evans[2], Michael Endres[3], Gyorgy Babnigg[3], Vivian Chu[1], Tri Do[1], Abigail Pfefferlen[1], Vincent Lombard[4], Asaf Salamov[2], Alex Copeland[2], Kerrie Barry[2], Hailan Piao[4], Roderick I Mackie[5,6], Scott Baker[4], Samuel Deutsch[2], Susannah G Tringe[2], Bernard P Henrissat[7], Igor V Grigoriev[2], Jan-Fang Cheng[2], Soichi Wakatsuki[8], Yasuo Yoshikuni[2], Trent Northen[9], Andrzej Joachimiak[3], Matthias Hess[1] [1]Dept of Animal Science, University of California, Davis, [2]DoE Joint Genome Institute, Lawrence Berkeley National Laboratory, [3]Argonne National Laboratory, [4]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, [5]Dept of Animal Sciences, University of Illinois, Urbana-Champaign, [6]Dept of Nutritional Sciences, University of Illinois, Urbana-Champaign, [7]Dept of Biotechnology and Biomedicine, Technical University of Denmark, [8]SLAC National Accelerator Laboratory, Stanford University, [9]Environmental Genomics and Systems Biology Division

Anaerobic fungi of the Neocallimastigomycota are important fibrolytic microbes in the gut of large herbivores, degrading plant biomass via mechanical and enzymatic means. Even though anaerobic fungi are known to be vital to biomass digestion, mechanisms underlying anaerobic fungal biomass degradation are poorly understood at the level of individual enzymes. To probe the extent of anaerobic fungal contributions to biomass degradation in the rumen and identify novel carbohydrate-active enzymes (CAZymes) participating in this process, as well as their potential role in in situ biomass conversion, we isolated anaerobic fungal enzyme sequences from rumen microbiome samples, then transformed them into expression vectors and evaluated their capacity to degrade a set of polysaccharide substrates. We introduced samples of switchgrass and corn stover into the rumen of cannulated cows for 48 hours, then isolated polyadenylated RNA from each substrate sample and sequenced it to obtain a polyadenylated metatranscriptome. After annotating metatranscriptomes for their phylogenetic composition and identifying putative CAZyme sequences, a subset of 21 of the 73 glycosyl hydrolase (GH) families probed was selected for further analysis based on increased abundance on either switchgrass or corn stover, relative to rumen fluid, or previously determined functions of importance to biomass degradation. Phylogenetic trees of these sequences were constructed, and a subset of these sequences representing the greatest sequence diversity, novelty, and potential CAZyme activity was selected for transformation into E. coli BL21(DE3) for functional characterization on carboxylmethylcellulose (CMC), as well as the insoluble chromogenic substrates AZCL-Amylose, AZCL-HE-Cellulose, AZCL-Pullulan, and AZCL-Xylan (Oat) (Sigma-Aldrich, St. Louis, MO) (Neogen, Lansing, MI). (something about Western blot analysis). 15 of 196 clones of these selected sequences exhibited activity on at least one substrate, representing members of GH6, GH8, GH9, GH11, and GH48; of these, seven came from the metatranscriptomes and were thus designated novel CAZymes. Two of these clones, IBT63 and IBT64, represent the first GH8s identified from anaerobic fungi. Two additional clones, IBT52 and IBT54, represent the first fungal CAZymes, of family GH6, to incorporate the carbohydrate-binding module CBM10; IBT54 additionally is the first identified fungal CAZyme to incorporate an Fn3-like domain, which form multi-domain complexes with CBM3. In addition, IBT84 and IBT85 contained enzymes combining GH11 and GH10 domains, suggesting a possible synergistic relationship between these two domains. These results further suggest possibilities for acquisition of some of these domains from bacteria via horizontal gene transfer, as well as synergy between some of these CAZymes as deployed in the rumen.

322A **Ergosterol is critical for sporogenesis in *Cryptococcus neoformans*** Amber Matha, Xiaofeng Xie, Xiaorong Lin University of Georgia

Microbes, both bacteria and fungi, produce spores to survive stressful environmental conditions. Spores produced by the fungal pathogen *Cryptococcus neoformans* serve as both surviving and infectious propagules. Because of their importance in disease transmission and pathogenesis, factors important for cryptococcal spore germination are being actively investigated. However, little is known about nutrients critical for sporogenesis in this pathogen. Here, we found that spores, relative to yeasts and hyphae, are enriched in ergosterol, the main sterol in fungal membranes. In *C. neoformans*, the ergosterol biosynthesis pathway (EBP) is upregulated by the transcription factor Sre1 in response to conditions that demand elevated biosynthesis of ergosterol. Although deletion of *SRE1* enhances the production of mating hyphae, the *sre1*Δ strain is deficient at producing spores. We found that the defect of the *sre1*Δ strain is specific to sporogenesis, not meiosis or basidium maturation proceeding sporulation. Consistent with the idea that sporulation demands heightened ergosterol biosynthesis, EBP mutants are also defective in sporulation. Moreover, overexpression of some EBP genes can largely rescue the sporulation defect of the *sre1*Δ strain. Collectively, we demonstrate that ergosterol is a critical component in cryptococcal preparation for sporulation.

323A **Investigating dormancy and its breaking in *Aspergillus fumigatus*** Justina M Stanislaw[1], Michelle Momany[2] [1]Plant Biology, University of Georgia, [2]University of Georgia
The initiation, maintenance, and breaking of dormancy across fungi is understudied and the biological mechanisms are ill defined. In the case of *Aspergillus fumigatus*, it is intriguing that a process so crucial to the survival of the conidium, the infectious propagule of invasive aspergillosis, remains unclear. In the present study, we investigate the germination process by analyzing *A. fumigatus* RNA-seq transcriptome data. We hypothesize that factors important in inhibiting germination will be proteins of unknown function that are highly differentially expressed in conidia. Transcripts that were found to be High In Conidia (HIC) relative to hyphae were selected as candidates for creation of a knockout collection, and HIC mutants were screened for their roles in dormancy and its breaking. Here we show data on HIC mutant Δ*hicA* suggesting that *hicA* may be an inhibitor of germination. Future work with this group of candidate genes will include phenotypic characterization of their roles in dormancy, germination and other aspects of development.

324A **Sex & Speciation: exploring the mechanism of sexual incompatibility by live-cell imaging of fertilization in *Podospora anserina*** Pierre Grognet[1], Sven Saupe[2], Fabienne Malagnac[1], Antoine Guichet[3], Sylvain Brun[4] [1]I2BC, Université Paris-Saclay, [2]IGBC, CNRS, [3]Institut Jacques Monod, CNRS, [4]Institut Jacques Monod, Université Paris Cité

Sexual reproduction in fungi and fruiting body development have attracted interest of researchers for centuries [1]. By setting up live-cell imaging of fertilization in *Sordariomycetes*, we have discovered the extraordinary manner with which male nuclei migrate across the trichogyne in *Neurospora crassa* [2] and we have shown that this phenomenon is conserved in the model fungus *Podospora anserina*. In order to elucidate the first steps of sexual reproduction, namely plasmogamy and better characterize the migration of male nuclei across the trichogyne towards the ascogonium in the core of the protoperithecium, we have started the functional analysis of both steps through live-cell imaging in *Podospora anserina*. In particular, the main questions we are addressing are, i) which components of the cytoskeleton are involved in male nuclei migration and how do they support the highest migration speeds ever observed for nuclei in eukaryotic cells, ii) how the envelope of these male nuclei (measuring 2 µm of diameter when they are embedded in conidia/spermatia) as well as the chromatin they contain, resist to the extreme stretching of more than 60 µm they face when they are submitted to pulling forces during migration, iii) identifying mutants of the process. Here, we present our first results identifying that microtubules support male nuclei migration. Furthermore, tagging of the nuclear envelope of male nuclei highlights its flexible and loose nature throughout the migration of male nuclei. Finally, in the context of the study of male sterile mutant, we have characterized, at plasmogamy, for the first time at the microscopy level, a cell death reaction related to sexual incompatibility. Since sexual incompatibility has a strong role in structuring the natural population in *Podospora*, resulting in a quasi-speciation effect[3], this approach amounts to the live exploration of the mechanistic basis of sexual isolation in that species.

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325A **Deciphering the roles of jumonji domain containing proteins in *Podospora anserina*** Mengyuan Li, Fabienne Malagnac, Pierre Grognet Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC)

In eukaryotic organisms, histone proteins are associated with DNA inside the nucleus. This structure called chromatin is associated with the epigenetic component of gene regulation. Indeed, epigenetic histone post-translational modifications or histone marks are set up by specialized enzymes, which lead to either an open conformation of chromatin, namely euchromatin, in which transcription occurs; or a close conformation of chromatin, namely heterochromatin, blocking transcription. However, the chromatin conformation needs to be dynamics to allow a fine regulation of gene expression. Thus, these histone marks have to be periodically removed for other marks to be deposited. The methylation of histone lysine is performed by histone methyltransferases (HMTs), whereas the removal of methyl group, or demethylation, is performed by histone demethylases (HDMs). HDMs containing a JmjC domain are widely conserved among Eukaryotes. In the model fungus *Podospora anserina*, it has been shown that the deletion of HMT coding genes caused defects in many aspects of the life cycle such as growth, differentiation, gamete production, sexual development, etc. (Carlier *et al*., 2021). However, compared to HMTs, there are only few studies on HDMs in fungi. We searched *P. anserina*'s genome for genes predicted to encode JmjC domain containing proteins. We detected 12 candidates for which knock-out mutants have been constructed. Here we present the analysis of this gene family and describe the phenotypes of the deletion mutants. These mutant are affected in a wide range of processes, such as sexual development, longevity and RIP. In addition, we have performed ChIP-seq and RNA-seq to get a genome wide insight on the changes in epigenetic landscape and transcription states.

This study, which complements our previous work (Carlier *et al*., 2021), provides a comprehensive view of the set of fungal enzymes that erase some of the histone marks that make up the histone code.

326A **Loss of female fertility may be beneficial for conidial dispersal and mycovirus elimination in the rice blast fungus** Momotaka Uchida[1], Kohtetsu Kita[1], Syun-ichi Urayama[2], Shin-ichi Fuji[3], Daisuke Hagiwara[2], Hiromitsu Moriyama[4], Tsutomu Arie[4], Takayuki Arazoe[1], Takashi Kamakura[1] [1]Tokyo University of Science, [2]University of Tsukuba, [3]Akita Prefectural University, [4]Tokyo University of Agriculture and Technology

The rice blast fungus *Pyricularia oryzae* (syn, *Magnaporthe oryzae*) is thought to have diverged from a *Setaria italica*-infecting strain in the Middle Yangtze Valley of China, during or shortly after rice domestication in East Asia. Although some isolates from

the region of origin retain their mating ability (female fertility), most field isolates are female sterile. Therefore, female fertility may have been lost during its geographical spread from the region of origin, and *P. oryzae* is an ideal biological model for studying why many filamentous ascomycete fungi lost sexual competence.

To identify the gene responsible for female sterility (female sterility gene), we performed recurrent backcrossing between two field isolates: CH598 (female fertile) and Kyu89-246 (female sterile). Genome sequencing and gene editing analyses revealed that the loss of function of Pro1, a global transcriptional regulator of mating-related genes in filamentous fungi, is one cause of loss of female fertility in Kyu89-246. RNA-seq analysis showed that Pro1 of *P. oryzae* upregulated a broad range of mating-related genes, including those involved in hyphal fusion and ascogonium formation, similar to *Sordaria macrospora*. Dysfunctional Pro1 variants were identified in several geographically distant isolates, including pandemic isolates of wheat blast fungus, suggesting a similar evolutionary significance. Therefore, we investigated whether the loss-of-function of Pro1 confers an advantage for asexual reproduction and found that Pro1-deficient mutants increased conidial detachment from conidiophores without affecting pathogenicity. Interestingly, all the ten field isolates infected with various combinations of mycoviruses retained their functional *Pro1* ORF. Among these, two isolates exhibiting significantly slower growth were selected for further analysis. By disrupting the *Pro1* gene in the isolates, mycelial growth and/or melanization were rapidly recovered in accordance with the reduction of mycoviral RNAs. This phenotype was consistent with that of Pro1-deficient mutants of the chestnut blight fungus *Cryphonectria parasitica*. Thus, female sterility evolution caused by the loss of Pro1 function in *P.* oryzae may be advantageous for asexual reproduction in nature in terms of conidial dispersal and mycovirus elimination.

327A **TORC 1 Signaling and Cell Growth Control in Budding Yeast** Andrew P Capaldi University of Arizona

Humans have harnessed the power of yeasts such as *Saccharomyces cerevisiae* for thousands of years, using them to make beer, wine, and bread, and more recently to produce pharmaceuticals and biofuels. At the same time, other yeasts are dangerous pathogens, including *Candida albicans*, *Candida glabrata,* and the newly emerging *Candida auris.* But how does each yeast species regulate its growth, stress responses, and morphological state so that it can survive, propagate, and/or infect another organism? Over the last ten years we have studied the main cell growth and stress response control circuits in yeast, including the TORC1 and PKA pathways, and discovered (i) that these highly conserved pathways have been dramatically remodeled in the yeasts compared to humans and (ii) that the pathways have also been rewired during the evolution of specific yeast species. Here we describe our current model of TORC1 and PKA signaling in *S. cerevisiae* including elements that are conserved in a subset of budding yeasts (the *Saccharomycetaceae/codaceae*) and elements that are conserved across all yeasts but not in worms, flies, or mammals. The main focus of the talk will be unpublished work where we used phosphoproteomics and other technologies to determine how the highly conserved TORC1 regulators Gtr1/2 (Rags in humans) cooperate with the yeast specific TORC1 regulator Pib2, to drive the cell into different stress and starvation states so that cells can grow on alternate nitrogen sources.

328A **A CRISPR/Cas9 system in *Neurospora crassa* – user-friendly, fast and efficient** Stefanie Gruettner, Frank Kempken Genetics and Molecular Biology in Botany, Botanical Institute of Kiel University

Due to its diverse biology and rapid growth, *Neurospora crassa* is a widely used eukaryotic model organism and especially offers advantages in genetic studies. Traditional genetic manipulation methods, such as homologous recombination, are time-consuming and labor-intensive[1]. Conversely, the RNA-guided CRISPR/Cas9 system offers high efficiency, is easy to handle and allows a precisely targeted mutagenesis. It has already been successfully applied in some filamentous fungi[2]. Here, we present a user-friendly CRIPSR/Cas9 system for *N. crassa*, by integrating the *cas9* sequence into the fungal genome and introducing guide RNA (gRNA) as naked RNA via electroporation into the cell. Our approach eliminates the need for constructing multiple vectors, speeding up the mutagenesis process. When editing the *cyclosporin-resistant-1* (*csr-1*) gene as a selectable marker, we achieved 100% editing efficiency under selection conditions. Furthermore, we successfully edited the non-selectable gene *N-acylethanolamine amidohydrolase-2* (*naa-2*), demonstrating the system's versatility. Combining gRNAs targeting *csr-1*and *naa-2* simultaneously increased the probability of finding mutants carrying the non-selectable mutation. The system is not only user-friendly but also effective, providing a rapid and efficient method for generating loss-of-function mutants in *N. crassa* compared to traditional methods.

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2. Schuster, M. & Kahmann, R. CRISPR-Cas9 genome editing approaches in filamentous fungi and oomycetes. *Fungal Genetics and Biology* vol. 130 43–53 Preprint at https://doi.org/10.1016/j.fgb.2019.04.016 (2019).

329A **UV induces translation in Fusarium species in a developmentally-regulated manner** Quyen Hoang[1], Shira Milo-Cochavi[1], Sapna Mishra[1], Shay Covo[2] [1]Hebrew University, [2]Plant Pathology and Microbiology, Hebrew U

Universally, DNA damage is known to inhibit translation. We previously reported that the same is true for Fusarium species inflicted with damage early on during germination. Here we show that UV irradiation induces translation when applied at a letter developmental stage where all spores were already germinated and established a filament. We observe doubling of the polysomes following UV as well as considerable increase in translation capacity. When the translation induction is suppressed, UV sensitivity is increased and DNA repair is decreased. The genes that are signifi cantly associated with polysomes after UV exposure belong to modules responsible for cell homeostasis and proteome health (protein folding). Surprisingly, the translation induction is at least partially independent of the TOR signaling.

330A **Modeling Asynchronous Nuclear Division in *Ashbya Gossypii*** Grace McLaughlin[1,2], Jay Newby[3], Veronica Ciocanel[4], Lauren Melfi[5], Benjamin Stormo[4], Therese Gerbich[6], Marcus Roper[7], Alexander Mayer[7], Timothy Elston[8], Amy Gladfelter[4] [1]Cell Biology, Duke University, [2]Biology, University of North Carolina at Chapel Hill, [3]University of Alberta, [4]Duke University, [5]Wentworth Institute of Technology, [6]Harvard University, [7]UCLA, [8]University of North Carolina at Chapel Hill

Multinucleate cells are common in biology, with examples including muscle cells, placenta, and fungi. Despite this, many aspects of their cell biology are not well understood. Dividing nuclei residing in a common cytosol would be expected to synchronize, as the oscillating levels of cell cycle regulators from each nucleus should in theory entrain neighbors. However, in the multinucleate fungus *Ashbya gossypii*, spatially neighboring nuclei have been observed to divide out of sync. Here we mathematically model *Ashbya* nuclei as a dynamically growing system of coupled phase oscillators to determine possible mechanisms that could lead to asynchronous division. Our goal is to study the effects of nuclear mobility, cytosolic compartmentalization, inhibitory signals, and noise on transient phase dynamics. To compare the model with experimental results, we develop a nuclear tracking pipeline with the aim of tracking nuclei during bypassing events, identifying nuclear division, and linking nuclei into hyphae. Initial results suggest a combination of locally and globally acting mechanisms are at play leading to the observed asynchrony in *Ashbya*.

331A **The molecular mechanisms of toxocyst development in oyster mushroom** Yi-Yun Lee[1,2], Sheng-Chian Juan[1,2], Yen-Ping Hsueh[1,2] [1]Institute of Molecular Biology, Academia Sinica, [2]Molecular and Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Sciences, National Defense Medical Center

Pleurotus ostreatus, the oyster mushroom, utilizes lollipop-shaped structures, toxocysts, to rapidly paralyze and kill nematodes under starvation. We have conducted forward genetic screens in *P. ostreatus* and isolated 22 *loss-of-toxicity* (*lot*) mutants that were incapable of paralyzing *C. elegans*. Whole genome sequencing and genetic mapping were employed to pinpoint the causative mutations in *lot1*, *lot2*, and *lot3* mutants to be pho85-cyclin 1 (*PCL1*), *HIS1*, and a transcription factor for toxocyst development 1 (*TTD1*), respectively. First, we explored the connection between Pho85, a cyclin-dependent protein kinase, and Pcl1. Through yeast two-hybrid assays, we demonstrated a physical interaction between Pho85 and Pcl1, suggesting that the Pho85-Pcl1 complex may influence the cytoskeleton and septin organization, leading to toxocyst formation. Subsequently, we developed a congenic strain, PC9.15, derived from the original mutagenized PC9 strain, and conducted bulked segregant analysis to map the remaining 19 *lot* mutants. Our analysis revealed that the *lot5*, *lot9*, *lot12*, *lot15*, and *lot21* mutants have mutations in tetrahydrofolate synthase (*MIS1*), *HIS4*, and *HIS5*. Furthermore, histidine supplementation in the growth medium successfully restored the deficiency in toxocyst development observed in these *lot* mutants, indicating the histidine biosynthetic pathway is required for toxocyst development.

332A **Structural and molecular investigation of secondary metabolite compartmentalization in fungal vesicles** Fabio Gherlone[1], Katarina Jojić[1], Slavica Janevska[2], Vito Valiante[1] [1]Leibniz-HKI Jena, [2]Centre for Innovation Competence Septomics

Fungal secondary metabolites (SMs) are a rich source of active compounds. The discovery of the chemical repertoire and biosynthesis of fungal SMs has increased over the past years, such as their chemical variability, the regulation and the organization of biosynthetic gene clusters (BGCs), as well as the comprehension of the producing organisms. Genome mining prediction and pathway biochemical elucidation are crucial in moving forward compound discovery. However, to have a full picture of biosynthetic pathways, the knowledge on enzymes and their activity needs to be coupled with the understanding of their spatial and temporal organization within the cell. Many subcellular sites of SM-associated proteins have been already described, and the fungal vesicular transport system was found to be is essential in the formation of compartments and the sorting of biosynthetic enzymes.

We studied the compartmentalization of the enzymes involved in the biosynthesis of sphingofungin (Aspergillus fumigatus) and fumonisin (Fusarium verticillioides), observing that it involves the endoplasmic reticulum (ER), ER-derived vesicles, and the cytosol.

However, all available prediction tools failed to detect the presence of so-called signal peptides (SPs) in their protein sequences. We thus tackled the problem from another angle. Batch alignment of gene orthologues in both BGCs exhibited conserved N-terminal regions for the sphingofungin and fumonisin biosynthetic enzymes SphH and Fum15, respectively (both P450 monooxygenases). Submission of these amino acid sequences to AlphaFold2 revealed that these peptides contain two alpha helixes spaced by a flexible loop. This was quite unexpected, since N-terminal helix-loop-helix (HLH) structures have been mainly found in DNA-binding proteins, such as transcriptional regulators.

These newly identified HLH domains of SphH and Fum15 were systematically mutated and tagged with GFP, and fluorescent protein localization was analyzed using confocal microscopy, first in Saccharomyces cerevisiae and then in Aspergillus *fumigatus*. 20-30 amino acids were found to be sufficient for correct localization of the fusion constructs, in perinuclear ER and ER-derived vesicles. Such HLH domain was also found C-terminally in SphF (a 3-ketoreductase in the sphingofungin BGC), and indeed was also alone sufficient for correct protein localization. This study gave us the first insights into this novel domain´s putative secondary structure and structure-related function, such as membrane topology.

333A **Septins Regulate Exocytosis through Physical Interactions with the Exocyst Complex during Fission Yeast Cytokinesis** Davinder Singh, Yajun Liu, Jian-Qiu WU The Ohio State University

Septins are GTP-binding proteins that can function as scaffolds for protein recruitment, membrane-bound diffusion barriers, or membrane curvature sensors. Septins play important roles in cytokinesis, but the nature of these roles is still obscure. In fission yeast, four septins (Spn1 to Spn4) accumulate at the rim of the division site as rings and last until cell separation. The octomeric exocyst complex, which tethers secretory vesicles to the plasma membrane, exhibits a similar localization pattern and is essential for plasma membrane deposition during cytokinesis. We find that the exocyst complex cannot maintain its ring localization without septins. Instead, the exocyst spreads across the whole division plane during furrow ingression in cytokinesis. Loss of the exocyst complex at the rim of the division plane results in mistargeting of secretory vesicles and their cargos at the division site. This contributes to a delayed cell separation in septin mutants. These results suggest that septins and the exocyst may physically interact. Indeed, we predicted several pairs of interactions between septin and exocyst subunits by AlphaFold2 ColabFold, which are confirmed by co-immunoprecipitation assays. Our results indicate that septins are important in regulating the exocyst localization on the plasma membrane for vesicle tethering during cytokinesis.

334A **VE-1 regulates the transcription and accumulation of components of the MAPK signalling pathway during sexual development in *Neurospora crassa*** Sara Cea-Sánchez, Sara Martín-Villanueva, Gabriel Gutiérrez, David Cánovas, Luis M Corrochano Dept of Genetics, University of Seville

The velvet complex is a fungal-specific protein complex that participates in the regulation of gene expression during development, pathogenesis, and secondary metabolism in response to environmental signals such as light. In *Neurospora crassa* the velvet complex is composed of VE-1, VE-2, and LAE-1. Strains with deletions in *ve-1* or *ve-2* have increased conidiation, and delayed and reduced sexual development. Alterations in the development of female structures (protoperithecia) in the *ve-1* and *ve-2* mutants suggested that a protein complex composed of VE-1/VE-2 regulates transcription during sexual development. The transcriptomes of the wild-type and the *ve-1* mutant strains were characterized in a time-course experiment during sexual development in dark and light. We identified 2,117 genes with different transcriptional profiles between the wild-type and the mutant strain in cultures kept in the dark, and 4,364 genes when cultures were kept in the light with an overlap of 1,648 genes. Among the misregulated genes, we detected genes that are known for their regulatory roles in sexual development, including genes in the mitogen-activated protein kinase (MAPK) signaling pathways, cell-cell fusion genes (*ham* genes) and transcription factor genes involved in fruiting body development. We detected in vitro binding of VE-1 and VE-2 to the promoter sequences of *mak-1*, *mak-2* and *mek-2* suggesting that VE-1/VE-2 plays a direct regulatory role in the transcription of MAPK genes. We also observed a reduction in the accumulation and phosphorylation of MAK-1 and MAK-2 during sexual development in the *ve-1* mutant. Furthermore, we detected transcription of *ve-1*, *ve-2,* and *lae-1* during all stages of sexual development, but the three proteins were not detected in the later stages of development (96 and 144 hours after fertilization). Our results suggest that the absence of VE-1 results in transcriptional changes that disrupt the MAPK signal transduction cascade regulating sexual development in *N. crassa*.

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335A **Interrupting the progression of an amphibian pandemic** David Firer[1,2], Don C Sheppard[1,2], Francois Le Mauff[2,3] [1]Microbiology and Immunology, McGill University, [2]Infectious Diseases and Immunity in Global Heath Program, Research Institute of the McGill University Health Center, [3]Microbial glycomics node, GlycoNET Integrated Services

Background: In recent decades, a mass extinction of amphibian populations has been driven by infection with the chytrid fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). These infections begin when motile chytrid zoospores adhere to the amphibian epidermis. These zoospores then mature into sessile zoosporangia which are supported by an invasive rhizome anchor that disrupts epithelium integrity, leading to amphibian death. Zoosporangia also serve as the sites of asexual reproduction, generating new zoospores which are then released via discharge tubes at the zoosporangial surface. These morphological changes require remodeling of the fungal cell wall, a structure that is currently uncharacterized in chytrids.

Hypothesis: Interfering with cell wall remodeling during discharge tube formation will prevent zoospore release.

Methods: *Bd* and *Bsal* cell wall polysaccharides were identified using mass spectrometry techniques. Exposed glycan motifs were localized across chytrid life cycles using lectin-assisted fluorescence confocal microscopy. The involvement of key cell wall polysaccharides in discharge tube formation was investigated by microscopy using glycan synthase inhibitors. Zoospore release and viability were assessed by flow cytometry.

Results: *Bd* and *Bsal* cell walls were found to contain predominately glucose and *N*-acetylglucosamine, with smaller quantities of mannose, xylose, and galactose. Sequential extraction of fungal polymers revealed that chytrids have a unique polysaccharide composition within the fungal kingdom, consisting largely of cellulose and chitin. This rare combination of polymers was confirmed by confocal microscopy and revealed to exhibit distinct distribution patterns. While cellulose was observed at the zoosporangium surface, chitin was found at rhizomes and at discharge tube formation sites. Inhibition of chitin synthesis by Nikkomycin Z during discharge tube formation resulted in abnormal discharge tube morphology and significantly decreased zoospore release.

Conclusion: This work reveals that *Bd* and *Bsal* share a similar cell wall composition, with cellulose and chitin at their core. More importantly, these data show that *Bd* and *Bsal* use a common strategy for zoospore release, providing great insight into this critical virulence process. This study paves the way for the discovery of novel universal antifungal targets which may be essential in limiting the further decline of amphibian populations.

336A **Correlation among nuclear increase, enzyme production and hyphal morphology in *Asperillus oryzae*** Ayaka Itani[1], Naoki Takaya[1], Oda Ken[2], Hideyuki Yamashita[3], Kanae Sakai[4], Kenichi Kusumoto[4], Norio Takeshita[1] [1]University of Tsukuba, [2]NRIB, [3]Higuchi Moyashi, [4]University of Osaka

Koji molds have long been used for fermentation and brewing due to their high secretion capacity of enzymes that break down carbohydrates, proteins, and other substances. We have discovered a phenomenon in which the number of nuclei of *Aspergillus oryzae* (RIB40) increases with the passage of incubation time. The increase in the number of nuclei is expected to increase the amount of transcription and translation. In fact, we found the correlation between the number of nuclei and enzyme activity. This phenotype was also observed in *Aspergillus sojae*, but not in the closely related *Aspergillus nidulans* or *Aspergillus flavus*. We also compared morphology and growth rates in hypha with and without increased nuclei. Live imaging showed that hypha with increased nuclei appeared by branching, grew very fast, and occupied the colony perimeter with incubation time. TEM imaging showed that hypha with increased nuclei had thicker cell wall and increased mitochondria. We searched for genes involved in the nuclear increase by comparing the genomes of *A. oryzae* strains with different phenotypes within the same clade. In addition, we collected hypha with and without increased nuclei by laser microdissection and analyzed changes in gene expression. In hypha with increased nuclei, the expression of genes involved in morphology such as the cytoskeleton and cell wall, nuclear and cell division, and Ca^{2+} transport was specifically increased.

337A **Generation and characterization of serial deletion- and point-mutants within the 5´-UTR region of *brlA* allow the identification of promoter sequences required and dispensable for *Aspergillus nidulans* conidiation** Ainara Otamendi[1], Alotz Bereziartu[1], Ziortza Agirrezabala[1], Eduardo A. Espeso[2], Oier Etxebeste[1] [1]Applied Chemistry, University of the Basque Country, [2]Dept of Cellular and Molecular Biology, CIB Margarita Salas - CSIC

In the genus *Aspergillus*, the transcription factor BrlA has a central role in the generation of the cell-types that form the conidiophore, multicellular structures each bearing thousands of conidia. Asexual development is induced in response to external and internal cues, and thus, it has been described that several transcriptional activators bind the promoter of *brlA* to determine its expression levels before and during development. Furthermore, transcriptional repressors also bind the 5´-UTR region of *brlA* 1)

to inhibit expression at late stages of conidiophore development, and 2) to activate sexual development. In this work, we followed a mutagenesis procedure to generate 1) *brlA::HA$_{3x}$* strains that included serial deletions of the promoter of *brlA* (*brlA^{p}*; approximately 3 Kb from the start codon of *brlAβ*), strains in which the hypothetic binding sites for *brlA^{p}*-binding transcription factors were deleted and 3) strains bearing point mutations in the first 23 codons of *brlAβ* (those that differentiate *brlAβ* from the isoform *brlAα*). None of the strains generated showed the *fluffy* phenotype characteristic of the null-*brlA* mutant and only deletion of the *brlA^{p}* region that includes an upstream open reading frame caused a decrease in conidia production. These results raise new hypotheses on the mechanisms that control *brlA* expression and the role of BrlA.

338A **On the evolution of clock mechanisms in fungal systems: a case of moonlighting functions of core-clock components?** Carlos Corrial[1], Consuelo Olivares-Yanez[2], Luis F Larrondo[1] [1]iBio, Pontificia Universidad Catolica de Chile, [2]Centro de Biotecnología Vegetal, Universidad Andrés Bello

During the past decade our lab has been studying how light and time shape fungal physiology and organismal interactions, aiming to dissect the molecular mechanisms underpinning circadian clocks and light perception.

Thus, for example, we provided for the first-time evidence of the importance of clock regulation in the interaction between a phytopathogenic fungus and a plant host. However, the relevance of circadian clocks in fungal-fungal interactions remains largely unexplored. We have now characterized a functional clock in the biocontrol agent *Trichoderma atroviride* to assess its importance its mycoparasitic action against the phytopathogen *Botrytis cinerea*. The results highlight the relevance of clock components, as well as dark/light conditions in the way organismal dynamics are established. By utilizing luciferase reporters to monitor the *T. atroviride* core-clock, we confirmed the existence of circadian oscillations that are temperature compensated and can be modulated by environmental cues such as light and temperature. Importantly, the presence of such rhythms appears to be highly dependent on the nutritional composition of the media, which suggest a conditional gating of clock mechanisms. Indeed, while we have identified defined nutritional cues that enable visualizing rhythms, several other ones appear to tamper with *bona fide* circadian oscillations.

Notably, our current evidence (as obtained in both in *B. cinerea* and *T. atroviride*) indicates that the main clock component (FREQUENCY) exhibits extra-circadian roles, particularly in the cross-roads of development, and metabolism, impinging Nitrogen assimilation and secondary metabolism, raising interesting questions about the origin and evolution of clock components in fungi, and suggesting potential moonlighting function for these clock proteins. Current effort concentrates on defining how FRQ converses with other regulators, and on how such interactions have emerged in the evolutionary history of this core-clock component.

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339A **Developing a new generation of antifungals: Identifying the targets of natural products with antifungal properties** Caroline Wang[1], Chien Der Lee[1], Yi Tang[2], Benjamin Tu[1] [1]University of Texas Southwestern Medical Center, [2]University of California, Los Angeles

Invasive fungal infections are becoming more widespread throughout the world in recent years. This is due to recent medical innovations resulting in an increased number of immunocompromised people as well as a lack of variety in classes of antifungal drugs, resulting in more widespread antifungal resistance. For these reasons, developing more classes of antifungal drugs with new mechanisms of action is imperative. The most common sources for new antifungal and antibiotic drugs throughout history have been natural products isolated from fungi or bacteria. The goal of this project is to screen dozens of natural products isolated from native fungal strains or engineered heterologous host strains for antifungal activity and elucidate their mechanisms of action to develop new classes of antifungal drugs. Using minimum inhibitory concentration analysis (MICs), we were able to show that 6 compounds have strong antifungal activity against *Saccharomyces cerevisiae* (AS2077715, aspterric acid, fischerin, rachel 400, parnafungin A, and parnafungin D). We performed mutagenesis on *S. cerevisiae* using in order to isolate resistant clones, and then used tetrad dissection to create pools of resistant and susceptible cells in order to isolate the mutation causing the antifungal resistance. Whole genome sequencing was performed on each pool to determine the genetic cause of the resistance. We are currently working on validating the targets of several of the compounds. Our next steps are to use CRISPR and plasmid-based-transformations to make knockouts or mutated versions of the genes of interest in order to determine if the mutations isolated are necessary and sufficient to convey resistance to each natural product.

340A ***Cryptococcus*** **employs alternative translation of a novel regulator *REF1* to produce isoforms with differential capacities for phase separation to control morphogenesis** Nathan K Glueck, Xiaorong Lin Microbiology, University of Georgia

Cryptococcus neoformans causes deadly meningoencephalitis in hundreds of thousands of immunocompromised individuals worldwide. *Cryptococcus* is a polymorphic fungus able to switch between yeast and hyphal growth as part of its sexual cycle, as a way of defending against natural predators, and as a mediator of fungal immunogenicity when interacting with mammalian hosts. The yeast-to-hypha transition is controlled by the transcription factor Znf2. Here, we have identified a new gene that antagonizes Znf2-mediated transcriptional activation and suppresses cryptococcal yeast-to-hypha transition. We named this gene repressor of filamentation 1 or *REF1*. Surprisingly, *REF1* transcription is controlled by Znf2 and is dramatically *increased* during hyphal morphogenesis. We discovered that transcriptional activation of *REF1* in response to filamentation-inducing stimuli produces 5'-truncated *REF1* transcripts. Transcription of these truncated transcripts starts downstream of the 5' splice site of the first intron in the *REF1* ORF, producing a larger Ref1 protein with an alternate N-terminus. Under nutrient-rich conditions favorable to yeast growth, basal level transcription of unmodified *REF1* transcripts results in the translation of Ref1 protein with a diffuse nucleoplasm localization. However, filamentation-inducing nutrient limitation causes a spike in 5'-truncated *REF1* transcripts (outnumbering basal level transcripts >20 fold) that are translated into a Ref1 isoform with a distinct nuclear punctate localization pattern indicative of phase separation. This nuclear punctate Ref1 isoform appears superior to the nuclear diffuse Ref1 in inhibiting filamentation but is more prone to proteolytic degradation. Our evidence points to a model in which Ref1 acts as a built-in negative regulator of Znf2-mediated transcriptional activation during the nascent stages of hyphal morphogenesis both as a bet-hedging strategy to avoid premature commitment of cellular resources to the yeast-to-hypha transition and to exert tight spatiotemporal control over the developmental process.

341A **Repair to survive: Tolerance to plant defence compounds involves several membrane repair strategies in the grey mould fungus *Botrytis cinerea*** Suraj Hassan Muralidhar[1], Linda Matz[2], Yaohua You[3], André Fleißner[2], Jan van Kan[1] [1]Lab. of phytopathology, Wageningen University and Research, [2]Institut für Genetik, Technische Universität Braunschweig, [3]Lab. of plant breeding, Wageningen University and Research

Fungal cells encounter potentially lethal plasma membrane injuries during their growth and development, and the survival of these injured cells depends on their ability to swiftly repair the damaged membranes. Despite fungal membranes being targeted by numerous clinical drugs and plant defense compounds, the molecular mechanisms governing membrane repair in fungi remain elusive. In this study, we investigated the mechanisms employed by the grey mould fungus *Botrytis cinerea* to tolerate the plant defense compound α-tomatine present in the vacuoles of tomato cells. *B. cinerea* utilizes a range of both pre-formed and induced membrane repair proteins to protect itself from the pore-forming activity of α-tomatine. The pre-formed calcium-binding proteins, Penta-EF-hand protein 1 (PEF1) and Annexins, along with the induced membrane repair proteins Glycosyl transferase family 28 (GT28) and Resistance to 7-aminocholesterol (RTA1), contribute to the tolerance to α-tomatine. Fluorescence microscopy revealed the recruitment of PEF1-GFP and GT28-GFP to the damaged sites following treatment with α-tomatine. GT28 is presumed to repair the membranes by glycosylating fungal sterols and thus preventing the interaction of α-tomatine with sterols, while the mode of action of PEF1 remains unknown. Staining with FM4-64 and filipin indicated that vesicles enriched in sterols swiftly accumulate at the damaged sites, suggesting a patching or plugging repair mechanism, as observed in animal models. These patches often co-localized with the membrane repair protein PEF1, providing instant protection against membrane damage. We also demonstrate that membrane repair is crucial for the pathogenicity of *B. cinerea*; both PEF1 and GT28 contributes to increased pathogenicity on tomatoes. Thus, this study highlights the crucial role of membrane repair during host-pathogen interaction.

342A **A novel chitin-binding apoplastic effector MoScp5 suppresses the chitin-triggered immunity by *Magnaporthe oryzae* is crucial for the rice blast disease** Zifeng Yang[1], Linwan Huang[1], Xinru Chen[1], Zonghua Wang[1,2], Wei Tang[1] [1]College of Plant Protection, State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, [2]Institue of Ocean Science, Minjiang University

Magnaporthe oryzae is a filamentous fungus that causes the blast disease of rice which results in severe losses of global rice production. Chitin is an important component of the phytopathogenic fungal cell wall and also functions as pathogen-associated molecular patterns (PMAPs) that can be recognized by plant pattern-recognition receptors to activate plant immunity. To escape chitin-triggered immunity, fungal pathogens have adopted several strategies, including the secretion of chitin-binding effectors to suppress plant immune recognition. Here, we identify a novel secreted protein containing a signal peptide but without any predicted domain in *M. oryzae*, named MoScp5 which is localized to the cell wall and can be secreted to the apoplastic space between the fungal cell wall and the rice plasma membrane during infection. Deletion of *MoSCP5* significantly impaired the pathogenicity of *M. oryzae*. Importantly, the C-terminal of MoScp5 can bind to chitin and is able to suppress chitin-induced plant

reactive oxygen species (ROS) production. Furthermore, we show that MoScp5 not only completes with rice chitin receptor OsCEBiP for binding chitin polymers but also directly interacts with OsCEBiP to evade the subsequent immune signaling. In conclusion, we characterized and functionally analyzed a novel chitin-binding apoplastic effector MoScp5 which can suppress chitin-induced ROS burst to establish successful infection of the host. Further detailed investigation on MoScp5 and OsCEBiP interaction sites and networks is warranted.

343A **Filamentous fungi release extracellular vesicles at log- and stationary phases** Shunichi Urayama[1], Mio Saito[1,1], Yuka Iwahashi[1], Ayaka Itani[1], Shunsuke Masuo[1], Akihiro Ninomiya[2], Hiromitsu Moriyama[3], Naoki Takaya[1], Nobuhiko Nomura[1], Norio Takeshita[1], Masanori Toyofuku[1], Daisuke Hagiwara[1] [1]University of Tsukuba, [2]The University of Tokyo, [3]Tokyo University of Agriculture and Technology

Extracellular vesicles (EVs) are small lipid bilayer vesicles released by various cells, influencing diverse biological traits and impacting both recipient and producing cells. The processes involved in EV production have been thoroughly examined in animals and bacteria, encompassing phenomena like plasma membrane blebbing and explosive cell lysis. These investigations contribute to a deeper comprehension of EV functions and their application in diverse biotechnologies. Over the past five years, an escalating number of studies on the role of fungal EVs, particularly in human pathogenic fungi and plant pathogenic fungi, have underscored their significance in the realms of fungal biology and ecology. Yet, the mechanisms governing the production of EVs in filamentous fungi remain unclear, lacking comprehensive cross-sectional and molecular genetic studies.

In this study, we explored EV production during the cultivation of Aspergillus oryzae and other filamentous fungi throu gh a time-course analysis. Our findings indicate that the occurrence of EV formation during the stationary phase is a common trait among filamentous fungi, with a few species capable of releasing EVs during the log phase. This discovery unveils the presence of previously undefined EV subpopulations. Our observations also suggested the relevance of the cell wall. In particular, the fact that most filamentous fungi released EVs during the stationary phase suggested the possibility that EV release was due to a common phenomenon in filamentous fungi, such as autolysis, which loosens the cell wall structure. Consistent with this hypothesis, cell wall-degradation or deletion of genes related to cell wall integrity induced EV formation. Microscopic examination further reveals explosive cell lysis and blebbing as mechanisms governing EV formation in filamentous fungi. In summary, our investigation proposes that filamentous fungi release EVs through explosive cell lysis and blebbing, processes influenced by cell wall attenuation and turgor pressure.

344A **Identification of an a-factor-like peptide mating pheromone secreted by the heterothallic ascomycete *Aspergillus fumigatus*** Sven Krappmann[1], Elisabeth Gabl[1], Tobias Pazen[1], Anna Heizmann[1], Stefanie Pöggeler[2], Minou Nowrousian[3] [1]University Hospital Erlangen, [2]University of Göttingen, [3]University of Bochum

Fungal sexuality accompanied by the formation of fruiting bodies that contain fertile meiospores relies on a complex sequence of events. In heterothallic ascomycetes, mating-type systems serve as regulatory means to secure that compatible isolates of opposite gender fuse to enter the sexual phase in their life cycle. This intricate process requires reciprocal secretion and recognition of pheromones, small peptides that are processed from precursors to become secreted into the cellular vicinity. Identification of mating pheromones of fungal origin with their cognate receptors is generally achieved by genome mining and homology searches, based on considerable conservation on the protein sequence level. In the taxonomic class of the Eurotiomycetes this approach had failed for peptides that would resemble a-factor-like pheromones due to their small size and low sequence conservation. Accordingly, the existence and nature of an a-factor-like peptide secreted by the heterothallic mould Aspergillus fumigatus had not been revealed to date. Sexuality of this opportunistic human pathogen is genetically determined by a bipolar mating-type system encoding master regulators in an exclusive manner. By making use of consistent transcriptional profiling data, we could identify an unannotated candidate gene *ppgB* (*p*heromone *p*recursor *g*ene *B*) encoding the presumed but so far elusive a-factor pheromone of *A. fumigatus*. The deduced peptide is 24 amino acids in length and comprises a canonical CaaX box motif at its C-terminus. Transcription patterns of *ppgB* and functional analyses of its hydrophobic product employing a suitable test system that is based on pheromone-sensitive yeast cells strongly support the hypothesis that PpgB serves as prototype for the long-sought a-factor like pheromone of the aspergilli. The identification of *A. fumigatus* PpgB closes a substantial knowledge gap with respect to cellular recognition and sexual propagation of Eurotiomycete fungi.

345A **Deter mining the cellular architecture of *Magnaporthe oryzae* appressoria through Cryo-Electron Tomography** Lauren S Ryder[1], Juan Carlos de La Concepcion[2], Yasin F Dagdas[2], David Haselbach Carlos de La Concepcion[2], Lucile Michels[3], Joris Sprakel[3], Alice B Eseola[4], Weibin S Ma[4], Nicholas Jose Talbot[5] [1]The Sainsbury Laboratory, [2]GMI, GMI, [3]Wageningen University, [4]TSL, The Sainsbury Laboratory, [5]TSL, TSL

Rice blast is among the most devastating diseases affecting global agriculture and is caused by the ascomycete fungus *Magnaporthe oryzae*. The blast fungus enters the plant using a dome-shaped infection structure called an appressorium. These infection cells generate enormous turgor pressure, which is directed towards the host, thereby breaching the plant cuticle and cell wall. Using fluorescence lifetime imaging of a membrane targeting molecular mechano-sensitive probe, we have visualised and quantified changes in membrane tension during appressorium development. This has revealed large-scale heterogeneity in appressorium membrane mechanics under extreme pressures, which are much greater than previously observed in any other cell type. Using this approach, we have characterised the role of a turgor-sensing histidine kinase signalling pathway in appressorium-dependent plant infection. To understand appressorium function in more detail, we are also taking advantage of recent advances in cryo-electron tomography (Cryo-ET) to visualise the cellular architecture of appressoria at unprecedented resolution. We have optimised a protocol to germinate fungal spores on Cryo-ET grids and induce development of appressoria on the surface of EM grids. This has allowed us, for the first time, to use techniques such as FIB-milling and Cryo-ET to study appressoria. We will report key cellular geometries and organisation associated with pressurised appressoria of the blast fungus. Our findings have the capacity to add new insight into appressorium-dependent plant infection, including the precise temporal and spatial regulation of melanin and septin-dependent re-polarisation during plant infection.

346A **Growth inhibition between filamentous fungal colonies of the same strain and its regulatory mechanism** Yuya Hamanaka[1], Takuya Katayama[1], Hiroki Kuroda[2], Jun-ichi Maruyama[1] [1]Dept of Biotechnology, The University of Tokyo, [2]Faculty of Environment and Information Studies, Keio University

Filamentous fungi form cell population as a colony and interact with adjacent cell populations. In particular, an interaction that inhibits growth between colonies is known as “antagonistic effect”. The growth inhibition between different species/strains has been analyzed for a hundred years. A number of mechanisms such as secretion of antibiotic metabolites have been suggested for the growth inhibition.

On the other hand, we found growth inhibition between colonies of the same strain in the filamentous fungus *Aspergillus oryzae*. Furthermore, growth inhibition between the same strain was observed in other filamentous fungal species. This phenomenon may reflect a fungal physiological interaction of self/nonself recognition among colonies of the same strain.

Based on RNA-seq and screening of transcription regulatory gene deletion library, we discovered that FlbA and LaeA were involved in the growth inhibition. To elucidate the regulatory mechanism, we first focused on RGS (Regulator of G protein signaling) protein FlbA, which accelerates GTPase activity of G protein α subunit FadA. As *flbA* deletion increases GTP-bound FadA, GTPase-deficient dominant active mutant of *fadA* was predicted to phenocopy Δ*flbA*. As expected, a dominant active FadA mutant did not show growth inhibition, suggesting the involvement of FadA-mediated G protein signaling in the growth inhibition. In addition, LaeA, another factor involved in the growth inhibition, is known to be positioned downstream of FadA-mediated G protein signaling, which regulates transition between vegetative growth and fungal development. Transcriptome analysis revealed that many downregulated genes at the confronted region of colonies overlapped in Δ*flbA* and Δ*laeA*, implying that FlbA and LaeA share a common signaling pathway. We will present the function of whole signaling pathway mediated by FadA G protein in the growth inhibition between colonies of the same strain.

347A **MoPce1, a CAP/PR domain containing effector is required for the pathogenicity of *Magnaporthe oryzae* by interacting with the OsDi19-5 in rice** Zhenyu Fang[1], Hunmin Bai[2], Xiaomin Chen[3], Guifang Lin[1], Zonghua Wang[4,5], Huakun Zheng[1] [1]Fujian University Key Laboratory for Plant Microbe Interaction, Fujian Agriculture and Forestry University, Fuzhou 350002, China., [2]State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002, China, [3]Fujian Agriculture and Forestry University, [4]State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002, China., [5]Fuzhou Institute of Oceanography, Minjiang University, Fuzhou 350108, China.

Rice blast fungus (*Magnaporthe oryzae*) causes massive yield losses annually worldwide. The fungus secreted bunch of effector proteins target different rice cellular compartments to facilitate its infection. However, most of intensively studied effectors are small secreted proteins with high presence/absence polymorphism in the fungus, and effectors conserved among different species

were rarely investigated. In this study, we identified MoPce1, a CAP/PR domain containing protein common in different species, as an important virulence factor from a screening of 145 putative core effectors (PCE). *MoPCE1* is required for pathogenicity but not the asexual development. Ectopic expression of MoPCE1$^{\Delta sp}$ in ZH11 background compromised the plant resistance. We also found that MoPce1 lacks the conserved cysteine residuals in CAP domain, and is BIC-localized in invasive hyphae and nuclei-localized when ectopically expressed in tobacco leaves and rice protoplasts. These results suggested that MoPce1 may bind novel ligand(s) rather than sterol. Indeed, we found that MoPce1 could interact with OsDi19-5, a transcription factor in rice. We inferred from these results that MoPce1 is required for pathogenicity by suppressing the immune response in rice, likely through the interaction with OsDi19-5.

348A **A nonclassically secreted effector of *Magnaporthe oryzae* targets host nuclei and regulates the rice immunity** Xiaomin Chen[1], Jiaxin Fan[2], Linwan Huang[2], Wei Tang[2], Zonghua Wang[2,3] [1]Fujian University Key Laboratory for Plant Microbe Interaction, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China, [2]State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China, [3]Institute of Oceanography, Minjiang University, Fuzhou 350108, China

Rice blast caused by *Magnaporthe oryzae* is one of the most destructive diseases and poses a growing threat to food security worldwide. Like many other filamentous pathogens, rice blast fungus releases multiple types of effector proteins to facilitate fungal infection and modulate host defence responses. However, most of the characterized effectors contain an N-terminal signal peptide. Here, we report the results of the functional characterization of a nonclassically secreted nuclear targeting effector in *M. oryzae* (MoNte1). We found that the MoNte1 encodes a nucleus-targeting, non-transcription factor type effector, which lacks an N-terminal signal peptide, but required for the pathogenicity of *M. oryzae.* In addition, we also aim to assess the role of MoNte1 and the NIPs (MoNte1 interacting proteins, NIPs) in the regulation of rice immunity, and dissect how the key NIP was regulated by MoNte1. Taken together, these findings reveal a novel effector secretion pathway and deepen our understanding of rice–*M. oryzae* interactions.

349A **Investigation of Differing Roles of Ammonium Transporters in the Nematode-trapping Fungus *Arthrobotrys oligospora*** Sheng-Chian Juan[1,2], Yen-Ping Hsueh[1,2] [1]Institute of Molecular Biology, Academia Sinica, [2]Molecular and Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Science, National Defense Medical Center

To adapt to environmental changes, cells require the ability to sense external signals. *Arthrobotrys oligospora*, a fungus belonging to a non-monophyletic group known as nematode-trapping fungus (NTF), senses prey signals and initiates trap morphogenesis under starvation. While a previous study has shown that ammonium suppresses trap formation in *A. oligospora* that the underlying mechanism is still unknown. Ammonium, a major nitrogen source that promotes growth for many microbes, is transported through the Ammonium transporter (Amt)/Methylammonium permease (Mep)/Rhesus protein (Rh) family. Therefore, to investigate how ammonium transport affects trap formation in *A. oligospora*, we first identified three potential ammonium transporters in *A. oligospora*, *MEP1, MEP2*, and *MEP3*. Phylogenetic analysis revealed that *MEP2* and *MEP3* are both high-affinity and low-capacity transporters, whereas *MEP1* is a low-affinity and high-capacity transporter. *MEP* gene expression is elevated in response to nematode exposure and subsequently downregulated upon prey capture, indicating their possible roles in the initiation of trap formation. Under low-nutrient conditions, single deletion mutants of *mep1* and *mep3* show decreased trap numbers, while traps numbers in *mep2* remain similar to wildtype. Double *mep* deletion mutants without enhanced phenotypes and the elevated gene expression of the remaining *MEP*(s) suggest functional redundancy among the three ammonium transporters. In contrast, upon addition of ammonium, *mep1* mutants were the only *mep* mutants to exhibit normal growth and suppression of trap formation, suggesting that Mep1 is the primary transporter involved in trap formation under ammonium treatment. Together, these results suggest that the Mep transporters are involved in trap formation initiation in *A. oligospora*. As nematode-trapping fungi (NTF) like *A. oligospora* have been proposed to be a biocontrol agent, an enhanced understanding of ammonium transport in this fungus is essential as agricultural environments are normally ammonium-rich.

350A **The NADPH-Oxidases NoxA and NoxB of *Arthrobotrys flagrans* are required for trap formation and functioning** Marius L Kriegler, Reinhard Fischer Dept of Microbiology, Institute for Applied Bioscience

Reactive oxygen species (ROS) control various aspects of the fungal lifestyle. One theory is that they act as second messengers promoting fruiting body development, hyphal fusion, symbiotic relationships and pathogenicity. However, their specific place of action remains elusive. One source for ROS are NADPH-oxidases, which form protein complexes at the cytoplasmic membrane. Here we studied the role of NoxA and NoxB in the nematode-trapping fungus *Arthrobotrys flagrans. A. flagrans* produces adhesive

trapping networks (Yu *et al*., 2021). The traps are differentiated hyphae which form a lasso-like structure where a hyphal branch forms a ring and fuses its tip with the original hyphae. This requires cell-to-cell communication and hyphal fusion (Wernet *et al*., 2023). A *ΔnoxA* strain was impaired in cell-to-cell communication and unable to close the trap ring. Deletion of NoxB did not impair trap formation or morphology. However, Δ*noxB* strains trapped only 10 % adult *Caenorhabditis elegans* in comparison to wild type. Larvae could be trapped much more efficiently than adult nematodes. This size-dependent trapping success may be explained by reduced stickiness of the traps. Deletion of the putative NoxB-interacting tetraspanin encoding gene *plsA* phenocopied the Δ*noxB* phenotype. The *noxB* gene was upregulated in traps, surprisingly only in two of the three cells. PlsA localized in small vesicles aligned at the inner rim of the trap. Our results demonstrate that NoxB and the tetraspanin PlsA are involved in pathogenicity of *A. flagrans* and appear to determine the stickiness of the traps. If and how they are related to the recently described *trap-enriched protein* TEP1 remains to be determined (Lin *et al*., 2023).

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351A **Vacuolar SNAREs-dependent retromer recruitment contributes to effective host invasion in *Magnaporthe oryzae*** Xin Chen, Haoming Zhong, Jiexiong Hu, Jie Zhou, Zonghua Wang, Wenhui Zheng State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350000, Fujian, China
The proper operation of vesicle transport depends on co-regulation of multiple proteins, including SNARE proteins and retromer complex. However, the functional relationship between the retromer complex and SNARE proteins in relation to vesicle trafficking still remain elusive. We have previously indicated that retromer complex modulates the autophagy and effectors related host infection events in rice blast fungus *Magnaporthe oryzae*. Here, we report that t-SNARE MoPep12 plays an important role in orchestrating the recruitment of MoVps35 onto the vacuolar membrane. To exert its functions, MoPep12 formed a vacuolar SNARE complex with MoVam7, MoVti1 and MoYkt6 and thereby mediate the recruitment of MoVps35 to vacuolar membrane to ensure its function, which further facilitate the autophagy-related lipids degradation and proper effectors secretion for successful host invasion. Altogether, our results provided insights into the precise mechanism of SNARE proteins and retromer complex in fungal development and pathogenicity of *M. oryzae*.

352B **Fungal COP9 signalosome assembly requires connection of two trimeric intermediates for integration of intrinsic deneddylase** Fruzsina Bakti[1], Emmanouil Stavros Xylakis[2], Helena Stupperich[1], Kerstin Schmitt[1], Oliver Valerius[1], Anna Maria Köhler[1], Cindy Meister[1], Anja Strohdiek[1], Rebekka Harting[1], Christoph Sasse[1], Kai Heimel[1], Piotr Neumann[3], Ralf Ficner[3], Gerhard Braus[1] [1]Molecular Microbiology and Genetics, Microbiology and Genetics, [2]Georg August Universitat, [3]Molecular Structural Biology, Microbiology and Genetics
The conserved eight-subunit COP9 signalosome (CSN) is required for multicellular fungal development. The CSN deneddylase cooperates with the Cand1 exchange factor to control replacements of E3 ubiquitin cullin RING ligase receptors, providing specificity to eukaryotic protein degradation. *Aspergillus nidulans* CSN assembles through a heptameric pre-CSN, which is activated by integration of the catalytic CsnE deneddylase. Combined genetic and biochemical approaches provided the assembly choreography within a eukaryotic cell for native fungal CSN. Interactomes of functional GFP-Csn subunit fusions in pre-CSN deficient fungal strains were compared by affinity purifications and mass spectrometry. Two distinct heterotrimeric CSN subcomplexes were identified as pre-CSN assembly intermediates. CsnA-C-H and CsnD-F-G form independently of CsnB, which connects the heterotrimers to a heptamer and enables subsequent integration of CsnE to form the enzymatically active CSN complex. Surveillance mechanisms control accurate Csn subunit amounts and correct cellular localization for sequential assembly since deprivation of Csn subunits changes the abundance and location of remaining Csn subunits.

353B **The *Neurospora crassa* JSN-1 protein binds multiple transcripts, including mRNA species required for proper conidiation** Anne Yenewodage[1], Zheng Wang[2], Jeffrey P Townsend[2], Oded Yarden[3] [1]Hebrew Univ of Jerusalem, [2]Yale University, [3]The Hebrew University of Jerusalem

RNA-binding proteins (RBPs) play crucial roles in cellular processes such as RNA transport, degradation, and translation. They are essential for the regulation of post-transcriptional gene expression and constitute hundreds of proteins in the eukaryotic genome. JSN-1 (NCU06199) is a member of the highly conserved Pomillio RBP family and is a member of the COT-1-GUL-1 complex, a kinase and RBP involved in the regulation of cell polarity, in *N. crassa*. The Δ*jsn-1* strain exhibits a block in the minor constriction phase of conidiation and phenotypically resembles the mutants *fl* and *acon-3*. To profile the repertoire of transcripts bound by JSN-1, we employed high-stringency RNA immunoprecipitation using a JSN-1::GFP-expressing culture, coupled with RNA sequencing. JSN-1 was found to bind a total of 1515 transcripts. 71 of them were also found to be bound by the co-complexing RBP GUL-1. Gene Ontology (GO) analysis revealed enrichment of organelle- and membrane-related genes. We also found enrichment in transcripts related to protein and nucleic-acid binding and a variety of protein kinases (e.g., *stk-32* and *stk-43*). Interestingly, JSN-1 exhibited binding affinity to several conidiation-related transcripts, including *con-6* (NCU08769), *con-8* (NCU09235), *con-10* (NCU07325), *acon-3* (NCU07617), *acon-4* (NCU03043), *fl* (NCU08726) and *fld* (NCU09739). To identify which genes exhibited altered expression in a Δ*jsn-1* mutant during conidiation, we profiled the transcriptome of wild-type and the Δ*jsn-1* strains at four time points (0, 4, 8, and 14 hours) after induction of conidiation. The major differences in the expression of genes involved in conidiation included a three-fold increase in the expression levels of *con-6* in the mutant 4 hours after induction of conidiation, a 60% reduction in the mRNA abundance of *acon-3* at the 8- and 14-hour time points, and a two-fold increase in the expression of *con-8* in the mutant at 14 hours post-induction. In addition, *csp-1* (NCU02713) expression was reduced two-fold at 8 hours post-induction. Furthermore, we found that expression of genes encoding for the hypothetical proteins NCU05529 and NCU09952 was completely abolished in the Δ*jsn-1* mutant. While JSN-1 is required for proper conidiation, the multiple mRNA species found to bind to this protein indicate its involvement in multiple cellular processes. It is likely that other RBPs (like GUL-1) can provide at least partial functional compensation when JSN-1 is impaired.

354B **The Right Place at the Right Time: Epigenetic control of sexual development and cell fate decisions in Neurospora crassa** Abigail M Deaven[1], Mallory R Lane[1], Ammal Abduljalil[2], Abigail J Ameri[1], Zachary A Lewis[1] [1]Microbiology, Unive rsity of Georgia, [2]Microbiology & Immunology, University of Nevada, Reno

Multicellularity is a complex process that arose independently in the plant, animal, and fungal kingdoms, and typically requires intricate genetic and epigenetic regulation. In most eukaryotes, Polycomb Repressive Complex 2 (PRC2) regulates multicellular development by establishing domains of histone H3 lysine 27 tri-methylation (H3K27me3) across conditionally activated genes. Unpublished analyses from our lab suggest that PRC2 functions as a repressor of sexual development in the filamentous fungus Neurospora crassa, because the majority of H327me3-enriched genes are exclusively expressed in fruiting bodies (perithecia). Furthermore, loss of H3K27me3 promotes the growth of engorged, melanized structures (false perithecia) resembling mature perithecia; however, these structures form in the absence of fertilization. Despite this relationship, the general mechanisms that control specific gene activation to promote perithecial fate are poorly understood. Using ChIP-seq to profile the distribution of H3K27me3 during sexual development, we observed several subtle, local changes to H3K27me3 patterns at conditionally expressed genes. Our analyses have also uncovered a predicted forkhead domain transcription factor (vsd-1), which is upregulated with a subtle loss of H3K27me3 across its gene body during sexual development. vsd-1 is necessary for female fertility and may be sufficient to drive the expression of genes involved in sexual development, indicated by the formation of false perithecia. Thus, we propose that vsd-1 acts as a pioneer factor to promote expression of genes in inaccessible chromatin domains during sexual development. Future work will profile the gene regulatory network controlling sexual development and identify regions of differential chromatin accessibility in vegetative and sexual tissue. This work will uncover novel mechanisms of epigenetic regulation of cell fate transitions in the fungal kingdom, which has important implications for the control of devastating fungal pathogens and the production of valuable secondary metabolites in medically and industrially relevant fungi.

355B **Characterization of parasexual DNA exchange in blast fungi.** Ryo Chiba, Momotaka Uchida, Kohtetsu Kita, Takayuki Arazoe, Takashi Kamakura Tokyo University of Science

Pyricularia (*Magnaporthe*) species are causal agents of blast diseases on a variety of monocots. Rice blast caused by *P. oryzae* is one of the most devastating plant disease, and *P. grisea* causes crabgrass blast on *Digitaria ciliaris* worldwide. As most rice-infecting field isolates lack a sexual reproduction (female fertility), parasexual DNA exchange may play an important role in the genetic variation of the rice blast fungus. Parasexual DNA exchange is thought to occur through hyphal fusion, nuclear fusion, and chromosomal segregation during mitosis without the production of sexual organs, such as perithecia and asci. However, the detailed mechanisms underlying parasexual DNA exchange in *P. oryzae* remain largely unknown.

To characterize parasexual DNA exchange in *Pyricularia* species, we generated two types of transformants, showing GFP fluorescence and hygromycin B resistance (GFP-Hph) or mCherry fluorescence and bialaphos resistance (mCherry-Bar), from rice blast and crabgrass blast isolates. GFP-Hph and mCherry-Bar transformants were co-cultured by placing them on the opposite sides of media, and mycelial plugs obtained from the contacted mycelial regions were transferred to selection media with hygromycein B and bialaphos. The capability for parasexual DNA exchange was evaluated based on the appearance of hygromycin B and bialaphos dual-resistant colonies with GFP and mCherry dual fluorescence. The appearance rate of the colonies differed between transformants and/or isolates, and the capability for parasexual recombination in crabgrass blast isolates tended to be higher than that in rice blast isolates. The combination of transformants that showed the highest recombination capability was used to screen putative parasexual recombination-related genes by generating disrupted mutants of the transformants and evaluating their recombination capability.

356B **Is there localized mRNA translation at the hyphal tip?** Domenico Modaffari, Edward W J Wallace, Kenneth E Sawin University of Edinburgh

Hyphal growth is driven by vesicle fusion at the cell tip. In many fungal species, a vesicle organizing center called the Spitzenkörper (SPK) forms at the cell tip. Electron micrographs show ribosomes at the base of the SPK. However, the molecular components of the SPK remain largely uncharacterized. The possible regulation of local protein translation at the SPK and hyphal cell tip has not been yet investigated.

Using the model mold *Aspergillus nidulans*, we show that the RNA-binding protein SsdA travels towards the hyphal tip on microtubules. SsdA is the ortholog of *S. cerevisiae* translational-repressor protein Ssd1 and *N. crassa* GUL-1. Ssd1 recognizes a conserved RNA motif. The motif is enriched on genes encoding cell wall proteins which localize at the hyphal tip. Overall, SsdA could be part of a greater system that regulates local protein production at the hyphal tip.

We also report a straightforward CRISPR-Cas9 system for scarless genetic engineering of *Aspergillus nidulans* and *Aspergillus* codon-optimized latest-generation fluorescent protein tags.

357B **Antioxidant Pathways that Protect the Plasma Membrane in *Candida albicans*** Kara Swenson Microbiology and Immunology, Stony Brook University

Candida albicans is a human fungal pathogen that causes invasive infections with high mortality rates in immunocompromised individuals. Current antifungal treatments for *C. albicans* infections can be toxic and ineffective, and the development of improved treatments requires a better understanding of *C. albicans* pathogenesis. A key characteristic of *C. albicans* is its ability to resist stress brought on by host immune attacks, including oxidative stress. Reactive oxygen species (ROS) generated during infection by host immune cells damage fungal cellular targets, including proteins, lipids, and DNA. The *C. albicans* plasma membrane (PM) is on the front line of attack by ROS, which can oxidize membrane lipids and cause lipid peroxidation, a chain reaction resulting in the formation of lipid radicals and peroxides, disruption of membrane integrity, and widespread PM damage. Therefore, the *C. albicans* PM must have special pathways to protect against oxidation. Our lab found that Flavodoxin-Like Proteins (Pst1, Pst2, Pst3, and Ycp4) protect against lipid peroxidation and are essential for virulence in mice, highlighting the importance of protecting membrane lipids during infection. In other organisms, such as mammals, Glutathione Peroxidases (GPxs) detoxify lipid peroxides to promote PM integrity and ROS resistance. *C. albicans* has four GPxs (Gpx3, Gpx31, Gpx32, and Gpx33); therefore, we created a quadruple mutant lacking all four GPx genes and found that it was very sensitive to organic peroxides, including lipid peroxides. However, Gpx3 is known to regulate Cap1, a major transcription factor for inducing ROS resistance genes. We therefore conducted stress sensitivity assays and found that a *cap1Δ* mutant had similar levels of sensitivity to organic peroxides as the *gpx3Δ* and quadruple mutant strains, and deleting Cap1 in the GPx quadruple mutant strain did not increase sensitivity to organic peroxides. This shows that GPxs primarily regulate Cap1 and do not play a prominent role in detoxification of peroxides. Assays with key antioxidant genes upregulated by Cap1, show that Glr1 (Glutathione reductase) is important for resistance to oxidized lipids, indicating a role for other members of the glutathione family of antioxidant proteins in preventing the deleterious effects of lipid peroxidation. Our current studies are aimed at identifying the key mediators of resistance to lipid peroxidation in *C. albicans* and evaluating their role in *Candida* pathogenesis.

358B **Comparing CRISPR-Cas9 Methods in *Candida auris*: a Challenging Conundrum** Dimitrios Sofras[1], Hans Carolus[1], Ana Subotić[1], Celia Lobo Romero[1], Craig L Ennis[2], Clarissa J Nobile[2,3], Jeffrey M Rybak[4], Patrick Van Dijck[1] [1]KU Leuven, [2]Dept of Molecular and Cell Biology, University of California, [3]Health Sciences Research Institute, [4]St. Jude Children's Research Hospital

Candida auris is an emergent fungal pathogen of significant interest for molecular research because of its recent inexplicable emergence, unique nosocomial persistence, high stress tolerance and common multidrug resistance. To investigate the molecular mechanisms of these interesting phenotypes, several CRISPR-Cas9 based genome editing tools have been optimized for *C. auris*. Nonetheless, genome editing in this species remains a significant challenge, and different systems have distinctive advantages and disadvantages.

In this work, we compare four different systems for their efficiency in introducing the same stop codon in the *ADE2* gene across isolates from five major clades of *C. auris*. In short, three of the systems have been designed to introduce the genetic elements necessary for the production of Cas9 and the appropriate guide RNA molecule into the genome of *C. auris* replacing the *ENO1*, *LEU2* and *HIS1* loci respectively. Meanwhile, the fourth system makes use of an episomal plasmid. For the methods requiring genomic integration, we additionally assessed the rates of correct targeting into the appropriate locus. Furthermore, we evaluated the increased efficacy of homologous recombination by deleting *KU70* and *LIG4*, which encode for proteins involved in the non-homologous end joining DNA repair pathway. Our research offers a comprehensive comparison of the current methods for precise genome editing in *C. auris* and sheds light on the advantages and limitations of several methods. This work aims to guide scientists in choosing the most appropriate tools for molecular work in this enigmatic fungal pathogen.

359B **Physiological adaptation to changing environments by the polyextremotolerant yeast Aureobasidium pullulans** Audrey Williams[1], Claudia Petrucco[2], Julian Liber[2], Alex Crocker[2,3], Amy Gladfelter[2] [1]Cell Biology, Duke University, [2]Duke University, [3]University of North Carolina Chapel Hill

Fungal life is found across a vast range of environments with extremes of pH, temperature, salinity, water availability, and other abiotic factors. Some fungi (termed *polyextremotolerant*), can grow in multiple extreme (as well as not-so-extreme) environments, and must adapt their physiology dramatically to maintain cell function in the face of these changes. A number of cellular adaptation mechanisms have been described, including changes in solute production, cell wall thickness, ion transport, membrane composition, and cell shape. However, we do not understand how these responses work together to sustain cell organization and biochemistry, the timescales on which different cellular adaptations occur, or how cells adapt to simultaneous changes in multiple environment features. To address these questions, we are developing the widespread, polyextremotolerant yeast *Aureobasidium pullulans* as a cell-biological model of adaptation to extremes. We are building a toolkit of genetically-encoded fluorescent probes with which to measure physiological and morphological traits including intracellular pH, ATP concentration, macromolecular crowding, and cell and vacuole size and shape. With these probes, we will compare the physiology of cells adapted to a range of temperatures, pH, and salinity, and map the dynamic responses of these cells to changes in these factors. We have also found that different *A. pullulans* isolates vary widely in their tolerance for high salinity and other culture conditions. We are sequencing the genomes of 200 isolates to identify genomic loci that correlate with the isolates' ability to grow in different environments. We will draw on these data to identify new candidate cellular processes that contribute to adaptation to extremes.

360B **Evolution of thermotolerance in *Cryptococcus* species** Vikas Yadav, Joseph Heitman Molecular Genetics and Microbiology, Duke University Medical Center

Calcineurin signaling is a highly conserved signaling cascade that governs the virulence of fungal pathogens in response to stress conditions that lead to calcium influx into cells. This calcium signal is sensed by calmodulin, which then binds to a series of targets including calcineurin, a serine-threonine specific phosphatase. Ca^{2+}-calmodulin binding to calcineurin triggers conformational changes that activate the phosphatase activity of the enzyme. In the human fungal pathogen *Cryptococcus neoformans*, calcineurin is activated by heat stress as well as in response to excess calcium. Previous studies established an essential role for calcineurin in growth at high temperature (37°C) and virulence. In this study, we identified genetic interactions with calcineurin to elucidate molecular mechanisms underlying thermotolerance in *C. neoformans* and its related species *C. deneoformans*. To achieve this, two genetic screens were performed. First, mutations were identified that restore the viability of calcineurin mutants during heat stress at 37°C. Second, mutations that confer resistance to a combination of calcineurin inhibitors (FK506 and cyclosporin A) at 37°C were identified. Combined together, these screens identified two distinct sets of mutations that bypass the requirement of calcineurin at 37°C in two *Cryptococcus* sister species suggesting functional divergence in the signaling cascades between the two species. Specifically, the screen in *C. deneoformans* identified a set of genes encoding proteins involved in cell wall modulation and budding whereas the screen in *C. neoformans* identified genes encoding a kinase as well as proteins involved in calcium signaling. A further extension of this screen with a kinase gene deletion collection identified three additional kinases that potentially act antagonistically to calcineurin during thermal stress. Further studies revealed that calcineurin function is bypassed via different

mechanisms in the two species and identified previously unknown components governing thermal stress responses in this critical group of human fungal pathogens.

361B **Cytoplasmic sequestering of a fungal stress-activated MAPK in response to a host plant phenolic acid** Rina Zuchman[1], Roni Koren[1], Tamar Ziv[1], Lupu-Haber Yael[1], Nitsan Dahan[1], Ofri Levi[1], Benjamin A Horwitz[2] [1]Biology, Technion, [2]Technion - IIT

The stress-activated MAP kinase Hog1 of *Cochliobolus heterostrophus* undergoes dephosphorylation rather than the expected stress-induced phosphorylation upon exposure to ferulic acid (FA), a phenolic compound abundant in the host plant, maize [1,2]. This unusual signaling mode is reflected in its subcellular localization as well. Unlike its nuclear localization during osmotic stress, a functional Hog1:GFP fusion protein forms cytoplasmic foci in response to FA, indicating its sequestering. These foci generally appear throughout a given hyphal compartment, within 10 min of the start of exposure to 1.5 mM or higher FA concentrations, and persist for an hour or more, depending on the FA concentration. Hog1:GFP did not colocalize with peroxisomes. In non-stressed cells, mitochondria are elongated, as typical for filamentous fungi, and become fragmented upon exposure to FA. Some, mostly larger, Hog1:GFP foci colocalized with fragmented mitochondria, however most of the smaller and more uniform-sized foci did not. The size, distribution and stress dependence of Hog1:GFP foci is reminiscent of heat-shock induced stress granules. The foci could, therefore, be liquid-liquid phase-separated granules. With Hog1:GFP as an affinity purification bait, we isolated an FA-dependent sub-proteome from a subcellular fraction enriched for fluorescent foci. The proteins identified include RNA-binding proteins, translation initiation factors and mitochondrial proteins. An example is the RNA recognition motif (RRM) and pumilio domain protein Puf2. A Puf2:tdTomato fusion protein, upon FA exposure, formed foci partially colocalizing with Hog1:GFP. Part of the mycelial population of some necrotrophic plant pathogens, including *C. heterostrophus*, is targeted for cell death in a specific time window during infection [3]. FA, a toxic plant metabolite, indeed promotes cell death [1]. Although Hog1/P38 orthologs in several fungal species are needed for optimal survival under stress, sustained activation can promote cell death. The sequestering and dephosphorylation of Hog1, therefore, may collectively attenuate cell death induced by defense compounds of the plant host.

[1] Shalaby et al. (2016) Environ. Microbiol.; [2] Zuchman et al. (2021) J. Fungi; [3] Shlezinger et al. (2011) PLoS Pathog.

362B **Elucidation of the *Cryptococcus neoformans* STRIPAK complex** Patricia P. Peterson[1], Jin-Tae Choi[2], Soojin Yu[2], Yong-Sun Bahn[2], Joseph Heitman[1] [1]Dept. of Molecular Genetics and Microbiology, Duke University Medical Center, [2]Dept. of Biotechnology, Yonsei University

The eukaryotic serine/threonine protein phosphatase PP2A is a heterotrimeric enzyme composed of a scaffold A subunit, a regulatory B subunit, and a catalytic C subunit. Of the four known B subunits, the B''' subunit (known as striatin) interacts with the multi-protein striatin-interacting phosphatase and kinase (STRIPAK) complex. Using protein sequences of known STRIPAK components from other species, protein orthologs were identified for STRIPAK complex subunits in *C. neoformans*, namely the A subunit, the C subunit, the B''' subunit striatin, as well as the striatin-interacting proteins. Distinct protein domains that mediate the structure and/or function of STRIPAK complex subunits in other species were also found to be conserved in the protein orthologs in *C. neoformans*. Protein-protein interactions, as detected through yeast two-hybrid analyses, have verified the presence of the STRIPAK complex in *C. neoformans* and suggests that it is assembled similarly to other fungi. Here, the PP2A catalytic subunit *PPH22* and the regulatory subunit *FAR8* were functionally characterized. We show that *PPH22* is a haploinsufficiency gene: heterozygous *PPH22/pph22*Δ mutant diploid strains exhibit defects in both hyphal growth and sporulation, and have a significant fitness disadvantage when grown in competition against a wild-type diploid strain. *pph22*Δ and *far8*Δ deletion mutant strains display decreased mating efficiency and are defective in producing hyphae, basidia, and basidiospores when crossed with a wild-type strain. Loss of either *PPH22* or *FAR8* leads to growth defects at 30^{o}C, severely impaired growth at elevated temperature, abnormal cell morphology, and reduced melanin and capsule production. *pph22*Δ and *far8*Δ mutants are also unable to grow in the presence of the calcineurin inhibitors cyclosporine A or FK506, suggesting these mutations are synthetically lethal with loss of calcineurin activity. Finally, whole-genome sequencing revealed that mutations in STRIPAK complex subunits lead to increased segmental and chromosomal aneuploidy, which suggests STRIPAK functions in maintaining genome stability. Taken together, these results reveal that the *C. neoformans* STRIPAK complex plays an important role in vegetative growth, sexual development, and virulence in this prominent human fungal pathogen.

363B **Evidence of phenotypic switching in *Fusarium oxysporum*** Pilar Gutiérrez-Escribano[1], Antonio Di Pietro[2] [1]University of Córdoba, [2]Genetics, University of Córdoba

Many microorganisms rely on nongenetic phenotypic variation to ensure rapid adaptation to continuously changing environments. Phenotypic plasticity is particularly relevant in the case of pathogenic organisms in which successful responses to adaptive

pressures set the basis for host infection and the emergence of drug resistance. *Fusarium oxysporum* is a large species complex (FOSC) of soilborne ascomycete fungi causing vascular wilt disease in over one hundred different plant hosts and is also an important emerging human pathogen. Using vital stains and selective media we found that the tomato wilt isolate *F. oxysporum* f. sp. *lycopersici* Fol4287 can stochastically activate hereditable and reversible switches between different phenotypic states. We also observed these high frequency phenotypic transitions in several other FOSC strains, including the human pathogenic isolate *F. oxysporum* MRL8996. Fol4287 switching variants showed differences in several morphological and functional properties linked to fungal virulence. Our data suggest that phenotypic switching is critical for understanding *F. oxysporum* biology and pathogenesis.

364B **Conserved Regulators of the Septation Initiation Network are required for *Aspergillus fumigatus* Echinocandin Resistance and Virulence** Harrison Thorn[1], Xabier Guruceaga[1], Adela Martin-Vicente[1], Ashley Nywening[1], Jinhong Xie[1], Wenbo Ge[2], Jarrod Fortwendel[1] [1]Clinical Pharmacy and Experimental Therapeutics, University of Tennessee Health Science Center, [2]Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital

Aspergillus fumigatus is a major invasive mold pathogen and the most frequent etiologic agent of invasive aspergillosis. The currently available treatments for invasive aspergillosis are limited in both number and efficacy. Our recent work has uncovered that the β-glucan synthase inhibitors, the echinocandins, are fungicidal against strains of *A. fumigatus* with defects in Septation Initiation Network (SIN) kinase activity. These drugs are known to be fungistatic against strains with normal septation. Surprisingly, SIN kinase mutant strains also failed to invade lung tissue and were significantly less virulent in immunosuppressed mouse models. Inhibiting septation in filamentous fungi is therefore an exciting therapeutic prospect to both reduce virulence and improve current antifungal therapy. However, the SIN remains understudied in pathogenic fungi. To address this knowledge gap, we characterized the putative regulatory components of the *A. fumigatus* SIN. These included the GTPase, SpgA, it's two-component GAP, ByrA/BubA, and the kinase activators, SepM and MobA. Deletion of *spgA*, *byrA* or *bubA* resulted in no overt septation or echinocandin susceptibility phenotypes. In contrast, our data show that deletion of *sepM* or *mobA* largely phenocopies disruption of their SIN kinase binding partners, *sepL* and *sidB*, respectively. Reduced septum formation, echinocandin hypersusceptibility, and reduced virulence were generated by loss of either gene. These findings provide strong supporting evidence that septa are essential not only for withstanding the cell wall disrupting effects of echinocandins, but are also critical for the establishment of invasive disease. Therefore, pharmacological SIN blockage may be an exciting strategy for future antifungal drug development.

365B **Identification of environmental and genetic regulators of apothecium development in *Sclerotinia sclerotiorum*** Jeffrey Rollins[1], Ulla A Benny[2], Chenggang Wang[2] [1]Plant Pathology, Univ Florida, [2]University of Florida
Multicellular fruiting body development by Ascomycota fungi requires canalized genetic pathways that remain responsive to environmental input. Additionally, cooperation between ascogenous and vegetative hyphae is required to produce the final, functional form. The stipitate apothecium of *Sclerotinia sclerotiorum* (Lib.) de Bary is a macroscopic, uni-parental sexual fruiting body that lends itself to manipulation via environmental and genetic experimentation. Both the undifferentiated stipe and the developing apothecial disc are photoresponsive. We have characterized the role of UV-A, red and blue wavebands of light in this developmental process as well as the effects of light deprivation at various phases of development on developmental fate. These studies have revealed a number of photoresponses including positive and negative phototropism, photomorphogenesis controlled by UV-A, de-etiolation of undifferentiated stipes and photo-determinacy. From a genetic perspective, we have employed random mutagenesis as well as targeted gene mutation to identify genes that affect patterns of development as well as tissue determinacy. Through a forward genetic screen, we have determined that a fungal-specific zinc binuclear cluster transcriptional regulator is required for apothecial disc expansion. Rather than developing mature discs, loss of function mutants reiteratively bifurcate at the stipe apex to produce a coralloid form, yet remains fertile. A second mutant identified through reverse genetics of a conserved MAPK-encoding gene produces elongated stipes with incompletely expanded discs indicative of a defect downstream of light perception. Collectively, these experiments utilizing environmental and genetic manipulations provide new insights into pattern development and tissue determinacy in Ascomycota fruiting body development.

366B **The role of peroxisome hitchhiking in secondary metabolism in *Aspergillus nidulans*** Livia D Songster[1], Gaurav Kumar[2], Valentin Wernet[2], Patreece Suen[2], Samara Reck-Peterson[1,2,3] [1]Cell and Developmental Biology, UC San Diego, [2]Cellular & Molecular Medicine, UC San Diego, [3]Howard Hughes Medical Institute

In the filamentous fungus *Aspergillus nidulans*, peroxisomes move long distances along microtubules by forming a transient contact with early endosomes through a non-canonical trafficking process termed "hitchhiking". Peroxisome hitchhiking requires the protein PxdA, which is conserved specifically within the Pezizomycotina subphylum of Ascomycota fungi. Peroxisomes are the only organelle demonstrated to hitchhike on endosomes in *A. nidulans*, but it is unclear why. Here, we investigated the physiological function of peroxisome hitchhiking by using bulk RNA sequencing to identify transcriptional changes in peroxisome hitchhiking

mutants. Mutants have mislocalized peroxisomes and altered expression of genes related to secondary metabolism (SM). SMs are organic molecules that are not strictly required for fungal growth but confer some ecological advantage. Our preliminary SM extraction results show that peroxisome hitchhiking mutants produce fewer SMs than wild type strains, despite widespread transcriptional upregulation of SM genes. SM pathways are compartmentalized into organelles to protect cells from toxic intermediates and provide regulatory control. While peroxisomes are known to be important to produce some SMs (such as penicillin, siderophores, or sterigmatocystin), the role of peroxisomes in other SM pathways is unknown. We hypothesize that peroxisome hitchhiking might be important for the production and/or secretion of certain SMs. To first estimate the relative importance of peroxisomes in SM, we bioinformatically predicted organelle localization of 1105 SM-associated genes in *A. nidulans* using a deep-learning approach. We found that 57 out of 75 predicted SM gene clusters possess >1 protein with a peroxisomal targeting sequence, supporting the idea that peroxisomes are a major site of SM production. Ongoing work is investigating the mechanism of peroxisome hitchhiking in coordinating SM across multiple organelles. Together, our data support the hypothesis that peroxisome hitchhiking might contribute to the production of some SMs. We also reveal how fungal cells compensate for loss of peroxisome hitchhiking and identify SM production and/or secretion as a potential physiological function for peroxisome hitchhiking across filamentous ascomycete fungi.

367B **Separation of life stages within anaerobic fungi highlights differences in global transcription and metabolism** Lazarina V Butkovich[1], Patrick A Leggieri[1], Stephen P Lillington[1], Tejas A Navaratna[1], Thea R Zalunardo[1], Anna Lipzen[2], Vivian Ng[2], Mei Wang[2], Juying Yan[2], Igor V Grigoriev[2], Michelle A O'Malley[1] [1]Chemical Engineering, University of California, Santa Barbara, [2]Lawrence Berkeley National Laboratory

Anaerobic gut fungi of the early-diverging fungal phylum Neocallimastigomycota are microbes proficient in valorizing low-cost but difficult-to-breakdown lignocellulosic plant biomass. In order to appreciate how different life stages contribute to biomass breakdown and production of enzymes relevant for biotechnological applications, we aim to better understand the life cycle of anaerobic gut fungi. In this study, we extracted RNA from culture samples of the anaerobic gut fungal strain *Neocallimastix californiae* G1 grown on rumen fluid-based media with glucose as the substrate. We used RNAseq to generate global gene expression profiles to compare two sample types: (1) cell pellets enriched in the young life stage of free-swimming, flagellated zoospores and (2) fungal mats with relatively more vegetative, encysted, mature sporangia. We find evidence that zoospores transcriptionally prime to encounter plant matter substrate, despite being grown on simple sugar substrate. For example, key catabolic carbohydrate-active enzymes are upregulated in zoospores when compared to fungal mats. Furthermore, we report significant differential gene expression for gene annotation groups, including transporters, transcription factors, and putative secondary metabolites. The described RNAseq dataset and analysis will inform future hypotheses and experiments regarding the different life stages of anaerobic gut fungi.

368B **Refining morphological models: branching and germination rate dynamics in early mycelial growth** Alexander G. Doan[1], Casey M. Douglas[1], Jessica E. Schafer[1], Steven D. Harris[2], Mark R. Marten[3] [1]University of Maryland Baltimore County, [2]IA State U., [3]Chem. Biochem. Environ. Engr., Univ Maryland,Baltimore County

We present a re-evaluation of filamentous fungal growth dynamics. Specifically, we bifurcate classical branching rate into two distinct rates: germination rate and true branching rate. Our results reveal that the inclusion of germination rate in the classical branching rate masks significant strain-specific variances in the true branching rate when germination rate is not considered separately. We support our approach with evidence gathered from experiments conducted on various strains of the model organism *Aspergillus nidulans*. Where classical branching rate remains constant, we find a marked variation in true branching rate. Our findings prompt a critical reconsideration of historical data where re-analysis would reveal previously unrecognized strain-specific morphologies. Our proposed method for calculating branching rate will enable a more complete understanding of filamentous fungal growth dynamics and morphology. By identifying previously overlooked subtleties in fungal growth patterns, our research offers renewed value in existing datasets that under classical methods have been exhausted.

369B **Uncovering important transcriptional regulations during conidiation and spore germination** Pin Wu, Fang Wang, Winnie Weng In Chong, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau

Filamentous fungi have the remarkable ability to undergo asexual reproduction, generating an enormous quantity of spores (a.k.a. conidia). These spores serve as the primary means of dissemination, acting as infectious propagules for human and plant pathogens, as well as significant contributors to food spoilage. The germination of spores and their subsequent growth as hyphae under favorable conditions are critical processes for infection and food spoilage. Therefore, understanding the mechanisms underlying conidiation and spore germination holds not only biological importance but also clinical and agricultural implications. To gain insight into the molecular mechanisms of conidiation and spore germination, we performed active transcription profiling

by ChIP-seq and transcriptome profiling by RNA-seq on *Aspergillus nidulans* at distinct stages of conidiation and germination with a high temporal resolution. Our results unveiled dynamic transcriptional and post-transcriptional changes throughout different stages of germination and conidiation. Each stage exhibited enrichment of distinct gene sets with specialized functions. For some of these gene sets, we have identified potential candidate transcriptional regulators that could serve as valuable drug targets for impeding conidiation and spore germination. Further characterization of these regulators holds great promise for the development of novel antifungal drugs for preventing human and plant fungal infections.

370B **Characterization of a Myb-like protein MylA in *Aspergillus flavus*** He jin Cho[1,1], Hee soo Park[2,2,3] [1]School of Food Science and Biotechnology, Kyungpook National University, [2]Dept of Integrative Biology, Kyungpook National University, [3]School of Food Science and Biotechnolog, Kyungpook National University

Aspergillus flavus is a major virulent fungus of seed crops such as peanuts and corn and is the major aflatoxin producer, which is one of the most carcinogenic mycotoxins. *A. flavus* mainly reproduces through asexual reproduction, producing the asexual spore called conidia. The process of conidia formation (conidiation) is regulated by various transcription factors. Among them, the myeloblastosis (Myb) transcription factor family is conserved in filamentous fungi and plays an essential role in various cellular processes. In this study, we characterized the role of Myb-like protein A, MylA, in *A. flavus*. First, we measured the expression of *mylA*during the life cycle of *A. flavus* and figured out that *mylA* was highly expressed in conidia. We then assessed the role of MylA in the fungal development of *A. flavus*. The *mylA*-deficient mutant showed reduced conidia production and colony growth compared to the control. Also, the Δ*AflmylA* strain produced a reduced amount of sclerotia. These results indicated that MylA plays a crucial role in both asexual and sexual development in *A. flavus*. To reveal the role of MylA in *A. flavus* conidia, RNA-seq analysis was carried out. RNA-seq analysis showed that the expressions of trehalose biosynthesis-related genes were downregulated in the Δ*AflmylA*conidia. By checking the trehalose content in each strain's conidia, the Δ*AflmylA* conidia showed a defect in trehalose biosynthesis. As trehalose acts as a cell protectant in fungi, the Δ*AflmylA* conidia showed reduced spore viability and stress tolerance. Also, the germination ability of each strain's conidia was tested. The Δ*AflmylA* conidia showed a delayed germination rate and also showed shorter germ tube length compared to the control. Lastly, we assessed the function of MylA in plant pathogenicity by performing kernel bioassay. When incubated with kernels, the *AflmylA* null mutant showed reduced conidia colonization and aflatoxin B1 production. Taken together, these results indicate that MylA plays a pivotal role in proper fungal development, conidial viability, germination ability, and plant pathogenicity of *A. flavus*.

371B **Heat resistant ascospores** Jan Dijksterhuis[1], Richard Van Leeuwen[1], Timon Wyatt[1], Luis Lugones[2], Han Wosten[2] [1]Westerdijk Fungal Biodiversity Institute, [2]Molecular Microbiology

Fungal ascospores can be remarkably stress-resistant eukaryotic cells. Many genera within the Eurotiales form ascospores with varying degrees of heat resistance and dormancy. Ascospores of the species *Talaromyces macrosporus* or *Aspergillus spinosus* survive over one-hour-heat treatments at 85 °C. These spores have a constitutive dormancy, meaning that even in rich nutrient media no germination occurs. However, after a short heat treatment (5 min, 85 °C) germination occurs. This is dubbed heat activation and can result in fungal outgrowth of products of food industry after pasteurization. ESR measurements show that the cytoplasm of the ascospores is dense, but that no glassy state occurs during heating in liquid. Drying or freezing of ascospores does not result in a change of the state of being activated, which suggest that once occurring, this state is permanent. In *T. macrosporus* a small molecular- weight protein is released upon heat activation, suggesting that dormancy is conveyed via an impermeability of the outer cell wall. Cryoplaning electron microscopy shows that rupture of the thick outer cell wall of these spores is necessary for germination.

Compatible solutes accumulate to a high concentration during maturation of ascospores. In *T. macrosporus* and *T. flavus,* trehalose is the sole solute accumulating up to over 1 M. In *Aspergillus fisheri* and *A. spinosus* novel compatible solutes are accumulated including a suite of trehalose-based oligosaccharides containing out of a α-1,1 trehalose core containing one, two or three α-1,6-glucose moieties defining isobemisiose, neosartose, and fischerose. Remarkably, in plants, sucrose, raffinose and stachyose are similarly sized oligosaccharides with a sucrose core and galactose moieties. This could suggest that (mixtures of) different compatible solutes result in variation in stress resistance. Indeed, *T. macrosporus* has higher liquid heat resistance as *A. fischeri*, but lower resistance against dry heat. Maturation and germination of ascospores are accompanied with changes in compatible solutes, which correlate precisely with microviscosity in the cytoplasm and a decrease in heat resistance.

372B **Regulated IRE1-dependent mRNA decay is induced by physiological ER stress associated with amylolytic enzyme production in *Aspergillus oryzae*** Mizuki Tanaka[1], Silai Zhang[2], Shun Sato[2], Jun-ichi Yokota[2], Yuko Sugiyama Sugiyama[2], Yasuaki

Kawarasaki[3], Youhei Yamagata[1], Katsuya Gomi[2], Takahiro Shintani[2] [1]Tokyo University of Agriculture and Technology, [2]Tohoku University, [3]University of Shizuoka

Regulated IRE1-dependent mRNA decay (RIDD) is a feedback mechanism in which the endoribonuclease, IRE1, cleaves endoplasmic reticulum (ER)-localized mRNAs encoding secretory and membrane proteins in eukaryotic cells under ER stress. RIDD is artificially induced by chemicals that generate ER stress; however, its importance under physiological conditions remains unclear. In this study, we found that RIDD is induced not only by chemicals but also upon induction of the production of endogenous secretory hydrolases in *Aspergillus oryzae*. α-Amylase mRNA was rapidly degraded by IreA, an Ire1 ortholog, when mycelia were treated with dithiothreitol (DTT), an ER-stress inducer. In *A. oryzae*, maltose uptake by the maltose permease, MalP, is a prerequisite for the activation of AmyR, a transcriptional activator of amylolytic gene expression. Notably, even without DTT, *malP* transcripts underwent RIDD when *A. oryzae* was grown in a maltose medium. Loss of the superkiller (Ski) complex (involved in 3′–5′ mRNA degradation) resulted in marked accumulation of short fragments of the *malP* mRNA resulting from cleavage by IreA and, to a lesser extent, of short fragments of the α-amylase mRNA. The decrease in the abundance of the full-length *malP* mRNA and appearance of its short fragments were suppressed in Δ*amyR* strain. These results indicate that RIDD occurs under physiological ER stress caused by the production of amylolytic enzymes. In addition, *amyR* deletion rescued the growth defect of *ski* mutants on maltose medium. Overall, these findings suggest that RIDD contributes to the maintenance of cellular homeostasis in *A. oryzae* under conditions that produce amylolytic enzymes.

373B **Characterization of spatio-temporal dynamics of the constrained network of the filamentous fungus *Podospora anserina* using a geomatics-based approach** Clara Ledoux, Cecilia Bobee, Eva Cabet, Pascal David, Frederic Filaine, Sabrina Hachimi, Christophe Lalanne, Gwenael Ruprich-Robert, Eric Herbert, Florence Chapeland-Leclerc LIED Universite Paris Cite

In their natural environment, fungi are subjected to a wide variety of environmental stresses which they must cope with by constantly adapting the architecture of their growing network. In this work, our objective was to finely characterize the thallus development of the filamentous fungus *Podospora anserina* subjected to different constraints that are simple to implement in vitro and that can be considered as relevant environmental stresses, such as a nutrient-poor environment or non-optimal temperatures. At the Petri dish scale, the observations showed that the fungal thallus is differentially affected according to the stresses but these observations remain qualitative. At the hyphal scale, we showed that the extraction of the usual quantities (i.e. apex, node, length) does not allow to distinguish the different thallus under stress, these quantities being globally affected by the application of a stress in comparison with a thallus having grown under optimal conditions.

Thanks to an original geomatics-based approach based on the use of automatized Geographic Information System (GIS) tools, we were able to produce maps and metrics characterizing the growth dynamics of the networks and then to highlight some very different dynamics of network densification according to the applied stresses. The fungal thallus is then considered as a map and we are no longer interested in the quantity of material (hyphae) produced but in the empty spaces between the hyphae, the intra-thallus surfaces. This study contributes to a better understanding of how filamentous fungi adapt the growth and densification of their network to potentially adverse environmental changes.

374B **The Role of Meiotic Factors in Ploidy Dynamics** María Angélica Bravo Núñez, Jimena M Luque, Athena Rogers, Andrew W Murray Molecular and Cellular Biology, Harvard University

Errors during chromosome segregation can lead to cells with the wrong number of chromosomes, a condition known as aneuploidy. Aneuploidy is a driving force in cancer progression and has been linked to the rapid emergence of drug resistance in fungal pathogens. For these reasons, it is important to understand the etiology of aneuploidy and the fitness advantage that aneuploidy may confer under environmental perturbations. Inappropriate expression of meiotic genes is a potential cause of aneuploidy as the normal function of these genes is to alter the ploidy of cells in order to generate haploid gametes (e.g., sperm or egg). Using the budding yeast *Saccharomyces cerevisiae*, we tested this idea by creating a library of meiotic genes (under the control of an inducible promoter), expressed them in mitotically-dividing cells, and determined their contribution to genome instability and their ability to adapt to exogenous stresses. Our work shows that genes involved in altering centromere behavior during meiosis can increase the levels of aneuploidy and faster adapt to stressful environments such as DNA replication stress. This work illustrates how meiotic genes may contribute to mitotic segregation errors and drive ploidy changes outside of gametogenesis. In addition, it showcases examples in which generating aneuploidies may be advantageous.

375B **Dark stipe mutants in fruiting body development of *Coprinopsis cinerea*** Shanta Subba[1], Botond Hegedüs[2], Vanada Pulusu[3], Le Chen[3], László Nagy[4], Ursula Kües[3] [1]Molecular Wood Biotechnology and Technical Mycology, University of

Göttingen, [2]Synthetic & Systems Biology Unit, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Science, [3]University of Göttingen, [4]Hungarian Academy of Science

Fruiting body development in the dung fungus *Coprinopsis cinerea* is strictly regulated by environmental conditions including light, lowering temperature (25 °C), and aeration. It follows a conserved scheme defined by day and night phases, with well predictable distinct morphological stages over the time. The differentiation process begins with the formation of primary hyphal knots (Pks) in the dark, which, when exposed to light, transform into compact aggregates, the secondary hyphal knots (Sks) in which stipe and cap tissues differentiate. Further light signals control tissue differentiation within the growing primordia. Primordium development (stages P1 to P5) takes five days to culminate on day 6 of the process in light-induced karyogamy within the basidia followed by meiosis and basidiospore production which parallels fruiting body maturation (stipe elongation and cap expansion). At several stages during hyphal knot and primordia development, failure in light signaling or in aeration leads to formation of so-called 'dark stipes', under unusual proliferation of stipe tissues and a block in cap development. Defects in formerly described genes *dst1* (*wc1*)*, dst2* (*wc2*) caused P1-induced ´dark-stipe´ phenotypes by defects in blue-light receptors and a FAD/FMN-binding dehydrogenase of the GlcD superfamily. Under block of aeration, scavenger experiments of CO_2 with KOH recovered the normal phenotypes in fruiting body development (Sk development, normal primordial P1 to P5 development, normal spore and fruiting body maturation). Accordingly, the increase in CO_2 content and not the lack of oxygen leads to abnormal morphological phenotypes. The complexity in the fruiting process and its regulation is reflected by our large mutant collection. Among are two mutants (*dst3*, *dst4*) with P3- and P4-induced 'dark stipe' phenotypes forming under standard fruiting conditions with light and aeration. Genome sequencing revealed missense and early stop codon mutations in enzymes associated with the citrate cycle and of branched amino acid production, as well as in the Zn-binding site of the Csn5 subunit of the Cop9 signalosome, and loss of the start codon in an Arf1-like GTPase, suggesting links to the CO_2 metabolism and also light signaling and regulation. Transformation of the mutants with wildtype genes (*pda1* and *csn5*) restored phenotypes.

376B **Evolution of chromosomal regions with *A* mating type loci in Agaricomycetes** Ming Fang[1], Shanta Subba[2], Fangjie Yao[3], Lixin Lu[3], Ursula Kües[4] [1]2 Laboratory of Genetic Breeding of Edible Mushrooms, Jilin Agricultural University, [2]University of Göttingen, [3]Jilin Agricultural University, [4]University of Goettingen

The Auriculariales with ear-shaped gelatinous fruiting bodies are an early-diverging clade of the Agaricomycetes, whereas the Agaricales with lamellate basidiocarps represent the largest euagaric clade of the Agaricomycetidae. Fruiting bodies typically form on a fertile dikaryotic mycelium obtained by mating of two compatible monokaryotic mycelia germinated from sexual basidiospores. Mating is controlled by mating-type genes which are subject to evolutionary forces like balancing selection, gene duplication, and gene conversion, which can further shape genetic diversity and dynamics of populations. Here we explore the evolution events on the mating-type loci of selected Auriculariales in comparison with species of the Agaricales. The *MAT-A* loci are defined by presence of two types of homeodomain transcription factor genes (*HD1* and *HD2*). Other genes in the chromosomal regions are structurally defined and annotated by homologies to genes of other species. Species of Auriculariales differed in number of *MAT-A* genes with two complete transversely transcribed *HD1-HD2* gene pairs in *Auricularia suglabrata*, one typical *HD1-HD*2 gene pair and one pair of genes with a transversed *HD2* gene in *Ausicularia heimuer*, and two complete gene pairs plus one extra *HD2* gene in *Exidia glandulosa*, suggesting duplications and inversions in the evolution of *MAT-A* in the Auriculariales. Duplications as well as sometimes inversions and deletions in *HD1-HD2* genes pairs were known before from some of the Agaricales. In these species, *MAT-A* loci are usually flanked by the conserved genes *mip* and *β-fg*. These genes are also found closely linked to each other in the vicinity of *MAT-A* genes in the Auriculariales, but in *E. glandulosa* in mirrored symmetry. Many other genes conserved in chromosomal *MAT-A* regions in the Agaricales were found also back in the chromosomal environment of the *MAT-A* genes in the Auriculariales, suggesting an ancient accumulation of these genes. Between species in the Auriculariales were blocks of synteny with inversions and translocations, as well as to species of the Agaricales. Phylogenetic analyses of *HD1* and *HD2* genes are performed to define temporal events in production of paralogous gene pairs to increase mating type numbers.

377B **Digital reconstruction and analysis of the growing and branching network of the filamentous fungus *Podospora anserina*** Thibault Chassereau, Florence Chapeland-Leclerc, Eric Herbert LIED Universite Paris Cite

Starting from a simple spore, fungi can create a very dense and interconnected network of hyphae. In this work, we show how we digitally reconstruct the mycelial network of *Podospora anserina* during its growth, from a single initial ascospore to a millimeter thallus around 20 hours after germination, using a series of greyscale images with a panorama every 18 minutes and a spatial resolution of 1.6 micrometers per pixel. The reconstruction of the dynamics of this network allows us to follow each apex over time (despite overlaps) and to identify each hypha individually, with the possibility of automatically categorizing the nature of the branching (apical or lateral) at its origin. This labelling makes it possible to automate several measurements that can then be applied rapidly to the entire network, such as measuring branching angles or elongation rates. The differences in dynamics and

temporality according to the type of hyphal branching also highlight the different roles of hyphae between exploration and exploitation of the environment.

378B **Collateral sensitivity prevents antifungal drug resistance evolution in Candida auris** Hans Carolus[1], Dimitrios Sofras[1], Giorgio Boccarella[1], Stef Jacobs[1], Louise Goossens[1], Alicia Chen[1], Ina Vantyghem[1], Celia Lobo Romero[1], Tibo Verbeeck[1], Siebe Pierson[1], Hans Steenackers[1], Pieter van den Berg[1], Katrien Lagrou[1], Toni Gabaldón[2], Judith Berman[3], Patrick Van Dijck[1] [1]KU Leuven, [2]Institute for Research in Biomedicine (IRB) Barcelona, [3]Shmunis School of Biomedical and Cancer Research
The increased prevalence of antifungal drug resistance and emergence of multidrug-resistant species such as *Candida auris* represent a global public health threat. By mapping drug susceptibility responses of experimentally evolved *C. auris* across diverse antifungals, we discovered stable and robust trends of cross-resistance (XR) and collateral sensitivity (CS). CS is the process in which drug resistance towards one drug confers an increased sensitivity to another drug as a fitness trade-off. Conversely, XR confers reduced susceptibility to multiple drugs upon exposure to a single antimicrobial. Neither CS nor XR have been explored extensively in pathogenic fungi, although they are well-studied I n bacteria and cancer, in which they have been implicated in treatment redesign to prevent or reduce resistance development. By using experimental evolution and mathematical modelling of population dynamics during treatment, we demonstrate that CS-based drug cycling can effectively prevent and reduce resistance development in *C. auris*. Additionally, we show that in competition experiments, a CS-exerting drug can eliminate existing resistant subpopulations, highlighting the capability of specific treatment switches to eliminate resistant infections. These findings provide a promising direction for future research that can improve treatment approaches for multidrug-resistant fungal pathogens like *C. auris.*

379B **Regulation of sexual development by IndB and IndD, the physical interactors of the NsdD GATA factor in *Aspergillus nidulans*.** Sang-Cheol Jun, Kap-Hoon Han Woosuk University
IndB and IndD of *Aspergillus nidulans* are interactors with the NsdD, a GATA type transcription factor, and this interaction appears to regulate the sexual developmental process of this fungus. However, not only has the regulatory mechanism of sexual development by IndB and IndD not yet been confirmed, but the functional domains of the two proteins have also not been characterized to date. We analyzed the intracellular localization of the interaction between NsdD and Ind proteins in *A. nidulans* using the Split-YFP/BiFC method. YFP signals of NsdD-IndB, NsdD-IndD and IndB-IndD were mainly observed in the vesicle domes and metulae of the conidiophore, an asexual reproductive organ, and the fluorescence signals were also observed in the septum and cell wall of hyphae. Additionally, to determine whether the IndB and IndD have a redundant function, we constructed single and double knock-out mutants of *indB* and *indD* and analyzed the characteristics of sexual developmental process. Single mutations of *indB* or *indD* underwent normal sexual development similar to the wild type, but double mutants resulted in uncontrolled formation of mature and immature cleistothecium during sexual development. Furthermore, the double mutant showed relative sensitivity to the reaction of cell wall lysis enzyme compared to the wild type or single mutants. These results suggest that the IndB/D proteins act as negative regulators in sexual development with redundant role, as well as the possibility that they are related to cell wall integrity signaling of *A. nidulans*.

380B **The apical endoplasmic reticulum in *Neurospora crassa*** Juan Manuel Martinez Andrade[1], Meritxell Riquelme[2], Robert Roberson[3] [1]Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), [2]Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), [3]Microbiology, School of Life Sciences

The apical organization of the endoplasmic reticulum (ER) in fungal hyphae, particularly in relation to polarized growth, remains largely unknown. In our recent research, we focused on YOP-1 and SEY-1, proteins predicted to form ER tubules. We observed their predominant expression at the peripheral ER (pER), specifically at apical and near-apical regions lacking nuclei (14.1 ± 2.2 µm) in *Neurospora crassa*. At the hyphal apex, YOP-1-GFP localized at the core of the Spitzenkörper (SPK), while SEY-1-GFP surrounded the outer layer of the SPK. To confirm this organization, the SPK was stained with FM4-64, and the Rab GTPases YPT-1 and SEC-4 labeled with mCherry were used as SPK markers. In addition, YOP-1-GFP was revealed at dynamic ER patches near the hyphal apex, similar to the membranes stained with ER-Tracker Blue-White DPX in a *N. crassa* WT strain. Three-dimensional reconstructions and near real-time recordings with spinning disk confocal microscopy revealed interconnected pER membranes emanating from the most proximal cortical nuclei in hyphal region II and extending toward the apex. Transmission electron microscopy confirmed the presence of smooth ER (sER) at the apex and rough ER at the subapex. The functional role of the apical ER in calcium regulation was explored. Cells growing in media without $CaCl_2$ exhibited fragmented apical ER (62%) compared to the control group (0.68 mM $CaCl_2$). Calcium inhibitors (EGTA, cyclosporin A, and neomycin) and knockout strains (Δ*yop-1*, Δ*sey-1* and Δ*yop-1*/Δ*sey-1*) resulted in significant growth colony reduction, particularly in Δ*sey-1* and Δ*yop-1*/Δ*sey-1* strains at various calcium inhibitors concentrations. Under sublethal concentrations of neomycin and cyclosporin A, the abundance of apical ER (YOP-1-GFP) and actin filaments (Lifeact-GFP) was reduced. Additionally, latrunculin A (10 µM) reduced the presence of YOP-1-GFP in the apical dome. These results collectively suggest that YOP-1 and SEY-1 positive ER membranes at the hyphal apex correspond to interconnected

tubular sER. This organization is essential for intracellular calcium regulation, influencing actin assembly and targeted exocytosis during hyphal tip growth.

381C **The putative translational repressor, SsdA, partially regulates carbon source-dependent roles of CotA signaling in *Aspergillus fumigatus*** Adela Martin-Vicente[1], Jinhong Xie[1], Harrison Thorn[1], Xabier Guruceaga[1], Ashley Nywening[2], Jarrod Fortwendel[3] [1]Clinical Pharmacy and Translational Science, University of Tennessee Health Science Center, [2]Integrated Program in Biomedical Sciences, University of Tennessee Health Science Center, [3]Clinical Pharmacy and Translational Science, Univeristy of Tennessee Health Science Center

Aspergillus fumigatus SsdA is a putative translational repressor regulating growth, cell wall integrity, and virulence. Orthologs of SsdA are known to be negatively regulated by the conserved Nuclear Dbf2-Related (NDR) kinases Cbk1 and COT-1 in *Saccharomyces cerevisiae* and *Neurospora crassa*, respectively. We previously identified the *A. fumigatus* Cbk1 ortholog, CotA, as an important virulence component in mouse models of invasive aspergillosis. Although the underlying virulence mechanism is unknown, we found CotA to regulate invasive hyphal growth in response to host-relevant carbon sources. Strains carrying *cotA* gene disruptions (*cotA*-1) fail to grow on non-sugar carbon sources that are readily available in the host lung environment, such as acetate and amino acids. The main objective of this work was to determine if CotA orchestrates growth in non-preferred carbon sources through the conserved downstream effector, SsdA.

In vitro media supplementation assays showed that loss of *ssdA* in the wild type strain (CEA10) (Δ*ssdA*) caused significant growth defects in both repressing and de-repressing conditions, reducing colony diameter of mature cultures by 30-50% depending on the carbon source. In contrast, deletion of *ssdA* in the *cotA*-1 disruption mutant (Δ*ssdA*/*cotA*-1) caused only a minor growth reduction in the presence of glucose. Strikingly, when compared to the parental *cotA*-1 disruption mutant, this double mutation resulted in significant growth recovery in non-sugar carbon sources. These results correlated with biomass accumulation observations, where the Δ*ssdA*/*cotA*-1 mutant displayed a significant increase in biomass in comparison to the *cotA*-1 parental strain under similar conditions. Therefore, loss of *ssdA* partially restored growth to the *cotA*-1 mutant in alternative carbon sources. As it is a putative translational repressor, we next sought to test if SsdA might function in a feedback loop to regulate CotA protein abundance in response to carbon source. In the wild type, we observed that culture in acetate resulted in only mild reductions in CotA protein abundance when compared to glucose culture conditions. Loss of *ssdA* did not impact CotA abundance in glucose, when compared to the wild type. In contrast, we observed a significant reduction of CotA abundance in Δ*ssdA* under acetate versus glucose culture.

Together, our findings support the hypothesis that the conserved translational repressor, SsdA, operates downstream of CotA to partially regulate the carbon source-dependent roles of CotA signaling in *A. fumigatus*. However, this regulatory mechanism is unlikely to be through direct feedback regulation of carbon source-responsive CotA translation.

382C **Sticky Business: Unraveling the Evolutionary Significance of Asymmetric Adhesion in *Colletotrichum* Species** Caleb O Bedsole[1], Mary Cowser[2], Jillian Hamilton[2], Lucia Gonzalez Rodriguez[2], Brian d Shaw[2] [1]Plant Pathology & Microbiology, Texas A&M University, [2]Texas A&M University

Colletotrichum is a globally significant genus of plant pathogens known for causing anthracnose across a diverse array of hosts. *Colletotrichum graminicola* is notable and is a pathogen affecting maize. Annually, the global economic impact of this pathogen reaches billions of dollars. *C. graminicola* conidia have a characteristic falcate shape and are dispersed by rain. Upon attachment to maize leaves, these conidia develop melanized appressoria that penetrate the leaf surface to initiate disease. Recent findings have brought attention to the existence of an adhesive strip that colocalizes with an actin array on one side of *C. graminicola* conidia, playing a crucial role in facilitating attachment and germination. This asymmetrical adhesive was postulated to enhance spore dispersal by assuring that some conidia do not attach to their initial deposition site. However, the extent of this asymmetric adhesive phenotype in other *Colletotrichum* species remains unknown, raising questions about its conservation within the genus. This study reveals the prevalence of an asymmetric adhesive in various *Colletotrichum* species despite morphological differences in spore shape. Notably, *Colletotrichum truncatum* is unique from other observed species by exhibiting a symmetric adhesive on both sides of its conidium. Furthermore, simultaneous development of the actin array and detachment from the conidiophore in *C. graminicola* was observed during spore development. The study of other *Colletotrichum* members holds promise in elucidating the evolutionary trajectory of this phenotype. Furthermore, these insights may prove instrumental in understanding spore dispersal dynamics across diverse hosts, shedding light on the intricate web of host specificity within the genus.

383C **Expanding the fluorescent toolbox in *Aspergillus fumigatus*** Isabelle S R Storer[1], Enrique V Sastré-Velásquez[2], Thomas J Easter[1], Birte Mertens[2], Michael J Bottery[1], Raveen Tank[3], Michael J Bromley[1], Fabio Gsaller[2], Norman van Rhijn[1] [1]Manchester Fungal Infection Group, University of Manchester, [2]Institute of Molecular Biology, Medical University of Innsbruck, [3]Microbial Evolution Research Manchester, University of Manchester

Fluorescent proteins are indispensable tools used to understand the spatio-temporal dynamics of molecular processes in living cells. Even though *Aspergillus fumigatus* causes more deaths globally than any other fungal disease, we lack a well-characterised tool kit of next-generation fluorophores, limiting our ability to probe fundamental biological processes of this critical human fungal pathogen. In this work, we chromosomally transform *A. fumigatus* with 18 fluorescent proteins with emissions covering the visible light spectrum and characterise their practical brightness during the different morphological stages. Through live cell imaging using fluorescence confocal microscopy and imaging flow cytometry, the relative intensity of each fluorophore was measured during hyphal growth and in spores. The fluorescent proteins mTagBFP2, mNeonGreen, Citrine, mKO2, mApple, and Katushka2S - green, yellow, orange, red and far-red respectively - displayed the highest relative fluorescent intensity in germlings. We demonstrate the utility of these reporters as inducible promoter systems, protein tagging, and pathogenicity. Finally, we generate a 4-colour strain by exploiting counter-selectable markers of the pyrimidine salvage pathway. This strain visualises the mitochondria, vacuoles, peroxisomes, and cell membrane to understand the dynamics of these subcellular structures in response to antifungal agents. This new resource will enable the community to conduct advanced live-cell imaging to gain a deeper understanding of subcellular localisation, quantify protein-protein interactions, elucidate novel druggable targets, and visualise host-pathogen interaction models.

384C **The sterol C-24 methyltransferase encoding gene, *erg6*, is essential for viability of *Aspergillus* species** Jinhong Xie[1], Jeffrey M Rybak[2], Adela Martin-Vicente[1], Xabier Guruceaga[1], Harrison I Thorn[1], Ashley V Nywening[1], Wenbo Ge[2], Josie E Parker[3], Steven L Kelly[4], David Rogers[2], Jarrod R Fortwendel[1] [1]University of Tennessee Health Science Center, [2]St. Jude Children's Research Hospital, [3]Cardiff University, [4]Swansea University

Ergosterol is a critical component of fungal plasma membranes. Triazoles, the most widely used antifungal drug class in the world, inhibit ergosterol biosynthesis for antifungal effect. Global increases in triazole resistance have threatened the continued use of these drugs and highlight the need for novel antifungals. Recent studies identified the fungus-specific sterol C-24 methyltransferase enzyme, Erg6, as a bona fide novel antifungal target in human pathogenic yeast. Unfortunately, Erg6 enzymes are largely unstudied in filamentous fungal pathogens like *Aspergillus fumigatus*. Here, we show for the first time that the lipid droplet-associated sterol C-24 methyltransferase, Erg6, is essential for *A. fumigatus* viability. We further show that this essentiality extends to additional *Aspergillus* species, including *A. lentulus, A. terreus,* and *A. nidulans*. Downregulation of *erg6* causes loss of sterol-rich membrane domains required for apical extension of hyphae and altered sterol profiles consistent with the Erg6 enzyme functioning upstream of the triazole drug target, *cyp51*A / *cyp51*B. Unexpectedly, *erg6* repressed strains demonstrate wild-type susceptibility against the ergosterol-active triazole and polyene antifungals. Finally, *erg6* repression reduces fungal burden accumulation in a murine model of invasive aspergillosis. Taken together with recent studies, our work supports Erg6 as an attractive and potentially pan-fungal novel drug target.

385C **The striatin-interacting protein phosphatase and kinase complex (STRIPAK complex) in Ustilago maydis** Julia Dennig[1], Lea Morbe[1], Kerstin Schmitt[2], Oliver Valerius[2], Gerhard Braus[2], Joerg Kaemper[1] [1]Genetics, Karlsruhe Institute of Technology, [2]Georg-August-University Göttingen

The striatin-interacting phosphatases and kinases (STRIPAK) complex is evolutionary highly conserved in eukaryotes. It functions as a node, by physical interaction with conserved signaling complexes to establish larger networks. In fungi, STRIPAK affects sexual development, growth and cell fusion. In fungi, investigation of STRIPAK has been focused on Ascomycetes as *S. macrospora*, *N. crassa* or *S. cerevisiae*. Here, we present characterization of STRIPAK components in the dimorphic basidiomycete *Ustilago maydis*, a pathogen of maize plants.

The main components of STRIPAK are kinases, tail-anchored proteins, developmental proteins and the phosphatase consisting of regulatory, catalytic and scaffolding subunits. The regulatory subunit, striatin, mediates interaction of the tail-anchored protein and the developmental protein, resembling the inner framework of the complex. The tail-anchored protein ensures localization at the membrane of mitochondria or nuclei, while the developmental protein is linked to internal membrane systems.

In contrast to Ascomycetes, both striatin (Far8, Umag03784) as well as the developmental protein (Far11, Umag10285) are essential in *U. maydis*, which might indicate supplementary pathways orchestrated by STRIPAK.

Deletion of the anchor protein (Far10, Umag04391) in *U. maydis* mirrors phenotypes observed in Ascomycetes, as cytokinesis defects, defects in hyphal fusion, premature release of pheromone-induced cell cycle arrest, altered cell wall and membrane integrity. *Δfar10*-strains are apathogenic as fungal cells are neither able to attach to the plant surface nor capable to form infection structures. Concomitantly with the observation in other systems, a complex of Far8, Far10 and Far11 localizes to the ER and mitochondria in sporidia (haploid cells that propagate by budding). However, colocalization of Far8 and Far10 or of Far11 with either Far8 or Far10 was lost after the switch from budding growth to filamentous growth or during filamentous growth. Only Far8 remains at mitochondria in filaments. The colocalization of each Far8, Far10 and Far11 with the nuclear envelope disappear during the dimorphic shift, pending on the migration of the nucleus from the initial sporidia to the emerging hypha. Our results indicate a restructuring of the STRIPAK complex during the dimorphic shift of *U. maydis* that precedes plant infection.

386C **Deciphering ploidy transitions of titan cells in *Cryptococcus neoformans*** Zhuyun Bian, Sheng Sun, Joseph Heitman Duke University School of Medicine

Cryptococcus neoformans is a prominent global fungal pathogen affecting primarily immunocompromised individuals and causing life-threatening meningoencephalitis. During the initial stage of pulmonary infection, cryptococcal cells produce large polyploid titan cells that exhibit heightened resistance to host immune defenses and potentially contribute to the persistence of cryptococcal infections. These titan cells divide to produce haploid and aneuploid (1N+1, 1N+2) daughter cells, potentially contributing to systemic infections. However, both the factors involved in triggering polyploidization in cryptococcal cells and the mechanisms mediating ploidy reduction remain elusive. In this investigation, we focused on diploid BH strains (fusion products of strains H99 and Bt63) as well as the AI187 strain (fusion product of strains M001 and JF99) to examine titan cell functions. Our findings reveal that titan cells that originate from diploid cells produce diploid daughter cells in most cases. Interestingly, we did identify several haploid daughter cells based on flow cytometry analysis. Moreover, distinct drug resistance profiles compared to the original diploid parental cells were observed among diploid daughter cells. Whole genome sequencing and comparison of both daughter and progenitor diploid cells revealed genetic variation, attributable to Loss of Heterozygosity (LOH) events or aneuploidy (2N+1) in the daughter cells. We are further exploring the possible involvement of meiosis-specific genes in titan cell biology. To this aim, we generated both haploid and diploid strains with deletions in the *SPO11* and *DMC1* genes, known for their central roles in canonical sexual reproduction. These studies will provide insight into whether meiotic genes, beyond their conventional functions in sexual reproduction, may also contribute to the adaptation of eukaryotic cells undergoing substantial genome changes in response to genotoxic stress.

387C **Post-Biogenesis Maturation of Pathogenic Fungal Spores Expands Germination Competence** Megan McKeon[1], Christina Hull[1,2] [1]Biomolecular Chemistry, University of Wisconsin-Madison, [2]Medical Microbiology & Immunology, University of Wisconsin-Madison

Under growth-limiting conditions, the human fungal pathogen *Cryptococcus* initiates sexual development and produces spores to aid in the colonization of new environments. To survive, these stress-resistant cells must remain dormant until they encounter favorable conditions for germination, an essential differentiation process in which spores transition into vegetatively growing yeast. While spores and yeast are both able to cause disease in mouse models of infection, the identity of infectious particle has a profound effect on disease progression and outcome, with spores causing higher fungal burdens in the brain. Despite the known of impact of spore-mediated infection, the molecular networks governing essential spore processes remain poorly understood. Based on observations that spore responses vary within a population, we hypothesized that spores undergo changes after biogenesis (maturation) that enable successful germination in diverse conditions. To determine the nature of *Cryptococcus* spore maturation, we carried out a series of quantitative germination assays with spores at different stages of development under a variety of environmental conditions and in the presence of inhibitors of specific biological processes. We discovered that immature spores are less efficient at germinating in complex carbon sources and that chromatin state and translational capacity differ between immature and mature spores. Transcriptomic analyses revealed distinct changes at the transcript level over the course of maturation, including an increase in transcripts associated with ribosome biogenesis and chromosome organization and a decrease in transcripts associated with transcriptional regulation and metabolic processes. Taken together, our data suggest that spore maturation includes establishing a suitable chromatin state and increasing ribosome biogenesis to poise spores to both sustain dormancy and germinate in diverse environments. These data also provide the first evidence for a maturation process in spores of a basidiomycete fungus. Defining the molecular mechanisms governing spore maturation promises to further our understanding of spore survival and germination and provide opportunities to identify spore-specific pathways to aid in the identification of targets for antifungal drug development.

388C **Characterization of exposed *Cryptococcus neoformans* cell wall components via fluorescence microscopy** Joseph G. Vasselli Molecular Genetics and Microbiology, Duke University

Cryptococcus neoformans is a basidiomycete fungus and the causal agent of cryptococcosis, an infection that largely affects immunocompromised individuals. The fungi are often found in the lungs and brains of affected patients, and over 100,000 die each year from infection. The fungal cell wall is an attractive target for developing antifungal therapies as it is essential for yeast and is absent in the mammalian host. Moreover, in Cryptococcus, the cell wall is critical for the expression of virulence-related traits, and it contains many pathogen-associated molecular patterns (PAMPs) that dictate the nature of the host immune response. Despite its importance, much of what is known about the molecular mechanisms of cell wall biosynthesis of *Cryptococcus*, its architecture, and the mechanisms by which it enables the yeast to survive under various stress conditions is assumed from research done in other model systems. Previous research has demonstrated that the cell wall's content and organization are particularly sensitive to the environment in which the fungal cells grow. Therefore, these media-dependent changes in the cell wall can alter the nature of the host immune response, with some media conditions causing a lethal immune response even to dead cells. Mutations in enzymes that remodel the cell wall also present immunologically relevant effects, as it has been previously shown that strains in which chitin deacetylases have been deleted induce a protective response in murine models that primes the immune system for defending against future infections. Understanding the architecture of the cell wall under these conditions is therefore essential to developing treatments and a potential fungal vaccine that protects against infection. My research seeks to target which cell wall components are necessary for these protective and lethal responses. Using fluorophores that bind to specific cell wall components, I have begun to label each component individually to create maps of the architecture of *C. neoformans* cells under immunologically relevant conditions, such as differing growth media or mutants with cell wall defects. Comparison of these fluorescent spatial maps of *C. neoformans* cell walls under lethal and protective conditions reveals which PAMPs are exposed on the cell surface. Elucidating which PAMPs are exposed and what remodeling is necessary for their exposure will present possible targets for future anti-fungal compounds and provide an understanding of which cell wall components induce a protective response in the host. This understanding would expedite the process of developing an anti-fungal vaccine.

389C **Novel microscopy tools reveal dynamic sub-cellular distributions of core clock components in *Neurospora crassa*** Ziyan Wang[1], Bradley M Bartholomai[1], Jennifer J Loros[2], Jay C Dunlap[1] [1]Dept of Molecular and System Biology, Geisel School of Medicine at Dartmouth, [2]Dept of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth

Circadian rhythms are endogenous daily oscillations driven by a molecular clock that helps organisms better coordinate with the environment. Organisms from fungi to animals share a similar phosphorylation-driven transcription/translation negative feedback loop as the core clock mechanism, an oscillator composed of positive and negative elements. Research on the filamentous fungus model organism, *Neurospora crassa*, has provided answers to many fundamental clock-related questions. In *Neurospora crassa*, the transcription factor White Collar Complex (WCC) serves as the positive element driving the transcription of *frequency* (*frq*). The intrinsically disordered protein Frequency (FRQ), with other regulators, forms the negative element complex that inhibits the function of WCC and stops *frq* transcription. Molecular components of the circadian clock have been described over decades of genetic and molecular biological studies. However, little is known about their dynamics and regulation at the subcellular level.

Neurospora crassa grows as a syncytium analogous to muscle cells, thus the subcellular distribution of molecules must facilitate precise temporal control throughout the syncytium. Live-cell imaging has emerged as a valuable tool in circadian research. We implemented novel strategies and microscopy tools for *Neurospora*, including 4-color imaging and microfluidics compatible with multi-day growth, to facilitate live-cell imaging of low-abundance circadian proteins. Through multi-color live-cell imaging in single cells, we tracked the circadian dynamics of the subcellular localization of WCC and FRQ in high spatiotemporal resolution. We also observed *in vivo* highly dynamic liquid–liquid phase separation (LLPS)-like behaviors of FRQ using the super-resolution SoRa microscope. Furthermore, by employing FRAP, we have unraveled the circadian-regulated nuclear import of FRQ and its underlying mechanism. We also optimized photoconvertible fluorescent proteins to facilitate further exploration of the nucleocytoplasmic transport of clock proteins.

Our work showcases the successful application of advanced microscopy techniques in a conventional fungal model organism to gain insights into the intricate subcellular dynamics of circadian proteins, paving the way for a deeper understanding of circadian rhythms.

390C **Role for Septins During High Temperature Stress Response in *Cryptococcus neoformans*** Tejas Mahendra Patel[1], Stephani Martinez Barrera[1], Piotr Stempinski[2], Lukasz Kozubowski[2] [1]Genetics and Biochemistry, Clemson University, [2]Clemson University

The fungal pathogen *Cryptococcus neoformans* adapts to the change in temperature upon entering its human host. *S*eptin proteins Cdc3 or Cdc12 are dispensable for growth of *C. neoformans* at 25°C but essential for proliferation at 37°C and therefore critical for virulence. Septins are a family of conserved filament-forming GTP-ases that bind to phosphoinositides and assemble as higher order complexes at the cell cortex to support cytokinesis and morphogenesis in fungal and animal cells. The exact contribution of septins to growth of *C. neoformans* at 37°C remains unclear. We find that upon temperature change to 37°C, septins accumulate at the plasma membrane (PM) as puncta, which likely represent the entire septin complex, including Cdc3, Cdc10, Cdc11, and Cdc12. To investigate possible contribution of the septin complex to cell wall and PM integrity, we compared the mutants lacking septin Cdc3 or Cdc12 with the wild type and two mutants that were expected to exhibit defects in either cell wall (*csr2*D) or plasma membrane (*erg3*D) integrity. We performed the following experiments: 1. Tested growth of the strains under conditions that compromise either cell wall or the PM. 2. To assess PM integrity, we evaluated the internalization of propidium iodide at 25 and 37°C. 3. We assessed the viability of the strains after treatment with cell wall digesting enzymes under hypotonic conditions. 4. We evaluated plasma membrane and cell wall morphology based on Transmission Electron Microscopy (TEM). In addition, we performed a mass spectrometry analysis of septin interactome at both 25 and 37°C. Our results suggest that septins play an important role in maintaining homeostasis of both the cell wall and the PM and emphasize how these two compartments are functionally interdependent and critical for adaptation to host temperature.

391C **The role of the GTPase Cdc42 in *Cryptococcus neoformans* stress response** Hannah Segbefiah Akahoho[1], Congyue Peng[2], Nabanita Saikia[3], Feng Ding[3], Lukasz Kozubowski[1] [1]Genetics and Biochemistry, Clemson University, [2]Clemson University, [3]Physics and Astronomy, Clemson University

In ascomycete yeast, *Saccharomyces cerevisiae*, the conserved Rho-family GTPase Cdc42 is essential for establishment of cell polarity and cytoskeletal organization. Cdc42 in basidiomycete pathogenic yeast, *Cryptococcus neoformans*, is not essential for establishment of cell polarity and growth at non-stress conditions. However, *C. neoformans* Cdc42 is essential for thermotolerance and dissemination in the murine inhalation model of cryptococcosis. It has been proposed that Cdc42 is involved in adaptation to 37°C by facilitating assembly of the complex composed of the filament-forming GTPases, septins, that contribute to cytokinesis and morphogenesis and are required for growth at 37°C. However, it remains unclear how Cdc42 impacts assembly of the septin complex and how it contributes to growth at 37°C. Here, we constructed a Cdc42-reconstitute strain, in which the activity of Cdc42 is controlled by the blue light-dependent switch. The molecular switch is based on the allosteric changes of a light, oxygen, voltage sensing domain (LOV2) of oat, *Avena sativa*. Blue light triggers allosteric configuration changes of LOV2 and inactivates Cdc42. The effectiveness of this light switch was confirmed. With this tool, we can control the activity of Cdc42 in living cells. Additionally, we are introducing a biotinylation domain (UltraID) to Cdc42 and based on this construct a mass spectrometry analysis of biotinylated proteins will be performed to identify Cdc42 binding partners that may be relevant to high temperature adaptation and virulence of *C. neoformans*.

392C **A fitness landscape instability determines the morphological diversity of tip growing organisms** Branka Zivanovic[1], Enrique Rojas[2], Maxim Ohairwe Ermoshkin[2] [1]Institute for Multidisciplinary Research, University of Belgrade, [2]Biology, New York University

Cellular morphology affects many aspects of cellular and organismal physiology. This makes it challenging to dissect the evolutionary basis for specific morphologies since various cellular functions may exert competing selective pressures on this trait, and the influence of these pressures will depend on the mechanisms of morphogenesis at play. To tackle this problem, we combined experiment and theory to investigate the mechanistic basis for morphological diversity among tip-growing cells from across the tree of life including fungal, plant and oomycete organisms. We discovered that an instability in the widespread mechanism of “inflationary” tip growth leads directly to a bifurcation in the common fitness landscape of tip-growing cells, which imposes a strict global constraint on their morphologies. We were able to test the predictions of shape instability on the highly tapered hyphae of the oomycete Achlya bisexualis. This result rationalizes the morphology of an enormous diversity of important fungal, plant, protistan, and bacterial systems. More broadly, our study describes a novel principle by which strong evolutionary constraints on complex traits, like biological form, may emerge from emergent instabilities within developmental systems.

393C **Defining sexual reproduction in *Coccidioides posadasii*** Marcus de Melo Teixeira[1], Klaire Laux[2], Matthew Morales[2], Ana Braga[2], Ashley Itogawa[2], Kaitlyn Parra[2], Heather Mead[2,3], Daniel Kollath[2], Bridget Barker[2] [1]University of Brasilia, [2]Northern Arizona University, [3]Translation Genomics Research Institute

Coccidioides spp. are dimorphic fungal pathogens endemic to arid regions across the Americas, responsible for causing coccidioidomycosis, commonly known as Valley Fever. Within the United States alone, an estimated ~350,000 human infections occur annually, with approximately 60% of cases manifesting as asymptomatic or exhibiting subclinical symptoms, while the remaining 40% develop acute respiratory infections. Of these, 1-5% progress to disseminated disease. It is known that *Coccidioides* exist as saprobic mycelia that reproduce asexually and forming infectious arthroconidia in the environment. When inhaled by a susceptible host, conidia transition to spherules inside which endospores are produced, released, and spread throughout the host. However, the sexual stages of *Coccidioides*' life cycle remain elusive, evading comprehensive understanding. Evolutionary analysis shows notable genetic diversity and admixture. Population genomics reveal allele sharing between divergent species, notably demonstrating introgression between *C. posadasii* and *C. immitis*, and can impact genetic diversity and adaptation. We hypothesize that the sexual cycle explains observed recombination patterns within *Coccidioides*, and meiospores (ascospores) with varied genotypic diversity in segregants could explain geographic expansion, broad host range, varied clinical phenotypes and antifungal resistance. Phylogenetic analyses show that *Coccidioides* is related to asci-producing species in the Onygenales that produce spherical or pear-shaped ascomata harboring uncinate, curved, or helical appendages. The ascomata consist of tightly interwoven hyphae and may contain multiple asci, each containing four to eight ascospores. Ascomycetes hinges upon two pivotal transcription factors: the MAT1-1 idiomorph, encoding an alpha domain transcription factor, and the MAT1-2 idiomorph, encoding a high-mobility-group (HMG)-domain transcription factor responsible for sexual identity determination. In *Coccidioides*, the *MAT1-1* and *MAT1-2* idiomorphs have been characterized. Additionally, genomic analyses of mating components support the existence of core sexual cycle machinery in *Coccidioides*. Finally, we provide data from *in vitro* mating crossings between *C. posadasii* strains, revealing the presence of fully developed ascomata, containing globose structures that resemble from young ascocarps covered with coiled appendages to asci containing globose cells consistent with ascospores. Both morphological and genomic evidence strongly supports the existence of a perfect life stage. Deciphering recombination mechanisms paves the way for probing long-term environmental survival, diverse pathogenesis, and the potential for host and niche expansions in future investigations.

394C **Re-routing of MAP kinase signaling for pentration peg formation in predator yeast** Mareike L Rij[1], Yeseren Kayacan[2], Florian Michling[3], Beatrice Bernardi[3], Juergen W Wendland[4] [1]Dept of Microbiology and Biochemistry, Hochschule Geisenheim University, [2]Vrije Universiteit Brussel, [3]Hochschule Geisenheim University, [4]Microbiology and Biochemistry, Hochschule Geisenheim University

Predator yeasts are either homothallic or heterothallic ascomycetes of the genus *Saccharomycopsis*. These yeasts represent a unique genus of necrotrophic mycoparasites that predate a wide range of yeasts and filamentous fungi. Their mycoparasitism can be divided into several phases including recognition of and adhesion to prey cells, penetration of prey cells, their killing and nutrient uptake. For the penetration of a prey cell a dedicated structure called penetration peg is formed. Penetration pegs grow in a polarized manner into the prey cells and these pegs show strong fluorescence with dyes recognizing carbohydrate moieties suggesting active protein secretion. In contrast to penetration hyphae of plant pathogenic fungi, predator yeast penetration pegs do not grow out of their prey cells, do not contain nuclei and thus do not become daughter cells. Each penetration peg is, therefore, a one-time investment for an attack of a single cell. Homologs of the mating and filamentation MAP kinase cascade of *Saccharomyces cerevisiae* have been shown to regulate appressorium formation e.g. in *Magnaporthe oryzae*. Similarly, deletion of the single *KSS1*/*FUS3* map kinase homolog *KIL1* in *Saccharomycopsis schoenii* generated non-predacious strains. *S. schoenii kil1* mutant cells were unable to form penetration pegs under nutrient-limiting and predation-promoting conditions. A downstream target of the *S. cerevisiae* Kss1/Fus3 MAP kinases is the transcription factor Ste12. We show that *S. schoenii ste12* mutants showed comparable phenotypes as *kil1* mutants indicating that Ste12 is a major target for Kil1-signaling. Comparative RNAseq transcriptomics identified genes involved in either hunger or predation. Particularly, amongst the predation response genes a shared promoter motif was identified with resemblance to the *S. cerevisiae* Ste12 DNA-binding site. Our data suggest a re-routing of MAP-kinase signaling in predator yeasts to regulate penetration peg formation in a similar way to what has been observed for *Magnaporthe,* even though these species are separated by >400 million years of evolution.

395C **Role of the cargo receptor CSE-8 in the intracellular trafficking of chitin synthases class I to the Spitzenkörper and septa in *Neurospora crassa*** Meritxell Riquelme[1], Samantha V González-Téllez[2] [1]Microbiology, Centro de Invstigación Científica y de Educación Superior de Ensenada, [2]Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada, B. C.

Chitin, a structural polysaccharide, plays a crucial role in maintaining cellular plasticity and integrity in most fungi. Chitin synthesis is orchestrated by chitin synthases (CHS), a major family of transmembrane proteins. Studies in*Saccharomyces cerevisiae*have identified Chs7 as the cargo receptor essential for the exit of Chs4 from the endoplasmic reticulum (ER). However, in filamentous fungi, the auxiliary machinery responsible for CHS trafficking remains poorly understood.*N. crassa*has two orthologues of Chs7: chitin synthase export (CSE) proteins, namely CSE-7 (NCU05720) and CSE-8 (NCU01814).Both CSE-7 and CSE-8 are highly conserved within filamentous Ascomycota, while in yeast, there is only one copy of these CHS export receptors. Previous research has highlighted the crucial role of CSE-7 in the localization of CHS-4 at cell wall synthesis sites, such as the Spitzenkörper (SPK) and septa[1]. In this study, CSE-8 was identified as a cargo receptor for CHS-3 (class I). In the*N. crassa*knockout strain*Δcse-8*, CHS-3-GFP fluorescence no longer localized at the SPK or septa, indicating the dependence of CHS-3 on CSE-8 for exiting the ER. In addition, the*Δcse-8*strain exhibited thinner hyphae and lower total chitin content than the wild-type strain. Sexual development was also affected in*Δcse-8*, as genetic crosses between *Δcse-8 mat a* and *mat A* resulted in perithecia abnormalities. Further studies are needed to comprehend the transport mechanisms of the other CHS, especially those unique to filamentous fungi (CHS classes 3, 5, 6 and 7).

[1]Rico-Ramírez, A. M., Roberson, R. W., Riquelme, M. (2018). Imaging the secretory compartments involved in the intracellular traffic of CHS-4, a class IV chitin synthase, in *Neurospora crassa*. Fungal Genetics and Biology, 117, 30-42.

396C **Investigating uniparental inheritance of mitochondrial DNA during sexual reproduction in *Cryptococcus neoformans*** Ran Shi Microbiology, UGA

Mitochondrial DNA uniparental inheritance (mtUPI) is prevalent in eukaryotic species, in which heteroplasmy can have a detrimental effect on the organisms. Still, the mechanism underlying mtUPI remains unclear, particularly in isogamic species like fungi. In anisogamic species like mammals where the size of the two gametes - egg and sperm - differ drastically, uniparental inheritance of mitochondria DNA is thought to be largely achieved by passive dilution. However, fungi or protists have gametes of similar cell size, indicating that other mechanisms are critical to ensure strict mtUPI. Given that isogamy is the presumed ancestral state of all eukaryotes, the mechanisms by which isogamic species achieve mtUPI will provide valuable insights into the evolution of mtUPI in eukaryotes. Since the meiotic progeny of the two model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* can inherit mtDNA from either parent, these species are ineffective for studying mtUPI. We propose to utilize *Cryptococcus neoformans* as a tractable eukaryotic model to study mtUPI. *C. neoformans* meiotic progeny inherits mtDNA from the **a** parent during **a**-α bisexual reproduction. Our preliminary data indicate that mitochondria from the α mating partner can enter the conjugation tube and zygote, and that α mtDNA gradually disappears after zygote formation. These observations refute the hypothesis that the exclusion of α mitochondria from migrating across the zygote is the reason for mtUPI in this fungus. We present evidence that elimination of α mitochondria or α mtDNA plays a major role in achieving mtUPI in *C. neoformans*. We are currently exploring key factors that control mtDNA stability and/or mitophagy for their potential roles in ensuring mtUPI.

397C **The peroxisome trafficking protein PxdA is required for secondary metabolite production and infection in the plant pathogenic fungus *Alternaria alternata*** Valentin Wernet, Livia D Songster, Gaurav Kumar, Swetha Mahesula, Patreece Suen, Samara Reck-Peterson University of California San Diego

The evolutionary success of fungi is partially based on their ability to produce secondary metabolites. Biosynthetic genes of these secondary metabolites are typically organized in gene clusters, and their production is often compartmentalized in different organelles (for example in peroxisomes) to protect the host from either toxic precursors or aid with complex biosynthesis. However, the role of organelle trafficking during secondary metabolite production remains largely unexplored. This is due to the specific conditions under which they are produced. In filamentous fungi, organelles are primarily moved long distances by the microtubule cytoskeleton and their associated motor proteins. In the mold *Aspergillus nidulans*, peroxisomes move along microtubules by hitchhiking on motile endosomes, a process that requires the endosome-associated protein PxdA (peroxisome distribution mutant A). However, the physiological reason for peroxisome hitchhiking is unclear. Based on our preliminary data we propose a role in secondary metabolite production.

Here we studied the role of peroxisome trafficking in the filamentous fungus *Alternaria alternata*, which produces toxic secondary metabolites during plant infection. To investigate the role of peroxisome trafficking during the fungal-plant interaction we

generated a mutant strain lacking the *A. alternata pxdA* gene using CRISPR-Cas9. The *A. alternata* Δ*pxdA* strain produces less aerial mycelium and has increased black pigmentation. Analysis of secondary metabolite production by thin-layer chromatography revealed the absence of at least two secondary metabolites in the Δ*pxdA* mutant, one being the mycotoxin Alternariol. Infection assays on tomatoes revealed a reduced virulence of the *pxdA* deletion strain. Investigating peroxisome localization in wild-type and the Δ*pxdA* mutant strain under varying conditions will unveil insights into trafficking mechanisms crucial for secondary metabolite production.

398C **Allocation of nuclei and growth potential among buds of the multi-budding yeast, *Aureobasidium pullulans*** Alison Wirshing[1], Claudia Petrucco[2], Analeigha Colarusso[1], Daniel Lew[1] [1]MIT, [2]Duke University

A. pullulans is a poly-extremotolerant yeast that thrives in diverse environments (both terrestrial and marine). In its yeast form, *A. pullulans* cells vary greatly in size and nuclear number, and they produce variable numbers of buds in each cell cycle. Here, we ask how equitably an *A. pullulans* mother cell distributes nuclei and growth potential amongst its multiple buds. Using live-cell imaging to track cell growth and nuclear partitioning, we find that a mother cell synchronously builds buds that grow at similar rates and attain similar volumes (~14% variability between daughters). The number of nuclei inherited by each daughter is more variable: most daughter cells inherit one nucleus, while ~10% inherit two. Mother cell nuclear number can increase, remain unchanged, or decrease in a given cell cycle. Daughters that inherit two nuclei have smaller nuclei than daughters that inherit only one, maintaining a relatively constant nuclear to cytoplasmic volume ratio (~10% variability between daughters). Interestingly, multi-budded cells appear to ensure each bud receives at least one nucleus, suggesting that nuclear segregation is coordinated among the daughters. Imaging mitotic spindle dynamics with a fluorescent tubulin probe, we found that metaphase spindles were not pre-aligned towards buds and had few astral microtubules. Anaphase was rapid and coincided with growth of astral microtubules and bending and rotation of the spindles. The fates of individual spindles in multinucleate cells was variable: in some cases the spindle deposited one pole in the mother and one in a bud as in typical budding yeasts, but in other cases both spindle poles remained in the mother, or both spindle poles entered buds. These observations do not indicate any obvious form of coordination between the spindles, and raise the possibility that cells may assure nuclear inheritance to each bud by post-mitotic error correction.

399C **Fungal Raincoats** Trine Aalborg[1], Marie Overgaard[2], Klaus Ringsborg Westphal[2], Thea L. Lunden[2], Reinhard Wimmer[2], Jens Laurids Sørensen[2], Teis Esben Sondergaard[1] [1]Chemistry and Bioscience, Aalborg University, [2]Aalborg University

We have identified a novel type of compound that may be utilized by fungi to fortify their cell walls, rendering them impermeable to water. Small hydrophobic molecules likely play a crucial role in binding proteins together. The fungal cell wall serves as a highly adaptable framework constructed from a robust network of diverse organic molecules, providing protection against various environmental challenges such as UV radiation, hydration, dehydration, chemical agents, mechanical stress, and predators. It has long been recognized that fungi produce hydrophobic components within their cell walls to thrive under varying humidity conditions. Currently, hydrophobins are credited with conferring hydrophobic properties to fungal cell walls, but our objective is to establish that cell wall constituents are joined by small secondary metabolites. The practical applications and significance of water-insoluble nanomaterials based on proteins are substantial, and this research would advance our comprehension of fungal biology. Our investigation has focused on these substances within two fungal genera, *Apiospora* and *Fusarium*; however, we propose that this mechanism is prevalent across the Ascomycota branch of the fungal kingdom.

400C **Cytoskeletal Mechanisms Driving 3D Cellularization of Multinucleated Chytrid Fungi** Edgar M Medina, Lillian Fritz-Laylin Dept of Biology, University of Massachusetts Amherst

Chytrids are the only members of the fungi that have motile flagellated cells without a cell wall—the zoospore. Zoospores are produced by 3D multinuclear cellularization, a specialized form of cytokinesis. in which a single multinucleated mother cell gives rise to multiple uninucleated daughter cells arranged in a packed 3D lattice. Although we understand many molecular mechanisms driving cellularization in one-dimensional rods (fission yeast sporulation) and two-dimensional sheets (Drosophila blastoderm cellularization), how these principles extrapolate to 3D cellularization programs is unclear. To address this problem, we have developed genetic tools for chytrid fungi, a lineage that undergoes synchronous 3D multinuclear cellularization during their development. Here we describe the dynamics and core mechanisms of 3D cellularization in the chytrid fungus Spizellomyces punctatus for the first time. By combining live cell fluorescence imaging, laser ablation, and pharmacological perturbations we address three core questions: how are cleavage furrows initiated, the sources of membrane used for building the cleavage furrows, and the cytoskeletal driving force underlying furrow formation, ingression, and establishment of the 3D cellularization lattice. We show that furrows are initiated by a combination of nuclear cortical migration and interaction between the nuclear microtubule organizing center (MTOC) and the plasma membrane. These furrows extend using membranes sourced from Golgi-derived vesicles.

While microtubules improve the accuracy and precision of the cleavage pattern, they do not play a role in furrow initiation, ingression, and formation of the cleavage network. Instead, we find that actomyosin networks provide the driving force of furrow initiation, ingression, and establishment of the cellularization lattice, forming a polymer with viscoelastic properties consistent with a contractile network. Together, this work suggests that the mechanisms underlying 3D cellularization in chytrids use the same machinery as that used for 1D and 2D cellularization in animals and yeast, but deploy them differently. Our findings reiterate the need to study fundamental cellular processes in diverse lineages and cell types rather than assuming the conservation of molecular mechanisms based on parts lists.

401C
Development of Efficient Base-Editing Systems with Versatile Applications in Fungi Guoliang Yuan, Jeffrey J. Czajka, Ziyu Dai, Kyle R. Pomraning, Joonhoon Kim, Beth A. Hofstad, Shuang Deng Chemical and Biological Processes Development Group, Pacific Northwest National Laboratory

The CRISPR/Cas system has revolutionized fungal genetic engineering and significantly advanced the exploration of bioproducts production. Traditional CRISPR/Cas9-based techniques, which involve DNA double-strand breaks (DSBs), often lead to unwanted mutations or off-target effects. To address these concerns, non-DSB single-base editing tools have been extensively developed across multiple species. Those base editing methods offer a safer approach to DNA modification, significantly reducing major risks associated with conventional DSB-mediated gene editing, such as the variability of insertions and deletions (INDELs) at target sites, potential chromosomal abnormalities, and cellular toxicity. However, the application of base editing in filamentous fungi remains relatively limited. Our study aims to develop efficient base-editing systems specifically for filamentous fungi, focusing on adenine base editors (ABEs) and cytosine base editors (CBEs). Here, for the first time, we constructed an adenine base editor in filamentous fungi and achieved an editing efficiency of up to 80% in *Aspergillus niger*. This ABE was used to induce A-to-G mutations, disrupting conserved intron sites and leading to pre-mRNA missplicing and a loss-of-function phenotype. As demonstration, we successfully obtained *A*. *niger* null mutants of the *albA* gene through this strategy. The per-mRNA missplicing mechanism was further investigated by cDNA sequencing of *albA* gene null mutants. We also developed a cytosine base editor, demonstrating editing efficiency ranging from 50% to 100% in *A*. *niger*. This CBE precisely converted two codons (TGG and CGA) into stop codons without forming DSBs. Furthermore, we demonstrated that CBE can induce pre-mRNA missplicing and gene disruption by introducing precise C-to-T mutations in highly conserved intron sites. Additionally, we're exploring an alternative approach to ABE/CBE-mediated gene disruption, including ABE-induced adenine to guanine conversion in start codon (ATG-to-ACG) and CBE-driven cytosine to thymine conversion in start codon (ATG-to-ATA). This study highlights the potential of base editing in fungal genetics, enabling precise gene disruption by creating premature stop codons, alternative mRNA splicing, and start codon mutations. This approach could significantly accelerate the development of fungal strains with desired traits, optimizing the yield, efficiency, and specificity of bioproducts.

402C **Understanding the loading and functions of mRNAs in Plant Extracellular Vesicles** Huaitong Wu[1], Shumei N Wang[2], Baoye N He[2], Hailing N Jin[2] [1]Plant Pathology and Microbiology, University of California at Riverside, [2]University of California at Riverside

This research aims to investigate the loading and functions of mRNAs in Plant Extracellular Vesicles. Like animal cells, plant cells can secrete bilayer lipid nanoparticles termed extracellular vesicles into extracellular environments. Recent encouraging findings showed plant EVs share similar properties with animal EVs and can play important roles in cargo secretion, immune response, and gene regulation. Our data show that mRNAs inside EVs likely play important roles in plant immune response during pathogen infection. We show that atHEL, an immune response gene in Arabidopsis, is present in plant EVs, especially after Botrytis infection, and can reduce virulence but not growth when expressed in Botrytis. Besides specific transcripts, we are also interested in the mechanisms of RNA sorting in plant EVs and hypothesize that RNA motifs can play an important role in RNA sorting into EVs. To further understand EV RNA sorting mechanisms and potential applications, we will identify and validate signature sequences in plant EV mRNA sorting and explore the effects of engineered EV RNAs in RNA delivery and plant protection. We believe the research will elucidate how RNAs are packed into EVs in plants and help develop RNA delivery platforms that could be used in the medical or agricultural field.

403C **Developmental Specific Effects of Key Plant Essential Oils against *Aspergillus fumigatus* in Pre- & Post-Infection Plate Models** William Holt[1], Arline Martinez[1], Eduarda Goncalves[1], Yainitza Hernandez-Rodriguez[2] [1]Biological Sciences, Florida Gulf Coast University, [2]Florida Gulf Coast University

With the rise of drug-resistant fungi, specifically *Aspergillus fumigatus*, alternative treatment research is crucial as this devastating opportunistic pathogen adapts to modern treatments. Currently, Aspergillosis has surpassed other fungal diseases in hospital

settings. For instance, Invasive Aspergillosis causes detrimental respiratory effects in immunocompromised patients and severely decreases respiratory function. *A. fumigatus* has demonstrated the ability to thrive under different immunological-defense environments and challenges the clearance and survival of infections. Alarmingly, there is a high incidence of resistance to the limited expensive treatments available, and depending on the patient's biology, toxic side effects have become an issue. Therefore, drug interactions, side effects, and the appearance of resistant strains urge the community to research additional therapeutic agents that can be used against these devastating fungal diseases. In the search for natural and safer resources able to inhibit the growth of *A. fumigatus*, we turned to the analysis and potential of Plant Essential Oils (PEOs) as possible antifungal agents. PEOs' potential as treatment can be a genuine and innovative way to treat fungal diseases. Previously, we analyzed the efficacy of 54 PEOs against *A. fumigatus* compared to the common antifungal Voriconazole by conducting Zone of Inhibition Assays in plate models. Assays were conducted at 37˚C (average human temperature). T-Test analysis of 54 different PEOs showed that 10 PEOs were able to outperform the preferred medical treatment Voriconazole. Here, we present the potential of these key PEOs as antifungals in different fundamental developmental stages: 1) before the spore breaks dormancy, pre-germ tube emergence, and pre-polarity establishment (or "pre-infection"), and 2) after polarity establishment, after fungal hyphal growth and development (or "post-infection"). We were able to identify PEOs that are effective at both developmental stages, in addition to PEOs that show developmental-stage specific inhibition. Here, we are excited to show preliminary results of PEOs as antifungal agents in key growth stages, their effect in the fungal cell wall and nucleus, as well as their ability to be fungistatic or fungicidal. This research opens the possibility of PEOs therapeutic use on their own or synergistically with current antifungals.

404C **Role of fungal transglutaminase domain-containing proteins in wound-related hyphal protection at the septal pore** Md Abdulla Al Mamun[1], Jun-ichi Maruyama[2] [1]Harvard Medical School, [2]The University of Tokyo

Filamentous fungi under the multicellular subdivision, Pezizomycotina of Ascomycota, possess a primitive morphological structure known as the septal pore, which mediates cell-to-cell connectivity between flanking cells. An interconnected array of cells in fungal hyphae are highly vulnerable to the risk of excessive cytoplasmic bleeding via the septal pore upon hyphal wounding. The Woronin body is a fungal-specific organelle that plugs the septal pore upon hyphal wounding, thereby protecting the flanking cells from excessive cytoplasmic loss via septal pores. We previously identified a series of septal pore plugging (SPP) proteins[1)], one of which, SppB, contains the transglutaminase domain. Transglutaminase, an enzyme that crosslinks substrates via the isopeptide bond formation, is known to participate in blood clotting and wound healing in humans, but the related functions of microbial transglutaminases are unknown.

In this study, we performed a functional characterization of the transglutaminase domain-containing proteins in the filamentous fungus *Aspergillus oryzae*[2)]. Here, the cytokinesis-related protein Cyk3 and peptide *N*-glycanase Png1 were also analyzed as the transglutaminase domain-containing proteins. SppB and AoPng1 accumulated at the septal pore upon wounding, whereas AoCyk3 and AoPng1 normally localized around the septal pore. All these proteins exhibited functional importance in wound-related hyphal protection at the septal pore. The putative catalytic triads of SppB and AoCyk3 were involved in the septal pore-related functions. Similar to typical transglutaminases, SppB was cleaved in response to wounding to remove the N-terminal region, which is required for its hyphal protective function. Finally, using a fluorescent-labeled artificial substrate, transglutaminase activity was detected *in vivo* at the septal pore of wounded hyphae, which functionally involves SppB and its putative catalytic triad. Our study suggests a conserved role for transglutaminase domain-containing proteins in wound-related cellular protection in fungi, similar to humans.

1) Mamun *et al.* (2023) *Nat. Commun.* 14:1418.

2) Mamun and Maruyama (2023) *Mol. Biol. Cell* 34:ar127.

405C **Deciphering the role of the SAM domain containing protein Vts1 during rice blast disease** Neftaly Cruz Mireles[1], Barakat Obadara[2], Prince Amoah[2], Paul Derbyshire[2], Lauren S Ryder[2], Mark-Jave Bautista[2], Alice Eseola[2], Xia Yan[2], Iris Eisermann[2], Weibin Ma[2], Frank L. H. Menke[2], Nick Talbot[2] [1]Norwich Research Park, [2]The Sainsbury Laboratory

Rice blast disease, caused by the filamentous fungus *Magnaporthe oryzae*, poses a significant threat to global food security, accounting for nearly 30% of loses in global rice production. To cause infection, *M. oryzae* needs to undergo a sequence of morphogenetic changes to develop a specialized infection structure called appressorium. It is well known that appressorium formation is regulated by the Pmk1 MAP kinase (MAPK) signalling cascade. However, there is little knowledge about the mechanism through which Pmk1 controls these intricate development-related morphogenetic changes. Recently, it has been discovered that the SAM domain containing protein Vts1 associates with the Pmk1 MAPK during early appressorium morphogenesis, and it is required for vegetative growth, appressorium development and pathogenicity. Here, we show that Vts1

is a highly conserved protein in filamentous fungi. In the blast fungus, Vts1 is a cytoplasmic protein that accumulates at the septal and appressorium pores where it co-localizes with the heteromeric septin ring. Additionally, the Vts1 interaction with core septins (Sep3, Sep4 and Sep5) and mis- localisation of these septins in a Δ*vts1* mutant, suggests a role for this protein in cytoskeleton reorganisation and re-polarisation. We also found that Pmk1 activation kinetics changes in Vts1 null mutant, suggesting a regulatory role of Vts1 on the Pmk1 MAPK cascade. Using Immunoprecipitation coupled to Mass Spectrometry (IP-MS) and a Yeast-Two-Hybrid (Y2H) screening approach, we defined Vts1 interactors at 0h, 4h and 24h during appressorium formation. Interestingly, we identified a different set of interactors for each timepoint, indicating a time-dependent role for Vts1 during appressorium morphogenesis. Furthermore, we discovered that the lack of Vts1 SAM domain impairs pathogenicity and germ tube formation. Our study provides new insight into the complex regulatory mechanisms of the Pmk1 MAPK signalling pathway during appressorium morphogenesis. Currently, we are characterising the role of Vts1 interactors as components of the Pmk1 pathway. By investigating Vts1 function, we aim to understand the physiological and regulatory processes governed by Pmk1 in the blast fungus.

406C **Pseudorabies virus upregulates low-density lipoprotein receptors to facilitate viral entry** ming shengli Henan Agricultural University

Pseudorabies virus (PRV) is the causative agent of Aujeszky's disease in pigs. The low-density lipoprotein receptor (LDLR) is a transcriptional target of the sterol-regulatory element-binding proteins (SREBPs) and participates in the uptake of LDL-derived cholesterol. However, the involvement of LDLR in PRV infection has not been well characterized. We observed an increased expression level of LDLR mRNA in PRV-infected 3D4/21, PK-15, HeLa, RAW264.7, and L929 cells. The LDLR protein level was also upregulated by PRV infection in PK-15 cells and in murine lung and brain. The treatment of cells with the SREBP inhibitor, fatostatin, or with SREBP2-specific small interfering RNA prevented the PRV-induced upregulation of LDLR expression as well as viral protein expression and progeny virus production. This suggested that PRV activated SREBPs to induce LDLR expression. Furthermore, interference in LDLR expression affected PRV proliferation, while LDLR overexpression promoted it. This indicated that LDLR was involved in PRV infection. The study also demonstrated that LDLR participated in PRV invasions. The overexpression of LDLR or inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9), which binds to LDLR and targets it for lysosomal degradation, significantly enhanced PRV attachment and entry. Mechanistically, LDLR interacted with PRV on the plasma membrane, and pretreatment of cells with LDLR antibodies was able to neutralize viral entry. An in vivo study indicated that the treatment of mice with the PCSK9 inhibitor SBC-115076 promoted PRV proliferation. The data from the study indicate that PRV hijacks LDLR for viral entry through the activation of SREBPs.

407C **The glycoprotein 5 of porcine reproductive and respiratory syndrome virus stimulates mitochondrial ROS to facilitate viral replication** wang jiang Henan Agricultural University

Viruses have evolved sophisticated mechanisms to manipulate host cell organelles to serve as niches for persistence and proliferation. In the present study, we aimed to investigate the role of cellular organelles in the replication of porcine reproductive and respiratory syndrome virus (PRRSV). We found that the morphology of mitochondria and the endoplasmic reticulum (ER) were both altered, and the contact between these two organelles was enhanced during PRRSV infection. By the overexpression of PRRSV-encoded open reading frames, we identified that only glycoprotein 5 (GP5) was essential for ER-mitochondria contact. Further investigation revealed that GP5 interacted with the ER inositol 1,4,5-triphosphate receptor (IP3R) and the mitochondrial voltage-dependent anion channel (VDAC1) to promote the Ca2+ efflux from ER into mitochondria. Excessive mitochondrial Ca2+ uptake resulted in mitochondrial dysfunction and substantial mitochondrial reactive oxygen species (mROS) production. Elevated mROS activated autophagy through the AMPK/mROR/ULK1 axis to facilitate PRRSV replication. GP5-induced mROS also triggered the NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) inflammasome. Inhibition of autophagy augmented NLRP3 inflammasome activation and exhibited an anti-PRRSV effect, suggesting autophagy counteracted the NLRP3-mediated innate immune response. Overall, our findings highlighted the importance of cellular organelles in virus-host interactions and provided new insights into the complex interplay between virus replication and innate immune responses.

408A**FungiDB: A free, web-based informatics resource for in silico hypothesis testing, data mining and exploration.** Evelina Basenko[1], Omar Harb[2], David Roos[2] [1]FungiDB, University of Liverpool, [2]FungiDB, University of Pennsylvania

FungiDB (www.fungidb.org) is an integral component of the Eukaryotic Pathogen, Vector and Host Bioinformatics Resource Center (VEuPathDB.org, a Global Core Biodata Resource). As an NIAID Bioinformatics Resources Center and a Global Core Biodata Resource). , VEuPathDB knowledgebases adhere to FAIR data-sharing principles, seeking to facilitate and expedite scientific discovery by enabling exploration, querying and mining of Omics-scale datasets across diverse species including hosts, invertebrate vectors of human disease, eukaryotic microbes, and also environmental and epidemiological studies.

VEuPathDB users can access to a wealth of data for fungal and oomycete species, including genome sequences and annotations; host and pathogen transcriptomics data; RNA-Seq/microarray co-expression and UMAP clustering; proteomics and post-translational modification records; genetic variation (polymorphisms and copy number variations); and phenotypes. Additionally, the resource provides insights into protein structure and predictions (e.g., SignalP, InterPro, AlphaFold), metabolic pathways, and more. All of these resources are conveniently accessible via a user-friendly web interface, enabling in silico hypothesis testing, data mining and cross-species inference.

Gene-specific information can be further perused on gene record pages, which may be supplemented through the addition of User Comments designed to capture expert knowledge from the community (functional characterization, publication citations, *etc*). Genome architecture may be explored using the JBrowse Genome Browser, and supplemented with expert knowledge *via* the Apollo platform for collaborative curation of both structural and functional gene annotation.

Users of FungiDB can also access the integrated private VEuPathDB Galaxy - My Workspace platform. The VEuPathDB Galaxy provides means for custom data analysis via pre-configured data analysis pipelines supplemented with pre-loaded reference genomes. FungiDB My Workspace facilitates further analysis and visualization of user data in the context of the integrated datasets in FungiDB.

Have questions, need help with navigating resources, want to nominate a dataset for integration, share data with us or invite us for a demo on Zoom? - Stop by the FungiDB Help desk during poster sessions, or email help@FungiDB.org

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*Authors are presenting on behalf of the entire FungiDB/VEuPathDB team.

409A **Genomic and Phenotypic variation in *Rhodotorula* species sampled from Extreme Environments** Xin-Zhan Liu[1,2], Eva Ottum[2], Cene Gostinčar[3], Benedetta Turchetti[4], Claudia Coleine[2,5], Laura Selbmann[5], Ian Wheeldon[2], Nina Gunde-Cimerman[3], Jason E Stajich[2] [1]Institute of Microbiology, Chinese Academy of Sciences, [2]Microbiology & Plant Pathology, Univ California, Riverside, [3]Biotechnical Faculty, University of Ljubljana, [4]Dept of Agriculture, Food and Environmental Sciences & DBVPG Industrial Yeasts Collection, University of Perugia, [5]Dept of Ecological and Biological Sciences, University of Tuscia

Rhodotorula are basidiomycete yeasts in the Pucciniomycotina subphylum, which are characterized by production of carotenoids and can be cultured from a broad range of temperature and harsh environments. They are found in spoiled food, in freshwater lakes, ocean and brackish waters, the human built environment, and from soils or rocks in arid lands including cold arctic/antarctic and hot deserts. Some lineages have been characterized exclusively from rock surface or endolithic samples. The species *Rhodotorula mucilaginosa* is capable of causing disease in mammals through skin and blood-borne infections. We characterized phenotypic traits of 288 strains grown in a range of laboratory conditions, including temperatures from 4 to 37 °C, carbon sources glycerol and xylose, pH and high and low salinity conditions. The results establish a phenotyping dataset consisting of survival and growth rates, carotenoid production, and morphological traits based on image analyses of colony growth on solid media. Using short-read Illumina sequencing, we have assembled and annotated draft genomes of 400 isolates of *R. mucilaginosa* and 100 across 15 other species in the genus collected from a diversity of environments from our collections and public genome datasets. The work reveals additional cryptic species and within *R. mucilaginosa*, a range sub-populations admixture. Several strains have copy number amplification of some chromosome and a few diploid strains are detected among the predominantly haploid isolates. We assembled reference genomes for type strains for 9 *Rhodotorula* species with Oxford Nanopore long reads to support comparative analysis of the *Rhodotorula* genus pangenome and focused population genomics study of *R. mucilaginosa*. The reference genomes, population genomics, and phenotyping support deep investigations of evolution, adaptation, link genotypes to phenotypes of these ubiquitous basidiomycete yeasts and test hypotheses about gene-level adaptation to cold, hot, and saline environments.

410A **Computational analysis and tRNA-sequencing reveal the diversity of tRNA across an entire fungal subphylum** Lauren Dineen[1], Abigail LaBella[2] [1]Bioinformatics and Genomics, University of North Carolina at Charlotte, [2]University of North Carolina at Charlotte

Over the past decade, the field of tRNA biology has emerged as a powerful tool in understanding novel eukaryotic processes. Traditionally, tRNA molecules have been placed at the centre of translation machinery as the key between codons and amino acids. Research now expands the role of tRNA as key components in mediating stress response in eukaryotes. Despite the major advances

in this field, we still know very little about tRNA dynamics and the role of tRNA gene diversity. Furthermore, there is a distinct lack of cross species data, leading to a gap in our fundamental understanding of tRNA. Here, we present an investigation into tRNA dynamics and gene diversity using a set of over 1000 budding yeast genomes. Initially, a computational analysis of tRNA genes revealed a wealth of diversity across species with tRNA genomic content ranging from 54-1183. These results were then used to guide the selection process of a set of yeast species representative of the range of tRNA diversity identified in our analyses. We then employ a direct RNA sequencing approach to carry out tRNA sequencing of this set of species in a range of rich and minimal media types. We observe the tRNA pool dynamics of several species in parallel for the first time, and as a result provide new insights into tRNA biology. Also, new questions are raised about the importance of tRNA as regulators of stress in eukaryotes.

411A **Codon usage and tRNA diversity in the subphylum Saccharomycotina** Colin Speer, Abigail LaBella University of North Carolina at Charlotte

The Saccharomycotina subphylum is home to vital yeast species in the biotechnology and medicinal industries. Despite hints at the interplay of selection and mutational bias in codon usage, the underlying evolution, physiological consequences, and molecular mechanisms of this dynamic remain unclear and are explored in this project. We have recently expanded the number of genomes in this fungal subphylum to 1,154. This has allowed us to characterize the genomic codon usage and tRNA content of an entire fungal subphylum. This analysis revealed heavy biases in some groups associated with drastic changes in tRNA sequences. The characterization of codon usage in this subphylum will aid studies in broad fields ranging from heterologous gene expression to cellular gene regulation.

412A **Rapid gain and loss of an aneuploid chromosome drives key morphology states and virulence in a fungal pathogen of humans** Sarah Heater, Rosa Rodriguez, Mark Voorhies, Anita Sil Immunology and Microbiology, UCSF

Environmental pathogens of humans must rapidly acclimate to thrive as they transition between the disparate conditions of environment and host. Little is known about the molecular regulation of this process in thermally dimorphic fungal pathogens such as *Histoplasma* species, which undergo a drastic transition in morphology and gene expression induced by the temperature of environment (where they form multicellular hyphae) or host (where they form unicellular yeast). Less is known about population genetics throughout these habitat transitions. We made the surprising discovery that a reversible aneuploidy helps *Histoplasma* thrive under environment but not host conditions. This aneuploidy is present in half of 25 clinical isolates tested, indicating that it appears in multiple lineages. It is rapidly gained and lost during laboratory passage, while other aneuploidies are very rare. The aneuploidy biases cells towards the environmental hyphal form: it increases the speed at which yeast transition to hyphae and pushes cells towards hyphal gene expression patterns even in conditions inducing yeast morphology. In a pooled competition, the aneuploidy provides a strong competitive advantage during the shift to environmental conditions. However, the aneuploidy downregulates genes associated with yeast morphology such as virulence factors, and confers a competitive disadvantage during shifts to the host form. Aneuploid *Histoplasma* is considerably less pathogenic than euploid *Histoplasma* in the mouse model of infection. In a competition experiment in mice, the aneuploid strain is at a disadvantage. To understand how the aneuploidy promotes and is advantageous for the environmental form while impeding the host form and decreasing virulence, we performed RNAseq analysis of euploid and aneuploid strains, revealing that the aneuploidy affects expression of key virulence factors and transcription factors (TFs). We identified two previously unstudied TFs within the aneuploid chromosome that, when overexpressed in the euploid strain, are each sufficient to yield phenotypes associated with the aneuploidy, such as hyphal growth. We are currently determining whether these TFs are necessary to promote these phenotypes. Taken together, these data strongly suggest that this aneuploidy benefits *Histoplasma* by rapidly increasing phenotypic diversity, thus helping these populations survive through frequent and abrupt transitions between the environment and mammalian hosts.

413A **Needles in fungal haystacks: Discovery of a putative a-factor pheromone and a unique mating strategy in the Leotiomycetes** Andi M Wilson, Martin PA Coetzee, Michael J Wingfield, Brenda D Wingfield Biochemistry, Genetics and Microbiology, University of Pretoria

The Leotiomycetes represent a hugely diverse group of fungi, which accommodates a wide variety of important plant and animal pathogens, ericoid mycorrhizal fungi, and producers of antibiotics. Despite their importance, these fungi remain relatively understudied and are not represented by any model species. In particular, sexual reproduction, the different mating behaviours, and the genetic mechanisms that underly these complex processes and traits are poorly understood in the Leotiomycetes.

We exploited publicly available genomic and transcriptomic resources to identify genes of the mating-type (*MAT*) locus and pheromone response pathway in an effort to characterize the mating strategies and behaviors of 124 Leotiomycete species.

Our analyses identified a putative a-factor mating pheromone in the Leotiomycetes. This finding represents the first-ever identification of this gene in Pezizomycotina species outside of the Sordariomycetes. However, the low inter-species diversity and

mating type-independent expression observed for this gene and the α-factor pheromone suggests that they may not be involved in mating, as they are known to be in the Sordariomycetes.

A unique mating strategy was also discovered in the genus *Lachnellula*, where species appear to have lost the need for the primary MAT1-1-1 protein. Instead, individuals harbouring the *MAT1-2-1* gene appear to be compatible with individuals having only the *MAT1-1-3* gene. Both the MAT1-1-3 and MAT1-2-1 proteins have the HMG box domain and consequently, these species may be exhibiting a sexual strategy comparable to basal fungi in the Zygomycota and Microsporidia.

This comprehensive catalog of mating-related genes in such a large group of fungi provides a rich resource from which in-depth, functional studies can be conducted in these economically and ecologically important species.

414A **Understanding the molecular mechanisms behind the fungal thermophilism** Andrei Stecca Steindorff[1], Maria V Aguilar-Pontes[2], Ronald De Vries[3], Don Natvig[4], Adrian Tsang[5], Amy Powell[6], Igor V Grigoriev[7] [1]DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, [2]Departamento de Genética, University of Cordoba, [3]Fungal Physiology, Utrecht University, [4]Dept of Biology, University of New Mexico, [5]Center for Structural and Functional Genomics, Concordia University, [6]Sandia National Laboratories, [7]DOE Joint Genome Institute, Lawrence Berkeley National Lab
A genome collection of over 30 thermophilic and thermotolerant fungi has been sequenced and assembled in the U.S. Dept of Energy Joint Genome Institute's MycoCosm portal (https://mycocosm.jgi.doe.gov) to understand the molecular basis of thermophily using comparative genomics. These fungal traits are scattered across the fungal tree of life, with their highest concentration within species of Chaetomicaceae, Thermoascoceae, and Trichomaceae, as well as some Mycoromycota. Using Mesquite to detect the ancestral state in the phylogeny, we found that thermophilism is the original lifestyle of all three prominent families of thermophilic fungi. Thermophilic genomes encode various thermostable enzymes, including carbohydrate-active enzymes such as xylanases, which are useful for many industrial applications. At the same time, the overall gene counts, especially in gene families responsible for microbial defense such as secondary metabolism, are reduced in thermophiles compared to mesophiles. We also found a reduction in the core genome in both the Chaetomiaceae family and Eurotiomycetes class. The GO terms lost in thermophilic fungi involve primary metabolism, transporters, UV response, and O-methyltransferases. Comparative genomics analysis also revealed higher GC content in the third base of the codon (GC3) and a lower effective number of codons (ENC) in fungal thermophiles than in thermotolerant and mesophilic fungi. Furthermore, using the Support Vector Machine classifier, we identified several Pfam domains capable of discriminating between genomes of thermophiles and mesophiles with an accuracy of F1 = 0.92. Using alphafold2 to detect protein structures of xylanases (GH10), we built a similarity network based on the structures. We found that the number of disulfide bonds is essential for the protein structure. The structure embeds the optima activity temperature with proteins with similar optima clustering in the network. Thus, comparative genomics offers new insights into thermophilic fungi's biology, adaptation, and evolutionary history while providing a parts list for bioengineering applications.

415A **Two-speed genomes drive the evolution of pathogenicity in amphibian-infecting chytrids** Theresa Wacker[1], Nicolas Helmstetter[1], Duncan Wilson[1], Matthew Fisher[2], David Studholme[3], Rhys Farrer[1] [1]Medical Research Council Centre for Medical Mycology, University of Exeter, [2]2MRC Centre for Global Infectious Disease Analysis, Imperial College London, [3]Biosciences, University of Exeter

Nearly half of all amphibian species are declining globally due to factors such as habitat loss and disease. Two related fungal pathogens (*Batrachochytrium dendrobatidis; Bd* and *B. salamandrivorans; Bsal*) have been attributed to these global declines and extinctions. However, the genetic mechanisms underlying host-specificity and pathology in the *Batrachochytrium* genus remain elusive and their evolution and origins of virulence are largely unknown. Using deep nanopore sequencing, we found that *Bsal* is extremely repeat-rich with high numbers of long terminal repeats, long interspersed nuclear elements and transposable elements. This repeat-driven genome expansion in *Bsal* has resulted in a tripling of its length compared with *Bd.* Key pathogenicity genes including M36 metalloprotease have expanded compared with *Bd*, and are enriched for flanking transposable elements, suggesting its genome expansion is connected to selective evolutionary processes. Both batrachochytrids had evidence of a two-speed genome architecture, including an enrichment of functional categories by repeat richness or sparsity. This is the first evidence for a two-speed genome in an animal pathogen, shedding new light on the role of repetitive sequences on the evolution of fungal pathogens driving global declines and extinctions of amphibians.

416A **Lessons from genomic and transcriptomic analysis of five marine-derived fungi** Abhishek Kumar, Pradeep Phule, Frank Kempken Botany, Christian-Albrechts University

Marine Fungi are potent secondary metabolite producers. However, limited genetic information are available their biosynthetic gene clusters (BGCs) and their biotechnological applications. To overcome this lack of information, herein, we used next-generation sequencing methods for genome sequencing of two marine fungi, isolated from the German Wadden Sea, namely *Calcarisporium sp.* KF525 and *Pestalotiopsis sp.* KF079. In addition, we sequenced a strain of Scopulariopsis brevicaulis isolated from a sponge, a Fusarium isolate from the Baltic Sea, and finally a strain of Rhodotorula isolated from the seafloor of the Mid-Atlantic Ridge. Many novel secondary metabolite gene (SMG) clusters of *Calcarisporium sp.* and *Pestalotiopsis sp.*, were detected, with the vast majority of all SMGs being unique for these two marine fungi. Only few of the SMGs were found to be expressed under laboratory conditions by RNA-seq analysis, but employing a marine strain of *Fusarium sp.* we also analyzed the effect of co-cultivation with marine bacterial populations from Baltic Sea, which led to activation of some SMGs. In addition, we observed specific activation of transporter proteins and certain transcription factors upon co-cultivation. These data include important clues for future genomic analyses of marine fungi.

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417A **Continual propagation of [D1,2] stwintrons in divergent *Xylariales*** Erzs?bet Fekete, Norbert Ág, Viktória Ág-Rácz, Alexandra Márton, Vivien Bíró, Michel Flipphi, Levente Karaffa University of Debrecen

Spliceosomal twin introns consist of two nested U2 introns excised consecutively. In a [D1,2] stwintron, an internal intron interrupts the 5'-donor of an external intron between the 1st and 2nd nucleotide (nt) (5'-$G_1|U_2$). For *Hypoxylon* sp. CO27-5, one can classify [D1,2]'s in two groups. Of these, sequence-similar "sister" stwintrons cross-identify by blastn, and occur at new intron positions in narrow taxa (species, variants). When reciprocal blastn screens were performed in genomes of other *Xylariales* species–using proven CO27-5 sister stwintrons as primary queries–258 new sequence-similar stwintrons were revealed in 12 species. Some species contain > 50 sister stwintrons, others < 10. All of them are integrated seamlessly in seemingly random exonic sequences, excluding transposon-driven mechanisms or splice-site co-option for their propagation. One observes essentially species-specific clades of sister stwintrons in maximum likelihood trees, implying vertical transmission of sequence-diverging duplication-competent [D1,2]'s. *Xylaria* sp. MSU SB201401 and *X. striata* RK1-1 are intimately related–like strains of the same species–albeit isolated from very different plant species, growing on different continents. 4 stwintrons unique to MSU SB201401 are phylogenetically clustered and the 3 most similar ones are >99 % identical, while the genes harboring them are completely unrelated in sequence, intron-exon structure and function. This lineage involves consecutive strain-specific stwintron duplication events, arguably the most recent duplications in our set. Although the continuous 11-nt near-terminal inverted repeat in these 4 MSU SB201401 stwintrons is not as long as those in prototypical CO27-5 sisters, the near-terminal stem structure is the stand-out common feature. We propose that this stem structure can bring the donor G_1 of the internal- and the acceptor G_3 of the external intron in close proximity, necessary for the rare duplication of [D1,2] stwintrons into double-stranded DNA breaks, including those with smaller or less perfect terminal inverted repeats. We also identified one potential lateral transfer: one MSU SB201401 stwintron is >93 % sequence-identical with two different [D1,2]'s in *Xylariaceae* sp. FL1651. The 3 genes harboring them are completely unrelated. This may imply that duplication-competent stwintrons can be (re)acquired by lateral transfer. Such rare events could contibute to the periodicity of overall (stw)intron gain and loss.

418A **Genomic and epigenomic variation in pathogenic *Cryptococcus* species** Tal Goodisman, Nicolas Helmstetter, Diana Tamayo, Elizabeth Ballou, Rhys A Farrer MRC Centre for Medical Mycology, University of Exeter

Cryptococcus species cause one of the deadliest fungal diseases in the world leading to 130,000 deaths annually, yet the cryptococcal epigenome governing important traits such as virulence and drug-resistance is poorly characterised. We studied the genomic loci of two histone modifications H3K4me2 and H3K9me2 in *C. neoformans* KN99, a H1 histone knock out strain (KN99ΔH1.5) and *C. gattii* R265. Our results indicate substantial genomic variation between H99/KN99 isolates and differences in genomic loci for H3K4me2 peaks between *Cryptococcus* species. Expression analysis suggest Histone H1.5 supresses the expression of genes involved in development transitions, indicative of a role as a gene-specific regulator rather than a global regulator. However, peak calling appears highly dependent on alignment software, peak-caller and peak-caller parameters. While Histone H1.5 has only a modest impact on gene expression, loss of H1.5 in *C. neoformans* yielded ~5X more H3K4me2 peaks and ~5X fewer H3K9me2 peaks, suggesting it governs both marks in opposing ways. Both H3K4me2 and H3K9me2 are predominantly enriched in sub-telomeric regions, while H3K4me2 is uniquely enriched in centromeric regions in *C. neoformans*. Within sub-telomeric regions, H3K4me2 and H3K9me2 marks are located either nearby or overlapping ergosterol synthesis (ERG2), chitin synthase (CHS8) and 1,3-β-glucan synthase genes (FKS1), implicating histone methylation with virulence and drug resistance. Orthologs of these genes in *C. gattii* reveal similar flanking histone modification sites, suggesting these marks may have been conserved for millions of years since the divergence of *Cryptococcus* species.

419A **Investigating the role of chromosomal rearrangements in adaptative evolution of the plant pathogenic fungus *Verticillium dahliae*** chen-yu Kuan[1], Andrea Doddi[2], Edgar A. Chavarro-Carrero[1], Alexander Mandel[1], Bart PHJ Thomma[1] [1]University of Cologne, [2]University of Rome "La Sapienza"

Verticillium dahliae is a presumed asexual soil-borne fungal pathogen that can infect a wide range of plant hosts. Previous explorations of the *V. dahliae* genome have revealed extensive chromosome rearrangements linked to the presence of plastic adaptive genomic regions (AGRs). These AGRs exhibit distinctive genomic features, including the enrichment of *in planta*-induced effector genes and genes associated with host adaptation, suggesting a crucial role for chromosomal rearrangements in adaptation to diverse plant hosts and environments. Despite these observations, when and how chromosomal rearrangements occur in *V. dahliae* remain poorly understood. We hypothesize that selective evolutionary pressure, such as persistent environmental stress including host plant-driven selection, may induce such rearrangements. To explore this hypothesis, we conducted artificial evolution experiments while screening for the occurrence of novel rearrangements. Our experiments involve subjecting *V. dahliae* to distinct stress treatments (heat stress, bacterial antagonism, nutrient stress, and UV radiation) or infections of *Arabidopsis thaliana*. Interestingly, we observed adaptive phenotypes in response to all stress treatments. Subsequent investigations will focus on utilizing Oxford Nanopore Technology DNA sequencing to determine whether novel rearrangements have occurred in the evolved *V. dahliae* strains.

420A **The roles of deubiquitination module and Rad6-Bre1 ubiquitin ligase complex in oxidative stress response and biofilm formation of *Candida glabrata*** Lee Yi Hang[1], Huang Yue-Han[1], Hsu Li-Hang[1], Lin Chi-Jan[2], Chen Ying-Lien[1] [1]Dept of Plant Pathology and Microbiology, National Taiwan University, 10617 Taipei, Taiwan, [2]Institute of Molecular Biology, National Chung Hsing University, 40227 Taichung, Taiwan

Candidiasis is one of the most important fungal diseases and generally refers to diseases of the skin or mucosal tissues caused by Candida species. *Candida glabrata* is an opportunistic human fungal pathogen. Infection with *C. glabrata* has significantly increased due to innate antifungal drug tolerance and the ability to adhere to mucocutaneous surfaces. Increasing evidence suggests that the epigenetic pathway may be an important factor in the development of drug resistance through existing or new mechanisms. Previous studies have shown that histone modifications, such as histone methylation and acetylation, play important roles in the virulence of *C. glabrata*. However, the ubiquitination and deubiquitination of histone H2B in *C. glabrata* have not been thoroughly investigated. In *Saccharomyces cerevisiae*, the ubiquitination of H2B is regulated by Rad6-Bre1 ubiquitin ligase complex, while deubiquitination is regulated by deubiquitination (DUB) module, a member of Spt-Ada-Gcn5 acetyltransferase (SAGA) complex, which comprised of Ubp8, Sgf11, Sgf73, Sus1. Previous research in our laboratory found that the HAT module Ada2 could regulate *C. glabrata* oxidative stress tolerance, drug tolerance, cell wall integrity, and virulence. However, the roles of the Rad6-Bre1 ubiquitin ligase complex and DUB module in those phenotypes are not yet understood. In this study, we found that *rad6* and *bre1* mutants exhibited sensitivity to antifungal drug amphotericin B and susceptible to oxidative stresses. DUB module genes *UBP8*, *SGF11*, and *SUS1*, but not *SGF73* positively regulate histone H2B DUB. Furthermore, *ubp8*, *sgf11*, and *sus1* mutants exhibited decreased biofilm formation and sensitivity to cell wall-perturbing agent sodium dodecyl sulfate and antifungal drug amphotericin B. In addition, the *sgf73* mutant showed increased biofilm formation but was susceptible to oxidative

stresses, antifungal drugs, and cell wall perturbing agents. The *ubp8*, *sgf11*, and *sus1* mutants showed marginal hypovirulence, whereas the *sgf73* mutant exhibited virulence similar to the wild type in a murine systemic infection model.

In conclusion, the *C. glabrata* Rad6-Bre1 ubiquitin ligase complex and DUB module plays distinct roles in H2B ubiquitination, oxidative stress response, biofilm formation, cell wall integrity, and drug tolerance, but exhibits minor roles in virulence.

421A **Pangenome graph uncovers signatures of rapid evolution in spinach downy mildew** Petros Skiadas[1,2], Sofía Riera Vidal[1], Joris Dommisse[1], Melanie Mendel[2,3], Joyce Elberse[2], Ronnie de Jonge[3], Guido Ackerveken[2], Michael F Seild[1] [1]Theoritical Biology and Bioinformatics, Utrecht University, [2]Translational Plant Biology, Utrecht University, [3]Plant-Microbe Interactions, Utrecht University

Chromosome-level genome assemblies are essential to study the biology and evolution of filamentous plant pathogens. However, traditional comparative genomics approaches typically focus solely on protein-coding genes and often do not take advantage of chromosome-level genome assemblies, thus failing to fully capture the genome diversity. Moreover, genome assemblies of different isolates often are annotated separately, leading to inconsistencies in gene annotation and protein-prediction, especially for highly variable and expanded effector gene families.

To address these challenges and to accurately compare multiple chromosome-level genome assemblies and their gene annotations, we created and annotated pangenome graphs for the oomycete pathogen *Peronospora effusa*, the economically most important disease in cultivated spinach worldwide. The pangenome of *P. effusa* is highly conserved, with 80% of the observed variation originating from large variants caused by transposons. The common annotation based on the pangenome graph revealed almost 10,000 genes and 400 predicted host-translocated effectors for each *P. effusa* isolate. Based on the structure and position of genes in the pangenome graph, we assigned genes to single-copy ortho-groups, which shows that genes in general are highly conserved between isolates, while the predicted effectors are much more variable. Most of the effector variation can be found in few effector gene clusters, mostly caused by gene copy number changes. The high-quality genome assemblies and the consistent annotation of genes and transposons enabled us to uncover examples of transposon insertion and duplication resulting in the expansion of effector genes. In summary, we here highlighted how we exploit pangenome graphs for detailed genome-wide comparisons of the spinach downy mildew *P. effusa*, which is essential to uncover the signatures of rapid evolution in filamentous plant pathogens.

422A **QTL Mapping and Bulk Segregant Analysis identifies CO_2 tolerance genes associated with virulence in the global pathogen *Cryptococcus neoformans*** Benjamin Chadwick[1], Xiaofeng Xie[1], Laura Ristow[2], Damian Krysan[2], Xiaorong Lin[1] [1]University of Georgia, [2]University of Iowa

Cryptococcus neoformans is a ubiquitous free-living soil yeast and opportunistic pathogen that causes ~223,100 cases of cryptococcal meningitis per year, killing over 180,000 people. The pathogenicity of *C. neoformans* relies on its adaptation to the host conditions. An important difference between its natural environment and the mammalian host is the concentration of CO_2. CO_2 levels in the host fluctuate around 5%, which is ~125-fold higher than in ambient air. We recently found that while clinical isolates are tolerant to host levels of CO_2, many environmental isolates are CO_2-sensitive and virulence-attenuated in animal models. The genetic basis responsible for cryptococcal adaptation to high levels of CO_2 is unknown. Here, we utilized quantitative trait loci (QTL) mapping with 374 progeny from a cross between a CO_2-tolerant clinical isolate and a CO_2-sensitive environmental isolate to identify genetic regions regulating CO_2 tolerance. To identify specific quantitative trait genes (QTGs), we applied fine mapping through backcrossing and bulk segregant analysis coupled with pooled genome sequencing of near-isogenic progeny but with distinct tolerance levels to CO_2. The roles of the identified QTGs in CO_2 tolerance were verified by targeted gene deletion. We further demonstrated that virulence levels among near-isogenic strains in a murine model of cryptococcosis correlate with their levels of CO_2 tolerance. Moreover, we discovered that sensitive strains may adapt *in vivo* to become more tolerant to increased CO_2 levels and more virulent. These findings highlight the underappreciated role of *C. neoformans* tolerance to host CO_2 levels and its importance in the ability of an opportunistic environmental pathogen to cause disease.

423A **A novel effector gene located in a selective sweep region plays an important role in virulence of a host-specific fungal pathogen** Wagner Calegari Fagundes[1], Frauke Caliebe[1], Rune Hansen[1], Janine Haueisen[1], Fatemeh Salimi[2], Alireza Alizadeh[3], Eva H. Stukenbrock[1] [1]Environmental Genomics, Max Planck Institute for Evolutionary Biology, Plön & Christian-Albrechts University Kiel, [2]Dept of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, [3]Dept of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University

Host adaptation can lead to strong footprints of selection in fungal plant pathogen genomes. Populations genomics analysis have the power to identify genes that have been under selection and therefore elucidate the possible pathways of adaptation and virulence of fungal pathogens to specific hosts. Using genome-wide scans of selection, we have identified distinct selective sweep regions in a host-specific lineage of the fungal pathogen *Zymoseptoria tritici* (causal agent of Septoria tritici blotch in wheat) infecting wild grasses from the genus *Aegilops* spp. Among these, one selective sweep comprised three candidate effector genes and one gene encoding a predicted carbohydrate-degrading enzyme (CAZyme), and showed high sequence divergence when compared to wheat-infecting *Z. tritici* isolates. In this study, we performed comparative genomics and transcriptomics to investigate its overall genomic architecture between *Aegilops*- and wheat-infecting *Z. tritici* isolates. Moreover, we conducted functional genetics analyses with the aim of identifying potential fitness effects *in planta*. We find that, in addition to distinct alleles in the *Z. tritici* lineages, the selective sweep region is characterized by genomic rearrangements and infection stage-specific transcriptional changes. Using a reverse genetics approach, we further confirm a pathogenicity-related role for one of the three candidate effector genes present in the selective sweep region in an *Aegilops*-infecting *Z. tritici* isolate. Three independent deletion mutants for the candidate effector gene Zt09_chr7_00299 exhibit a significant reduction in virulence in *Aegilops* plants when compared to the wild-type strain, suggesting a pivotal role during infection. Taken together, our results indicate that the identified selective sweep region harbors important traits involved in host adaptation in *Z. tritici* and highlight a key effector gene contributing for host-specific virulence.

424A **Network-based approach for discovering transcription factors associated with fungal plant biomass conversion** Ferry Hagen[1], Joanna E. Kowalczyk[2], Astrid E. Mueller[2], Ronald P. de Vries[2] [1]Fungal Physiology, Westerdijk Fungal Biodiversity Institute, [2]Westerdijk Fungal Biodiversity Institute

Fungal plant biomass conversion (FPBC) is an important component of the global carbon cycle and has been widely applied for the production of biofuel, enzymes and biochemicals. Identification of transcription factors (TFs) governing the FPBC process is important for genetic engineering of fungi towards sustainable production of high-value bioproducts from renewable lignocellulose. However, the functional characterization of new TFs is challenging due to the difficulties in computational prediction and labor consuming experimental validation.

Here, we developed a bioinformatics framework for screening of FPBC related TFs based on reconstructing gene regulatory networks from transcriptome data and enrichment analysis of manually curated FPBC gene sets. Applying this method on model fungi *Aspergillus niger* and *Neurospora crassa,* and the less-studied Basidiomycete *Dichomitus squalens*, we successfully identified both known TFs and promising candidates. The function of one identified TF, HapX, has been experimentally validated, and several candidates were supported by literatures and transcriptome data. Our new method will accelerate the identification of novel TFs involved in FPBC, and facilitate the further improvement of fungal cell factories.

425A **Mating Pheromone and Receptor Genes in the *Ceratocystidaceae*: Insights into Diverse Mating Strategies** Frances Lane, Brenda Wingfield, Michael Wingfield, Markus Wilken University of Pretoria

The pheromone-receptor communication system plays a pivotal role in fungal mating interactions. In heterothallic fungi, distinctive pheromones identify compatible mating partners, allowing them to interact and initiate the sexual cycle. Conversely, in homothallic fungi, which are capable of sexual reproduction without a mating partner, evidence exists for pheromone chemical signals remaining important in functions beyond mate recognition. Species in the *Ceratocystidaceae* (*Ascomycota*) display a broad spectrum of mating-type behaviours, including heterothallism and various forms of homothallism. In this study, we identified the pheromone and receptor genes from the genomes of 35 species across 13 genera in the *Ceratocystidaceae*. Our analyses successfully identified the a- and α-pheromone receptor genes in all species. However, the α-pheromone receptor in *Ambrosiella* species lacked crucial transmembrane domains, potentially rendering it non-functional. The α-pheromone gene could also not be identified in the *Ambrosiella* species, but was present in two copies in both *Berkeleyomyces* species. The a-pheromone gene was also identified in all *Ceratocystidaceae* genomes, with many species containing homologs of this gene across multiple loci. This study is the first to identify these genes in *Ceratocystidaceae* species other than in the genus *Huntiella*. Given the diversity of sexual strategies found in this family, our findings provide a foundational platform to delve more deeply into the evolutionary dynamics of sexual reproduction in this distinct fungal lineage.

426A **Genome-wide identification of effectors and variant effects from across the breadth of diversity of *Fusarium*** Hye-Seon Kim[1], Stephen Harding[2], Guixia Hao[2], Robert Proctor[2], Olivia Haley[3], Carson Andorf[3] [1]USDA-Agricultural Research Service, [2]Mycotoxin Prevention and Applied Microbiology Research Unit, USDA-Agricultural Research Service, [3]Corn Insects and Crop Genetics Research Unit, USDA-Agricultural Research Service

Fusarium is a large genus that includes many economically important plant pathogens and mycotoxin-producing species. Like other plant pathogenic fungi, *Fusarium* species can secrete small effector proteins that overcome plant defenses. Genomic data from fungal plant pathogens has been used to identify effectors and determine the underlying mechanism by which they impact plant defenses. Information on the 3D structure of proteins can aid the understanding their functions. This can be especially true for effectors that occur in diverse species and have potential as effective targets in control of crop diseases. In addition, understanding the structural variation of proteins that is revealed by genomic data across *Fusarium* is crucial for deciphering its biology, evolution, pathogenicity, and virulence phenotypes. Advances in Artificial Intelligence (AI) and Machine Learning have markedly improved our ability to identify, predict 3D structures, and assess genome-wide variant effects. Using these tools and an in-house genome sequence database, we identified 2,916 genes encoding putative effectors in 199 genome sequences that represent 23 *Fusarium* species complexes. Examination of the putative effectors using AI AlphaFold and ESMFold provided insight into the diversity of 3D structures of the effectors. Expression analysis of 50 of these effectors in the cereal head blight pathogen *Fusarium graminearum* revealed that twelve are highly expressed during wheat head infection. The predicted 3D structures of effectors as well as other proteins from the *Fusarium* species can be viewed at https://fusarium.maizegdb.org/protein_structure. We also used the protein data to develop a resource called "Fusarium PanEffect", https://www.maizegdb.org/effect/fusarium_v2 that can compare predicted 3D structures and genetic variants in 22 well-annotated *Fusarium* proteomes. This resource includes a missense variant function that predicts whether amino acid substitutions have benign, mild, or strong phenotypic consequences. Thus, Fusarium PanEffect provides information on variations in the structure and function of homologous proteins as well as on distribution of proteins among *Fusarium* species. This information will aid identification of genetic targets for developing control strategies that reduce crop diseases and mycotoxin contamination caused by *Fusarium*.

427A **A deep learning strategy for biosynthetic gene cluster prediction in fungal genomes** Stephen F Harding, Robert Proctor, Hye-Seon Kim ARS, U.S. Dept of Agriculture

Fungi produce numerous secondary metabolites (SMs) that can function as plant hormones, pigments, or toxins, including mycotoxins (e.g., fumonisins and trichothecenes) that are of concern to food and feed safety. Genes directly involved in synthesis of the same SM are typically adjacent to one another in a biosynthetic gene cluster (BGC). Multiple programs have been developed to predict fungal BGCs from genome sequence data. However, some are constrained to detect specific BGC types (e.g., Ribosomal synthesized and post-translationally modified peptides) while others yield inaccurate predictions by over or underestimating the numbers of genes in the predicted BGC or fail to detect or identify known clusters. The application of machine learning (ML) based programs to fungal BGC discovery addressed these limitations and demonstrated increased BGC detection accuracy relative to fungiSMASH and DeepBGC; however, so far, published analyses with those platforms have been limited to the *Aspergillus* species *A. niger and A. nidulans*. Thus, ML based model performance with other fungi is unreported. Therefore, the application of ML methods for fungal BGC discovery is an emerging area of study. We developed a ML model for BGC discovery and classification by combining deep learning and other ML methods. Our model adapted the PFAM2Vec embedding method, bidirectional long-short term memory network, and ML classification approach implemented by DeepBGC. In addition, because the original DeepBGC's dictionary was constructed using bacterial genomes, we constructed a fungal PFAM dictionary using >4,000 fungal genomes. We also incorporated sequence similarity networks (SSNs) in our BGC classification methods to help infer the chemical structure and biological activity of metabolic products of the novel BGCs. Through our research, we aim to provide a ML-based fungal BGC discovery model that is readily applied to all fungal genera while also addressing limitations of other BGC mining programs.

428A **A smut hybrid provides insights in the regulation of effector genes contributing to tumor formation of *Ustilago maydis*** Janina Werner, Weiliang Zuo, Gunther Doehlemann Institute for Plant Sciences and Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne

Smut fungi infect economically important crops including barley, sorghum, wheat and maize. The majority of the smuts infect their host systemically and replace the inflorescences by teliospores, i.e. *Sporisorium reilianum f. zeae* infecting maize systemically to cause head smut disease. In contrast, *Ustilago maydis*, the model organism within the smuts and close relative of *S. reilianum*, can form distinct tumors locally at sites of infection on both maize leaves and inflorescences. *U. maydis* and *S. reilianum* have similar genomes in size and synteny and infect the same host, *Zea mays*, providing a promising basis for interspecific hybridization.

We exchanged the mating type genes between *U. maydis* and *S. reilianum* and generated a recombinant hybrid to investigate, how the different effector gene sets of the two species contribute to virulence. The hybrid successfully colonized maize and revealed a *S. reilianum*-like phenotype without tumor formation. We used RNAseq to get insights in genome compatibility and gene expression levels in the binuclear hybrid strain. Thereby, we discovered 219 differentially expressed 1:1 effector orthologs in the hybrid with distinct gene expression patterns. While one group orthologs showed the similar expression in the wild type and the hybrid, another set of orthologs showed reversed regulation in the hybrid compared to the wild type. Notably, the majority of the reverse expressed effector genes remain in gene clusters associated to virulence. We hypothesized that *U. maydis* effector genes downregulated in the non-tumor forming hybrid could play a role in tumor formation. To test this hypothesis, we performed infection assays with knock-out mutants of the respective effector genes and identified two novel virulence factors with a role in tumor formation.

In a next step, we overexpressed transcription factors (TF) which are activated during host infection by *U. maydis.* Strikingly, overexpression of one conserved TF triggered the hybrid strain to induce tumor formation. Using RNAseq, we are identifying the effector orthologs regulated by this TF to unravel the molecular basis of *U. maydis* induced tumorigenesis.

429A **Identification of essential components for protein secretion in the phytopathogen *Zymoseptoria tritici*** Alexander Featherstone[1], Megan McDonald[2], Sian Deller[3], Graeme Kettles[2] [1]School of Biosciences, The University of Birmingham, [2]The University of Birmingham, [3]Syngenta

Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici*, is one of Europe's most devastating diseases of wheat. With increased levels of resistance to fungicides now common, new fungicide targets involved in essential *Z. tritici* processes are required. Secretory pathways represent attractive targets as they are important for fungi to interact with their environment throughout their life cycle. This includes the secretion of adhesins and digestive enzymes during early colonisation, secretion of polysaccharides that allow production of the cell wall, and secretion of virulence proteins (effectors) throughout infection. Essential components for protein secretion are well known in model yeasts, however, this information is lacking for *Z. tritici* and other phytopathogenic fungi.

To identify essential secretory pathway components, this project first aims to develop a reporter assay for *Z. tritici* protein secretion using a secreted luciferase system previously employed in yeast, where protein secretion is sensitively measured as luminescence (Kanjou *et al*., 2007). To transform *Z. tritici* with the NanoLuc luciferase gene, a 'soft' selectable landing site will be used in which the succinate dehydrogenase locus gains a point mutation that confers carboxin resistance (Kilaru *et al*., 2015). This reporter system will be used in large-scale forward genetics screens by UV mutagenesis to identify mutants impaired in protein secretion. Our recent progress towards this aim will be reported.

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430A **High-throughput genetics, essential gene discovery, and fluconazole resistance in *Cryptococcus neoformans*** Joshua Lyon[1,2], Caroline Craig[3], Michael Eickbush[3], SaraH Zanders[3], Blake Billmyre[1,2] [1]Pharmaceutical and Biomedical Sciences, University of Georgia, [2]Infectious Diseases, University of Georgia, [3]Stowers Institute for Medical Research

Cryptococcus neoformans causes nearly 200,000 deaths annually. Treating Cryptococcosis is complicated by the limited set of effective antifungal drugs and by antifungal drug resistance. Development of novel targeted therapies will be facilitated by a better understanding of which genes are required for growth in order to enable prioritization of targeted drug development. We have developed a high-throughput quantitative genetics approach in *Cryptococcus neoformans* using massively parallel insertional mutagenesis coupled with targeted sequencing to identify genes that tolerate (nonessential) or do not tolerate (essential) insertional disruption. The *C. neoformans* genome includes dozens of genes whose orthologs are nonessential in other fungi but required in *C. neoformans* and vice versa. In addition, we have used this same high-throughput approach to map genes that affect susceptibility to fluconazole. The identified hits include genes from pathways with previously identified roles in drug resistance, including the HOG pathway and *PKA1/NRG1*. In addition, we have identified novel contributors to drug susceptibility and resistance, including the *RIM101* pathway and multiple individual genes of unknown function. Transposon inserts in regulatory regions of essential genes generate sensitized strains that enable genome-wide analysis of the contribution of previously

intractable essential genes to stress responses including fluconazole resistance. Taken together, this work produces a global understanding of fluconazole resistance and a map of essential genome function to guide future drug development in *C. neoformans*. We are currently expanding our TN-seq approach in order to establish the *Cryptococcus* genus as a model fungal genus to understand the evolution of virulence-related traits.

431A **Functional characterization of a novel sugar transporter in *Trichoderma reesei* and its role in cellulase induction** Lucas Matheus Soares Pereira, David B. Maués, Roberto do Nascimento Silva Dept of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil.

The filamentous fungus *Trichoderma reesei* is widely used for the industrial production of cellulases due to its ability to secrete a large amount of proteins (up to 100 g/L on an industrial scale). The expression of lignocellulose-degrading enzymes is dependent on the carbon source and is regulated by various transcription factors and membrane proteins such as sugar transporters and receptors. Sugar transporters are crucial for the recognition of cellulose, which triggers the expression of CAZymes, and for the uptake of soluble inducing sugars released during saccharification and their downstream signaling. However, the role of most sugar transporters encoded in the genome of *T. reesei* is still unknown. Previous studies have shown that the sugar transporter Tr44175 was upregulated in the early stages of cultivation on cellulose and is able to transport the inducing sugars cellobiose and sophorose. In this work, the role of *Tr44175* in the *T. reesei* TU-6 strain was investigated. Deletion of the gene encoding *Tr44175* in *T. reesei* reduced growth on cellobiose, L-arabinose and lactose in solid media and growth on glucose and CarboxyMethyl-cellulose in submerged media. The role of *Tr44175* in the degradation of cellulose by *T. reesei* was also investigated, and the results showed that deletion of *Tr44175* affected the transcription of cellulase-encoding genes. The expression of cellobiohydrolase *cel7a*, endoglucanase *cel7b* and β-glucosidase *cel3a* had almost completely disappeared in the mutant strain at 12 hours of culture. At 24 hours of culture, the expression of these cellulases was equal between the Δ*Tr44175* and the WT strain, except for β-glucosidase *cel3a*, which showed lower expression in the mutant. The expression of the transcriptional activator *xyr1* and the sugar transporter *crt1*, which are essential for cellulase induction, were lower in the mutant at 12 hours and higher at 24 hours of cultivation than in the wild strain. However, the Δ*Tr44175* strain showed a higher amount of secreted total protein, CMCase, xylanase and β-xylosidase activities compared to the WT strain during 96 hours of cultivation in cellulose. The β-glucosidase activity was the same as in the WT strain, except at 48 hours of cultivation when pNPGase was higher in the mutant strain. These studies suggest that the sugar transporter Tr44175 is important for cellulase induction in the early stages of cultivation on cellulose and for the growth of *T. reesei* on different carbon sources

432A **A near-complete genome assembly and annotation of the dothideomycete marine fungus, *Neophaeotheca triangularis,* the first from the order Neophaeothecales** Faith A Martin[1], Mareike Moeller[2], M. Dolores Camacho-López[3], Meritxell Riquelme[3], Michael Freitag[1] [1]Oregon State University, [2]Australian National University, [3]Center for Scientific Research and Higher Education of Ensenada

The term “marine fungi” represents a diverse group of both obligately aquatic and sometimes terrestrial species isolated from marine environments that have been historically underrepresented in studies, in part due to biased sampling methods and often time-intensive culturing. However, with accelerated climate change, more resources are being devoted to increase our understanding of aquatic microbiomes, helping to uncover the large fungal diversity in marine systems. Current estimates for the total number of fungal species in marine habitats are likely too low, but new advancements in sequencing technologies and molecular biology methods allow us to uncover new species, and will aid in understanding how these organisms adapt and grow in harsh environments. *Neophaeotheca triangularis* is a saprophytic marine fungus that was originally classified within the polyphyletic order Capnodiales, but has now been moved to the Neophaeothecales [1]. There are few DNA sequences, let alone genomic, data for species in the Neophaeothecales, and to address this knowledge gap, we assembled a near-complete genome of *N. triangularis,* by employing both Oxford Nanopore and Illlumina DNA sequencing. Our assembly resulted in 22 chromosomes spanning approximately 25 Mb. Transcriptomics allowed identification of more than 9,000 genes, with nearly 4,000 orthologs (BUSCO). Like other marine black yeasts, *N. triangularis* can use unconventional methods of growth, e.g. meristematic division, and these may be responses to varied media conditions [2]. To explore mechanisms of cell division in this species on a genetic level, we are combining phenotypic analyses, transcriptomics data (RNA-seq) and chromatin modification maps (ChIP-seq) under different growth conditions.

433A **Chromosomal engineering in the plant pathogenic fungus *Verticillium dahliae*** Yukiyo Sato, Bart PHJ Thomma Institute for Plant Sciences, University of Cologne, Germany

Plants and pathogens are engaged in everlasting molecular arms-races in which genes encoding host immune receptors and pathogen-secreted virulence factors play central roles. Fungal virulence factors are typically encoded in highly variable genomic compartments that drive pathogen adaptation. In the ascomycete fungus *Verticillium dahliae* such dynamic genomic compartments are called “adaptive genomic regions” (AGRs) that are the result of large-scale genomic rearrangements that have

generated segmental duplications that underwent reciprocal gene losses. AGRs are enriched in *in planta* induced virulence genes, active transposable elements, histone H3K27me3, and structural variation. Recently, AGRs were found to physically co-localize in nuclei despite their distribution throughout the genome. However, causal relationships among the various traits that are linked to AGRs remain unclear. To address this, we established CRISPR-Cas9-based chromosomal engineering in *V. dahliae* to artificially engineer large-scale chromosomal rearrangements. We first engineered a reciprocal chromosomal translocation between a large AGR cluster and a core genomic compartment between two chromosomes. RNA-Seq analysis on this chromosomal translocation mutant revealed that genome-wide approximately 2% of genes were differentially expressed when compared with the wild-type strain. This result suggests that the chromosomal translocation impacts gene expression. We are now generating additional chromosomal translocation mutants over different AGR and core regions to collectively analyze whether and how these change the transcriptome, H3K27me3 profile, and nuclear co-localization profiles. These experiments will reveal which of the traits follow after the large-scale chromosomal rearrangements, providing insight into causal relationships.

434A **Convergent genome expansion in fungi linked to evolution of root-endophyte symbiosis** Yi-Hong Ke[1], Gregory Bonito[2], Hui-Ling Liao[3,4], Brian Looney[1], Alejandro Rojas-Flechas[2], Jake Nash[1], Khalid Hameed[1], Christopher Schadt[5], Francis Martin[6], Pedro W Crous[7], Otto Miettinen[8], Jon K Magnuson[9], Jessy Labbé[5], Daniel Jacobson[5], Mitchel J Doktycz[5], Claire Veneault-Fourrey[6], Alan Kuo[10], Stephen Mondo[10], Sara Calhoun[10], Robert Riley[10], Robin Ohm[10], Kurt LaButti[10], William Andreopoulos[10], Jasmyn Pangilinan[10], Matt Nolan[10], Andrew Tritt[10], Alicia Clum[10], Anna Lipzen[10], Chris Daum[10], Kerrie Barry[10], Igor V Grigoriev[10,11], Rytas Vilgalys[1] [1]Duke University, [2]Michigan State University, [3]North Florida Research and Education Center, [4]University of Florida, [5]Oak Ridge National Laboratory, [6]Interactions Arbres-Microorganismes, INRA Centre de Nancy, [7]Westerdijk Fungal Biodiversity Institute, [8]University of Helsinki, [9]Pacific Northwest National Laboratory, [10]U.S. Dept of Energy Joint Genome Institute, [11]University of California Berkeley

A highly diverse community of fungal endophytes exist within the intercellular apoplast of plants, yet genomic features associated with endophytism remain largely unknown. To identify genomic features associated with evolution of endophytic symbiosis, we sequenced genomes of 23 fungal root endophytes from a single host tree genus (*Populus*) representing 19 phylogenetically diverse lineages across the fungal tree of life. Compared with closely related fungi belonging to other ecological guilds, poplar root endophytic fungi had significantly greater genome size and gene counts, including higher numbers of CAZymes, protease, lipase, small secreted proteins, and antibiotic resistance genes. We identified orthogroups, Pfam domains and CAZymes that discriminate poplar root endophytes from other fungi. Pectinase GH88 was consistently found enriched associated with endophytism across different annotation methods. Other pectinases were also enriched as has been observed in root endophyte communities of the model plant species *Arabidopsis thaliana*. Other discriminative genes included proteins functioning in transporters, carbon metabolism, and transcription factors. Contrasts comparing multiple genomic features indicate the intermediate placement of pathogenic fungi between endophytes and saprobic fungi genomes, suggesting a distinctive state of endophytism. Our observations suggest that the expanded genomic content of fungal endophytes enables them to exploit a wider range of nutritional sources and symbiosis-related genes which are required for growth in-planta. The identification of key genomic features associated with endophytic lifestyle in poplar root endophytes provides new insights into the universal genetic components in endophytism and offers abundant genome resources for further studies of their functional significance in plant-fungal symbiosis.

435A **Genome-wide functional analysis of WD40 proteins in the fungal pathogen *Cryptococcus neoformans*** Jin-Tae Choi[1], Seong-Ryong Yu[1], Yu-Byeong Jang[1], Yujin Lee[1], Seok-Hwan Jung[1], Jeeseok Oh[1], Hyunjin Cha[1], Kyung-Tae Lee[2], Yong-Sun Bahn[1] [1]Yonsei University, [2]Jeonbuk National University

Protein-protein interaction (PPI) is crucial for biological functionality, with the WD40 domain playing a pivotal role. Yet, the function of WD40 proteins in the pathogenicity of human fungal pathogens is still unclear. Our study scrutinized the roles of canonical WD40 proteins in *Cryptococcus neoformans*, a global fungal pathogen causing fatal meningoencephalitis. We identified 94 canonical WD40 proteins in the *C. neoformans* genome and constructed 105 signature-tagged gene deletion strains for 53 WD40 proteins. Our *in vitro* and *in vivo* analyses revealed WD40 proteins significantly contributing to virulence and characterized their interaction networks. Furthermore, the potential essentiality of 36 WD40 proteins was verified through conditional inhibition or sporulation analysis. Our finding enriches the understanding of WD40 protein-dependent PPI networks in fungal pathogenicity and offers insight into potential antifungal drug development through PPI inhibitors.

436A **High-resolution assemblies for oat crown rust enable the detection of somatic hybridization and cryptic recombination** Eva C Henningsen[1,2], David Lewis[1], Eric Nazareno[3], Yung-Fen Huang[4], Brian J Steffenson[3], Shahryar F Kianian[3,5], Eric Stone[2,6], Peter N Dodds[1], Jana Sperschneider[1], Melania Figueroa[1] [1]Agriculture and Food, CSIRO, [2]Research School of Biology, The Australian National University, [3]Dept of Plant Pathology, University of Minnesota, [4]National Taiwan University, [5]Cereal Disease Laboratory, USDA-ARS, [6]Biological Data Science Institute, The Australian National University

Puccinia coronata f. sp. *avenae* (*Pca*), the causal agent of crown rust disease, is the most damaging foliar pathogen of oats worldwide. Virulence traits in a biotrophic fungus like *Pca* are controlled by complex molecular interactions between the pathogen and host that follow the gene-for-gene concept. Known drivers of virulence evolution in rusts include mutation, genetic recombination, and somatic hybridization. The obligate biotrophic lifestyle and dikaryotic nature of *Pca* traditionally presented challenges to develop resources for studying virulence evolution. However, recent advances in genome sequencing and assembly, combined with analysis of existing *Pca* collections, have been key for the construction of one haplotype phased chromosome-level reference genome, two partially phased references, and the release of publicly available short read sequencing data from *Pca* populations in the USA and South Africa. Based on virulence phenotypic analysis of the Australian *Pca* population, researchers have proposed that the pathogen evolves primarily by mutation and clonality; however, this hypothesis has not been examined closely at the genotypic level. Here, we characterise a *Pca* population collected from 2020-2022 in Australia and a small collection of isolates from Taiwan. Phylogenetic analysis indicates that the Australian *Pca* population is comprised of 17 lineages which are vastly distinct from those in the US and South Africa. We chose 15 isolates from the US and Australia as pangenome representatives and constructed their fully phased, chromosome-level haplotypes. Consistent to previous observations in other rust fungal species, multiple somatic hybridization events were detected in the Australian and US populations. One haplotype present in Australia is also shared with isolates from Taiwan, demonstrating the impact of nuclear exchange in the epidemiology of *Pca*. By characterizing the mating type loci, we demonstrate that *Pca* is highly likely to be tetrapolar; all individuals are heterozygous at the unlinked *PR* and *HD* loci. Interestingly, the haplotype-resolved pangenome enabled the detection of cryptic recombination between Australian haplotypes as revealed by a parent-F1 relationship from field isolates. These observations could be explained by yet undetected sexual cycles in *Rhamnus* species already present in Australia. In summary, clonality, recombination, and nuclear exchanges are all factors influencing the evolution of oat crown rust in Australia.

437A **Systematic Analysis of Host-derived Cues for the Regulation of Pathogenicity-linked Transcription factors in Human Fungal Pathogen *Cryptococcus neoformans*** Seong-Ryong Yu[1], Minjae Lee[1], Kyung-Tae Lee[2], Yong-Sun Bahn[1] [1]Dept of Biotechnology, College of Life Science and Biotechnology, Yonsei University, [2]Dept of Biotechnology, Korea Zoonosis Research Institute, Jeonbuk National University

Cryptococcus neoformans is a causative agent of global fungal meningoencephalitis, responsible for over 180,000 annual deaths. In analyzing this pathogen, we performed *in vivo* transcription profiling to monitor 180 transcription factors (TFs) during infection. Our focus was on 12 TFs that were notably induced in host-mimicking conditions (HMC). To determine which host factors contribute to gene induction during infection, we dissected HMC signals into components of temperature, carbon, and nitrogen starvation. Remarkably, we identified three distinct cues significantly influencing gene regulation. Temperature upshift markedly induced the expression of six genes. Similarly, glucose starvation and nitrogen starvation highly induced the expression of six and nine genes, respectively. Furthermore, deleting *MLN1* resulted in growth defects under carbon starvation conditions with alternative carbon sources, excluding glucose. Transcriptome analysis of the *mln1*Δ mutant under carbon starvation suggested *MLN1*'s involvement in the L-leucine degradation pathway. *In vivo* studies using a mouse model demonstrated attenuated virulence and reduced lung fungal burdens in mice inoculated with *mln1*Δ. In conclusion, our systematic dissection of host-signaling cues provides deeper insight into the complex signaling pathways that modulate host-pathogen interactions in *C. neoformans.*

438A **Unravelling the *MAT1* Locus: Insights Into Sexual Reproduction Across Diverse *Sclerotinia* Species** Sikelela Buthelezi, Chanel Thomas, Brenda Wingfield, Nicky Creux, Markus Wilken University of Pretoria

Sclerotinia, a fungal genus in the family *Sclerotiniaceae*, is best known for its type species *S. sclerotiorum*. Predominantly, research efforts have centered on three primary species, *S. minor*, *S. sclerotiorum*, and *S. trifoliorum*. Such studies delved into aspects including host range, distribution, and reproductive strategies. However, a critical gap persists in our understanding of the lesser-explored members of this genus. In filamentous ascomycetes like *Sclerotinia*, sexual reproduction is governed by the mating-type (*MAT*) genes situated at the *MAT1* locus. This study examined the *MAT1* locus of nine isolates representing various *Sclerotinia* species (*S. asari*, *S. bulborum*, *S. sulcata*, *S. sativa*, *S. matthiolae*, *S. spermophila*, *S. subarctica*, *S. nivalis*, and *S. pseudotuberosa*). Combining publicly available and newly generated draft genome sequences, our analysis identified the four

known *Sclerotinia MAT* genes (*MAT1-1-1*, *MAT1-1-5*, *MAT1-2-1*, and *MAT1-2-10*) among these species. Notable findings include the absence of the *MAT1-1-5* gene in *S. spermophila* and *S. sulcata*, hinting at a potential heterothallic mating strategy. Conversely, *S. pseudotuberosa*, *S. sativa*, *S. nivalis*, *S. bulborum*, *S. asari*, and *S. matthiolae* exhibited the presence of all four *MAT* genes, indicative of homothallism. Structural variations in the *MAT1* region, including the presence of repeat sequences, suggested a form of secondary homothallism in some of these species. This study presents draft genome sequences for seven lesser-studied *Sclerotinia* species, while shedding light on the diversity of sexual reproduction and the structure of the *MAT1* locus within this group. However, it is important to note that the taxonomy of many of these species remain unresolved, posing challenges for interpreting our findings. This further highlights the need for a robust taxonomic framework for *Sclerotinia*.

439A **Investigating Toxicity Through Fungal Genomes: A Case Study on *Pseudopithomyces chartarum*, the Causal Agent of Facial Eczema in Cattle** Neriman Yilmaz[1], Jérôme Collemare[2], Cobus Meyer Visagie[1] [1]University of Pretoria, FABI, [2]Westerdijk Fungal Biodiversity Institute

Facial eczema (FE) or pithomycotoxicosis is a secondary photosensitisation disease in ruminants attributed to sporidesmin A — an epipolythiodioxopiperazine (ETP) toxin produced by the fungus *Pseudopithomyces chartarum* (formerly *Pithomyces chartarum*), which is predominantly found in the leaf litter of pastures. FE was discovered in New Zealand in 1894 and has spread worldwide. Regions affected include Argentina, Australia, France, the Netherlands, Portugal, South Africa, Spain, Turkey, the United States, Uruguay and recently China. The occurrence of outbreaks depends on climatic factors and typically occurs during warm, humid periods in summer and autumn. In an earlier study in New Zealand, an ETP biosynthesis gene cluster (BGC) was discovered in the genome of *P. chartarum*, which codes for enzymes that are crucial for the biosynthesis of sporidesmin.

In this study, additional *P. chartarum* isolates from South Africa are analysed and compared with the non-sporidesmin-producing species *P. sacchari* and *P. maydicus* as well as with the New Zealand *P. chartarum*genome. The research involves the phylogenetic dereplication of non-ribosomal peptide synthetases (NRPSs) in the genomes of *Pseudopithomyces*. A maximum likelihood phylogenetic tree using characterised fungal NRPSs from the MIBiG database and the literature as reference values describes the candidate BGC for sporidesmin production. Comparative analysis of the locus across different genomes reveals a partially conserved ETP BGC. This is the first case of a cross-genus investigation of potential sporidesmin BGCs in *Pseudopithomyces*. Validation of the role of the BGC in sporidesmin biosynthesis requires further functional characterisation.

440A **The next dimension of CAZymes - Inferring the functional interplay between fungal carbohydrate-active enzymes for biomass conversion** Kristian Barrett[1,2], Lene Lange[3], Igor Grigoriev[4,5], Anne Meyer[1] [1]DTU Bioengineering, Technical University of Denmark, [2]Joint Genome Institute, Lawrence Berkeley National Laboratory, [3]LLa Bioeconomy, [4]Dept of Energy, Joint Genome Institute, Lawrence Berkeley National Laboratory, [5]Dept of Plant and Microbial Biology, University of California Berkeley
The next dimension of CAZymes - Prediction of functional interplay between fungal carbohydrate-active enzymes. Inferring the interplay between groups of different CAZy families
Fungal biomass degradation is a huge collaboration exercise for the genome encoded enzymes. Particularly the secreted fiber-active enzymes have an incredibly complicated interplay during which some enzymes may have the direct catalytic effect on the polysaccharides whereas other enzymes may only assist in the conversion indirectly as accessory enzymes. Such accessory enzymes have been greatly overlooked, as the effect is not apparent with the enzyme alone but only in combination with one or more other enzymes. The aim of my work is to identify such putative enzymatic combinations, by systematic identification of co-occuring/co-regulated CAZymes through genome comparisons. Hence, unraveling enzyme interplay for fungal biomass conversion while understanding their deployed biological strategies. This method relies on our classification of all CAZy families into functional subgroupings powered by our webserver CUPP.INFO [1].

[1] Kristian Barrett, Cameron J Hunt, Lene Lange, Igor V Grigoriev & Anne S Meyer. Conserved unique peptide patterns (CUPP) online platform 2.0: implementation of +1000 JGI fungal genomes, Nucleic Acids Research, gkad385 (2023).

441A **LCR differ among fungal phyla and from proteome background** Aleksander Kossakowski[1], Kamil Steczkiewicz[1], Stanislaw Janik[1,2], Anna Muszewska[1] [1]Institute of Biochemistry and Biophysics, PAS, [2]Faculty of Mathematics, Informatics and Mechanics, University of Warsaw

Low complexity regions (LCR) perform multiple, biologically relevant functions in every cell. Up to date, the diversity and distribution of LCR have been studied mostly in Prokaryota. Little is known about LCRs within fungi, since most studies concerned model yeast which barely represents the diversity of its kingdom. At the same time, fungi are grateful objects for studying genome-to-ecology relationships. They differ greatly in terms of genomic compositions, metabolic pathways, and living environments. We

performed a survey of LCR regions in proteins across all Fungal Tree of Life branches. We show that the abundance of proteins with LCR regions and the count of LCR regions themselves are positively correlated with proteome size. We observed that most of LCR are present in proteins that contain protein domains but do not overlap with the domain region. Observed amino acid distribution in LCR deviates from the background frequency for the proteome, with a clear overrepresentation of amino acids with functional groups, predominantly negatively charged. Moreover, we discovered that each lineage of fungi favors distinct LCR expansions pointing at a different evolutionary trajectory of each fungal group.

442A **Unravelling the 3D Architecture of *Batrachochytrium* genomes by Hi-C analysis** Nicolas Helmstetter, Tal Goodisman, Jamie Harrison, Rhys Farrer University of Exeter

Batrachochytrium dendrobatidis (Bd) and *Batrachochytrium salamandrivorans (Bsal)* are aquatic fungal pathogens causing the chytridiomycosis disease in amphibians. They are the only species belonging to the early diverging Chytridiomycota known to infect vertebrates. Their recent expansion in geographical distribution and host range has led to sharp amphibian declines and extinctions, contributing markedly to Earth's sixth mass extinction.
Since diverging, *Bd* and *Bsal* have evolved markedly different host species range, with *Bd* infecting a very wide range of hosts, while infection by *Bsal* is restricted to the Caudata (salamanders and newts). Furthermore, *Bd* causes hyperkeratosis (thickening of the hosts skin) compared with *Bsal* that causes deep ulcerations in the skin of the host.
Analysis of their genomes also revealed notable differences including notably a threefold transposable element-driven expansion in *Bsal* compared with *Bd*, with specific expansion of pathogenicity genes and signatures of two-speed genomes. The genome assemblies of *Bd* (24Mb, 69 scaffolds, 348 contigs) and *Bsal* (74Mb, 165 supercontigs) remain fragmented, and 3D interactions unknown, limiting our knowledge of how the genome is organised and shaped by evolution. Through conformation chromatin capture followed by high throughput sequencing (Hi-C), we have further scaffolded the assemblies into more complete reference genomes and begun to gain insight into their 3D organisation including the identification of topologically associating domains and loops. We hypothesise the genome structure is a key regulator and determinant of pathogenicity mechanisms in the batrachochytrids including host range and pathology.

443A **Gene expression patterns reveal the ability of *Trichoderma reesei* RUT-C30 to utilize *Hyaloscypha bicolor* melanized necromass** Irshad Ul Haq, Peter Kennedy, Jonathan Schilling Plant and Microbial Biology, University of Minnesota, Twin Cities

Microorganisms have been increasingly recognized as significant contributors to the accumulation of organic matter in soil through the inputs of their own dead cells, collectively referred to as 'necromass'. Fungal necromass makes up a large portion of the carbon stocks deposited in the soil of boreal forest ecosystems. Although ecological studies have indicated a role for melanin in controlling rates of necromass decomposition, how fungi drive this turnover is poorly understood. However, several fungal species, including the saprotrophs, have been found to abundantly associate with necromass in soil and it is hypothesized that they might be able to recycle necromass using their large repertoire of carbohydrate active enzymes. Here, we grew *Trichoderma reesei* RUT-C30 in shaking liquid culture on melanized necromass from *Hyaloscypha bicolor* and glucose and determined *T. reesei* RUT-C30 gene expression patterns using RNA seq. Gene expression data revealed that *T. reesei* RUT-C30 upregulated genes involved in the utilization of fungal cell wall components including chitin, glucan and mannan. We also observed differential expression of protease-encoding genes in the presence of melanized necromass, suggesting a possible role of proteases in melanin deconstruction. We found that *T. reesei* RUT-C30 can utilize necromass from *H. bicolor* as a substrate and could play essential roles in the recycling of the carbon deposited in soils of boreal forest ecosystems.

444B **Exposure to agricultural DHODH inhibitors result in cross-resistance to the novel antifungal olorofim in *A. fumigatus*** Norman van Rhijn[1], Michael Bottery[2], Isabelle Storer[2], Johanna Rhodes[3], Mike Bromley[2] [1]Manchester Fungal Infection Group, University of Manchester, [2]Manchester Fungal Infection Group, [3]Radboud UMC

Pesticides, including fungicides, are extensively used in agricultural practice to protect plants from unwanted growth of weeds, plant pathogens and other pests. Dual use of antifungals in the environment and in the clinic, with similar mode of actions, has been shown to drive the development of resistance. Although not a plant pathogen, *A. fumigatus*is ubiquitous in the environment and therefore exposed to agricultural fungicides. Extensive use of triazoles in the environment has led to high rates of resistance found in clinical *A. fumigatus* isolates. The development of novel antifungals is paramount to be able to treat azole-resistant aspergillosis. Olorofim is a novel antifungal for clinical use, targeting the essential protein DHODH, for which resistance is rare. Recently, several agricultural DHODH inhibitors, including ipflufenoquin, quinofumelin and tetflupyrolimet, have gone through the approval process. We show that these DHODH inhibitors are active against *A. fumigatus*, and have the same mode of action as olorofim. Spontaneous mutation analysis revealed we can select for ipflufenoquin resistant *A. fumigatus* isolates. These

ipflufenoquin resistant mutants show cross-resistance to olorofim. Furthermore, other agricultural DHODH inhibitors recently approved as herbicide have the potential to result in cross-resistance to olorofim. Lastly, we show that *A. fumigatus* isolates which are multi-drug resistant to a range of agricultural fungicides and clinically used antifungals are more fit under exposure to sub-inhibitory concentrations of ipflufenoquin and olorofim. Our results highlight the potential dangers of using DHODH inhibitors in agriculture and the future threat of resistance development to novel antifungals by selection in the environment.

445B **Crosstalk Between *Aspergillus nidulans* CWIS and SIN Pathways Under Cell Wall Stress** Alexander G Doan[1], Jessica E Schafer[1], Meredith E Morse[1], Matthew S Quintanilla[1], Dela-Joshua K Dayie[1], Kay T Latt[1], Julianna C Wasiuta[1], Steven D Harris[2], Mark R Marten[1] [1]Chemical, Biochemical, and Environmental Engineering, University of Maryland, Baltimore County, [2]Plant Pathology, Entomology, and Microbiology, Iowa State University

This study investigates the complex interaction between the Cell Wall Integrity Signaling (CWIS) and Septation Initiation Network (SIN) pathways in *Aspergillus nidulans*. We used a microscopy-based assay to measure septation phenotypes, aiming to understand *A. nidulans'* response to cell wall stress. Micafungin, a known inhibitor of β-1,3-gluca n synthesis, was employed to induce this stress. We observed a strong correlation between micafungin treatment and normalized septation in wild-type strains. This finding suggests that the activation of the CWIS pathway under cell wall stress triggers a crosstalk signal that activates the SIN pathway, increasing septation. Further investigations using recombinant strains with targeted knockouts of CWIS and SIN signaling proteins enabled us to identify crosstalk between MpkA (CWIS) and SepH (SIN) kinases. Following this discovery, we broadened our study to screen the *A. nidulans* kinase deletion library for other regulatory genes connected to these pathways. The results of this study advance our understanding of fungal biology and provides insight regarding development of possible antifungal strategies.

446B ***Fusarium proliferatum* in different host plants: evolution and comparative genomics** Alessandra Villani[1], Antonia Susca[1], Miriam Haidukowski[1], Stefania Somma[1], Marthe De Boevre[2,2], HyeSeon Kim[3], Stephen Harding[3], Sarah De Saeger[2], Robert H. Proctor[3], Antonio Moretti[1] [1]National Research Council, Institute of Sciences of Food Production, [2]Center of Excellence in Mycotoxicology and Public Health, [3]USDA-ARS, NCAUR, Mycotoxin Prevention and Applied Microbiology Research Unit

Fusarium proliferatum is an important plant pathogen that produces multiple mycotoxins, including fumonisins. The wide geographical range of *F. proliferatum* can be attributed to its occurrence on an extraordinarily wide range of crops (i.e., asparagus, date palm, fig, garlic, onion, rice, sorghum, and wheat). Although several studies have examined the genetic diversity among and within *Fusarium* species, in-depth analyses of genomic and phenotypic variability among *F. proliferatum* isolates collected from different geographic regions, climatic zones, and hosts are lacking. Our previous studies showed that *F. proliferatum* isolates recovered from fig in Turkey and date palm in Iran do not produce fumonisins in maize kernel culture. To further investigate the nonproduction phenotype, we did a more in-depth investigation of *F. proliferatum* using (un)targeted metabolomic approach. Data processing revealed the presence of fumonisin biosynthetic intermediaries in extracts of cultures from fumonisin-nonproducing isolates. Moreover, we gained more insight into genetic variability by analyzing 47 genomes of *F. proliferatum* for the presence and variation in genes and gene clusters responsible for the synthesis of mycotoxins and other secondary metabolites. The gene content and organization of the fumonisin gene cluster revealed no significant differences in gene occurrence, orientation, and genome location among fumonisin-producing and nonproducing *F. proliferatum* strains, except in the coding region of some *fum* genes, including *FUM14*, involved in the esterification of the tricarballylic moieties to the hydroxyls at C-14 and C-15 of fumonisins. Overall, the results of this research will be a reference for further studies aimed at building the mycotoxin risk knowledge base of agricultural products contaminated by *F. proliferatum* and identifying genes involved in the pathogenesis, and therefore fumonisin contamination, in certain geographic/climatic regions or on a plant host.

447B **Diverse signatures of convergent evolution in cacti-associated yeasts** Carla Gonçalves[1,2,3], Marie-Claire Harrison[4], Jacob L. Steenwyk[4,5], Dana A. Opulente[6], Abigail Leavitt LaBella[4,7], John F Wolters[8], Xiaofan Zhou[4,9], Xing-Xing Shen[4,10], Marizeth Groenewald[11], Chris Todd Hittinger[8], Antonis Rokas[4] [1]Dept of Biological Sciences & Evolutionary Studies Initiative, Vanderbilt University, [2]Associate Laboratory i4HB—Institute for Health and Bioeconomy and UCIBIO—Applied Molecular Biosciences Unit, Dept of Life Sciences, NOVA School of Science and Technology, Universidade NOVA de Lisboa, [3]UCIBIO-i4HB, Departamento de Ciências da Vida,Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, [4]Dept of Biological Sciences, Vanderbilt University, [5]Howards Hughes Medical Institute and the Dept of Molecular and Cell Biology, University of California, [6]Biology Dept Villanova University, Villanova, [7]Dept of Bioinformatics and Genomics, University of North Carolina at Charlotte, [8]Laboratory of Genetics, DOE Great Lakes Bioenergy Research Center, Center for Genomic Science Innovation, J. F. Crow Institute for the Study of Evolution, Wisconsin Energy Institute, University of Wisconsin-Madison, [9]Guangdong Province Key Laboratory of Microbial Signals and Disease Control, Integrative Microbiology Research Center, South China Agricultural University, [10]College of

Agriculture and Biotechnology and Centre for Evolutionary & Organismal Biology, Zhejiang University, [11]Westerdijk Fungal Biodiversity Institute

Many distantly related organisms have convergently evolved traits and lifestyles that enable them to live in similar ecological environments.

For instance, yeasts (Subphylum Saccharomycotina, Phylum Ascomycota) that evolved into ecological specialists associated with cacti (cactophilic yeasts) are sparsely distributed across almost the entire yeast phylogeny. However, the phenotypic traits as well as the genetic changes that facilitated this ecological association remain elusive.

In this work we benefited from genomic, phenotypic, and ecological data obtained for more than 1,000 yeast species, representing nearly all species belonging to the subphylum Saccharomycotina, to explore signatures of convergent evolution in cactophilic yeasts. We inferred that the ecological association of yeasts with cacti arose independently at least 17 times at distinct time points across the subphylum. Using a machine-learning algorithm, we found that cactophily can be accurately predicted from both genomic and phenotypic data. The most informative feature for predicting cactophily was thermotolerance, which might be associated with duplication and altered evolutionary rates of genes impacting the integrity of the cell envelope. We also identified horizontal gene transfer and duplication events of plant cell wall degrading enzymes in distantly related cactophilic clades, suggesting that putatively adaptive traits evolved through disparate molecular mechanisms. Remarkably, we found multiple instances of cactophilic yeasts and their close relatives that are emerging human opportunistic pathogens, suggesting that the cactophilic lifestyle, and perhaps more generally lifestyles favoring thermotolerance, might preadapt yeasts to human pathogenicity. This work underscores the potential of combining a multifaceted approach involving high throughput genomic and phenotypic data to shed light onto ecological adaptation and highlights how convergent evolution to wild environments can facilitate the transition to human pathogenicity.

448B **Fast-tracking metabolism: insights into the mechanisms and evolution of high glycolytic rates in non-conventional yeasts** Linda C Horianopoulos[1], Antonis Rokas[2], Chris Todd Hittinger[3] [1]Wisconsin Energy Institute, University of Wisconsin-Madison, [2]Dept of Biological Sciences, Vanderbilt University, [3]Laboratory of Genetics, Wisconsin Energy Institute, University of Wisconsin-Madison

Yeasts are famous for their fermentative capacity, a property which has elevated them to an important place in human society. The whole genome duplication, which resulted in the retention of extra copies of glycolysis genes and hexose transporters, is often offered as an explanation for the high glycolytic rate and robust fermentation in *Saccharomyces cerevisiae*. To better understand the range of fermentative capacity across yeast species and the genetic mechanisms underlying this variation, we assessed the glycolytic rates of diverse species within the yeast subphylum by measuring extracellular acidification rates (ECAR) upon the addition of glucose in the assay media. Through this approach, we confirmed that those species which have undergone a whole genome duplication have rapid glycolytic rates. Furthermore, through comparing phenotypes across the subphylum, we identified several species within the genus *Saturnispora* with high ECAR and rapid glycolytic rates. These high-ECAR yeasts rapidly consumed glucose and produced ethanol even under aerobic conditions. The last common ancestor between *Saturnispora* spp. and *S. cerevisiae* would have existed over 200 million years ago, well before the whole genome duplication. Interestingly, the high-ECAR *Saturnispora* spp. have no duplications in core glycolytic genes, nor in hexose transporters when compared to closely related species that have much slower glycolytic rates. Therefore, we employed comparative transcriptomics to identify upregulated genes in the high glycolytic rate species. We found a hexose transporter that had significantly higher expression in species with rapid glycolytic rates and, through targeted genetic manipulations, functionally confirmed that this hexose transporter is required for the high glycolytic rate phenotype. Taken together, these results not only identify a novel clade of yeasts with rapid glycolytic rates, but also suggest that there is an independently evolved mechanism to increase glycolytic flux.

449B **Host adaptation mechanisms in fungal pathogens: harnessing GWAS to explore host associated genomic traits in natural infections of fungal pathogens** Cecile Lorrain[1], Alice Feurtey[1,2], Julien Alassimone[1], Bruce A McDonald[1] [1]Plant Pathology, ETH Zurich, [2]Laboratory of Evolutionary Genetics, University of Neuchatel

Plant-pathogenic microbes, including the wheat fungal pathogen *Zymoseptoria tritici*, need to adapt to their host environment[1]. Despite extensive research efforts, the mechanisms underlying specific host environment adaptation of fungal pathogens are still largely unknown. In plants, genome-wide association studies (GWAS) have been extensively used to uncover the complexity of local adaptation and disease resistance genetic architecture. However, the application of GWAS in deciphering fungal pathogenicity and host adaptation is trailing behind**.** The main limitation for large-scale GWAS in plant pathogens remains the phenotyping of

several hundreds to thousands of strains in multiple hosts. Here, we leverage the power of GWAS to identify host-associated genomic traits in *Z. tritici* using natural infection data and whole-genome sequencing of 900 fungal strains from twelve different host cultivars. For this we compared one group of strains from a focal wheat cultivar against randomly sub-sampled groups of strains from different hosts, likewise correcting for unbalanced group sizes with a bootstrapping approach. We identified from one to twelve candidate genes associated with specific wheat cultivars. Among these, we found the effector *Avr3D1*, one of the few *Z. tritici* characterized effectors, which provides a proof-of-concept for our host-associated GWAS approach. Additionally, we identified a diversity of gene functions from predicted effector candidates to transcription factors, highlighting the complexity of the genetic basis underlying natural infections. By tapping into natural infection data, our study provides a novel outlook for GWAS in fungal plant-pathogens, transcending the limitations imposed by traditional phenotyping methods.

1. Feurtey, A. *et al.* A thousand-genome panel retraces the global spread and adaptation of a major fungal crop pathogen. *Nat. Commun. 2023 141* **14**, 1–15 (2023).

450B **Identification and characterization of histone modifying genes in the *Coprinopsis cinerea* genome** Emma Valcourt, Grace Clemens, Marilee A Ramesh Biology, Roanoke College

Histones, highly conserved proteins found in all eukaryotes, are fundamental for packaging and organizing DNA within the nucleus. They are regulated by a group of modifying enzymes that mediate access to the DNA sequence for gene expression and replication. Previously, canonical histones and histone variants have been identified in the *Coprinopsis cinerea* genome and shown to be distributed in three clusters on chromosomes two, seven, and nine. Our current study has focused on identifying the histone modifiers in the genome. An initial search identified 21 gene predictions that had potential roles in histone modification. Further evaluation classified histone acetylases (7), histone deacetylases (7), histone methylases (5), histone demethylase (1) and histone chaperonin (1). Each potential modifier is being explored for possible protein-protein networks utilizing Stringdb to identify other partners in the *C. cinerea* genome that work in conjunction with these genes to regulate histone behavior.

451B **Predicting fungicide tolerance: defining mutations in the *CyP51* and *CytB* genes among septoria leaf spot populations in Canada** Mohamed Hafez Abdel-Fattah, Mouldi Zid, Dianevys Gonzalez-Peña Fundora, Ryan Gourlie, Thomas K Turkington, Reem Aboukhaddour Agriculture and Agri-Food Canada

Sterol demethylation inhibitor (DMI) and Quinone outside inhibitor (QoI) fungicides play a pivotal role in managing wheat fungal pathogens in North America, however, their efficacy diminished with the emergence of fungicide-tolerant populations. The aim of this study is to investigate DMI and QoI fungicide tolerance among populations of septoria blotch causing species: *Parastagonospora nodorum* and *Parastagonospora pseudonodorum* in western Canada. A five-year microbiome investigation in several locations across western Canada identified the prevalence of *P. nodorum* and *P. pseudonodorum,* with a higher propiconazole (DMI) tolerance in *P. nodorum*. Sequencing of the *CyP51* and *CytB* genes (encodes the target protein of DMI and QoI fungicides, respectively) was compared utilizing 133 isolates (102 *P. nodorum* and 31 *P. pseudonodorum*) recovered between 2018 and 2022. Results identified 26 and eight nonsynonymous mutations between the two species in the coding region of *CyP51* and *CytB* genes, respectively. These mutations may indicate higher fungicide tolerance for *P. nodorum* compared to *P. pseudonodorum*. This is the first report on *CyP51* and *CytB* gene polymorphism in Canadian *P. nodorum* populations, and the first report on genetic diversity within these genes in *P. pseudonodorum*. This study presents the inaugural findings on *CyP51* and *CytB* gene polymorphism in Canadian *P. nodorum* populations and sheds light on genetic diversity within these genes in *P. pseudonodorum*. It may also provide a molecular approach to predict fungicides tolerance in fungal pathogen populations.

452B **Exploring the genetic basis of interaction between the filamentous fungus *Trichoderma atroviride* and bacteria using a genome-wide loss-of-function approach** José Manuel Villalobos-Escobedo[1], Lori Huberman[2], Catharine Adams[1], Adam M. Deutschbauer[3], Louise M Glass[1] [1]PMB, University of California, Berkeley, [2]Cornell University, [3]The Environmental Genomics and Systems Biology Division, The Lawrence Berkeley National Laboratory, Berkeley

Trichoderma spp. can establish beneficial interactions with plants. It has been observed that when these fungi colonize the roots of various crops, there is an increase in biomass and fruit production**[1]**. Interestingly, these fungi also can modify the microbiota in the rhizosphere, primarily inhibiting the growth of different species of bacteria**[2]**. In this study, we employed Random Barcode Transposon-Site Sequencing (RB-TnSeq)**[3]** to identify bacterial genes important for fitness in the presence of *Trichoderma atroviride* exometabolites**[4]**. Three rhizosphere bacteria—*Klebsiella michiganensis*, *Herbaspirillum seropedicae*, and *Pseudomonas simiae*—known for promoting plant growth, were studied alongside the non-rhizosphere species *Pseudomonas putida* KT2440. We found that nitrogen-fixing bacteria primarily competed for iron and relied on the TonB/ExbB siderophore transport system for

optimal fitness in *T. atroviride* exudates. Conversely, *P. simiae* and *P. putida* depended on mechanisms associated with membrane lipid modification for cationic antimicrobial peptide (CAMP) resistance. Unexpectedly, exudates from *T. atroviride* rescued purine auxotrophic mutants of microbial strains that establish symbiosis with plants. These data suggest that beneficial plant-associated bacteria may be promoted in their growth by *T. atroviride* in the tripartite interaction.

Using an analogous approach, we are identifying essential genes in *T. atroviride* for interacting with soil bacteria. To achieve this, we created a Random Barcoded T-DNA insertional library (RB-TDNA)[5] in *T. atroviride*, allowing us to conduct experiments on fungal-bacteria interactions and determine essential mechanisms in the fungus associated with these interactions. With this comprehensive approach, our goal is to contribute valuable insights into the complex relationships between fungi and root-associated bacteria, which will serve as a foundation to enhance agricultural crops.

[1] doi: 10.1038/nrmicro2637.
[2] doi: 10.1371/journal.pone.0227228
[3] doi: 10.1128/mBio.00306-15.
[4] doi: 10.1371/journal.pgen.1010909
[5] doi: 10.7554/eLife.32110

453B **Small but mighty: genome analysis of a basidiomycetous species in the genus *Meira* isolated from North American *Catalpa* seed pods** Catalina Salgado-Salazar, Lisa A Castlebury Agriculture Research Service, United States Dept of Agriculture

Meira is a genus of basidiomycetous yeast in the *Exobasidiomycetes* (*Ustilaginomycotina*) with less than a dozen species described to date. Species in this genus have been found associated with plants, living as endophytes or inhabiting the surface of leaves and fruits, or associated with dead mites and powdery mildew fungi. Some species may have the potential to be used as biological control agents. In this study, a single strain (M1555) of an unknown *Meira* sp. was isolated from the seed pod of *Catalpa* (*Bignoniaceae,* Angiosperms) in Prince George's County in Maryland in September 2018. Maximum likelihood analysis of the nuclear large subunit rDNA (LSU) of this isolate showed it is closely related to *M. argovae*, a species previously found on mites affecting *Ricinus communis* leaves. Isolate M1555 was grown on potato dextrose agar (PDA) and corn meal agar (CMA), and genomic DNA was used to generate Illumina and Oxford Nanopore sequencing reads. Preliminary analysis of Illumina data resulted in a draft genome assembly size of 16.8 Mb organized in 83 scaffolds and containing 7,430 predicted protein genes. Only two secondary metabolite clusters were identified, and local BLAST searches indicated the entire mitochondrial genome sequence was contained within a single contig, with a size of 23.4 kb. Initial BUSCO completeness indicated 97% of universal single-copy genes are present in the genome assembly. Long sequencing reads obtained using Oxford Nanopore sequences will be used to improve the draft genome assembly by scaffolding contigs to fill the gaps resulting from high coverage Illumina reads and improve phylogenetic placement within the order *Exobasidiales*. Genomic analyses of previously under sampled and rare lineages of basidomycetous fungi are necessary not only to explore species diversity and to increase phylogenetic resolution, but also for novel gene discovery to harness the potential pest-fighting abilities of these fungi.

454B **Gene expression and zinc tolerance in *Suillus luteus*** Jessica Fletcher[1], Alexander Smith[1], Janne Swinnen[2], Karl Jonckheere[2], Anna Bazzicalupo[3], Hui-Ling Liao[4], Greg Ragland[1], Jan Colpaert[5], Anna Lipzen[6], Sravanthi Tejomurtula[6], Kerrie Barry[6], Igor Grigoriev[6], Igor Grigoriev[6,7], Joske Ruytinx[2], Sara Branco[1] [1]University of Colorado Denver, [2]Vrije Universiteit Brussel, [3]Royal Botanic Gardens, Kew, [4]University of Florida, [5]Hasselt University, [6]DOE Joint Genome Institute, [7]University of California Berkeley

Zinc (Zn) is a micronutrient required for metabolism, growth, and reproduction. However, in excess it disrupts cell function and can lead to death. We investigated the genetic basis of Zn tolerance in *Suillus luteus*, an ectomycorrhizal fungus associated with pine trees. Previous research found tolerant and sensitive isolates, and candidate genes for metal tolerance.

We compared the transcriptomes of a Zn-tolerant and a sensitive isolate in the presence/absence of Zn stress to investigate genes actively involved in tolerance. The Zn-sensitive isolate showed significant gene expression changes upon Zn exposure. The Zn-tolerant isolate showed minimal differences between Zn and no-Zn conditions, indicating tolerance is likely constitutive and expressed independently of metal exposure.

We also examined each isolate's response to Zn exposure. The sensitive isolate downregulated genes involved in transmembrane transport and metal binding, and upregulated genes related to stress responses. Overall, Zn treatment did not alter the transcriptome of the tolerant isolate, but some genes were significantly affected including upregulation of Zn ion binding and

transport genes. Genes upregulated in both isolates included stress response and Zn binding genes. Genes downregulated in both isolates when exposed to Zn were involved in transport, including a zinc transporter (SlZRT1).

To investigate the existence of constitutive tolerance, we compared the transcriptomes of the Zn-tolerant and sensitive isolate in no Zn conditions. We found that genes downregulated in the sensitive isolate Zn response were already being expressed at significantly lower levels in the tolerant isolate in no Zn conditions. Transporters, including efflux pumps and a ZIP transporter (SlZRT2), were highly expressed in the tolerant isolate in no Zn conditions, suggesting their role in constitutive tolerance. In addition, expression of chromatin rearrangement and DNA binding genes in the tolerant isolate suggest epigenetic regulation of constitutive Zn tolerance. Conversely, the sensitive isolate showed higher levels of Zn redistribution/storage transcript expression, indicating stricter control of internal Zn in the absence of extracellular Zn.

This research deepens our understanding of the molecular mechanisms involved in Zn tolerance in *Suillus* species and paves the way for further studies highlighting the importance of ectomycorrhizal fungi in host plant symbiosis and environmental stress.

455B **Unique Expansion of PARP Family Proteins in the *Fusarium oxysporum* species complex** Cecelia Murphy[1], Shira Milo[1,2], Daniel Norment[1], Marina Rocha[2], Houlin Yu[1], Domingo Martinez-Soto[1], Neta Shlezinger[2], Li-Jun Ma[1] [1]University of Massachusetts Amherst, [2]Hebrew University of Jerusalem

Fusarium oxysporum is a cross-kingdom fungal pathogen known for its devastation of economically important agricultural crops and contributing to disseminated fusariosis and fusarium keratitis. The *F. oxysporum* species complex (FOSC) contains over a hundred different *forma speciales* that describe unique host specific plant-fungal interactions, determined by host-specific accessory chromosomes. No specific *forma specialis* has been reported for human pathogenic *F. oxysporum*; however, distinct sets of accessory chromosomes were reported among human pathogenic *F. oxysporum*. Understanding the regulatory mechanisms that govern pathogenicity is essential for the development of effective agricultural and medical methods to control *F. oxysporum* infections. This study focuses on the poly-ADP ribose polymerase (PARP) protein family, which participates in numerous regulatory cellular functions, including DNA repair, apoptosis, chromatin remodeling, and cell cycle regulation by synthesizing chains of ADP-ribose molecules. Comparative genomic analysis revealed its unique expansion in FOSC, ranging from three to twenty copies across strains and the expansion copies exclusively located on accessory chromosomes. The catalytic triad H-Y-E motif responsible for poly-ADP ribosylation, characterized in the human PARP1 protein, was identified in the *F. oxysporum* ortholog, located in the core genome. Several variant motifs which suggest mono-ADP ribosylation activity were identified in the expansion copies. Utilizing two plant pathogens, a biocontrol strain, and a human keratitis strain as a comparative system, we discovered that PARP copy number is positively correlated with survival rates in the presence of DNA damage agents. Knocking out the *Parp1* gene, which is the primary DNA repair PARP, in all four strains, significantly reduced infection severity in *Arabidopsis thaliana* and tomato plants, and increased sensitivity to human macrophages. This suggests that the PARP family in *F. oxysporum* not only plays a role in the response to DNA damage, it may also be involved in host-pathogen interactions. We believe that these findings open future possibilities for further investigating the precise mechanisms of this host-microbe interaction.

456B **Investigating fungicide mode of action using high-throughput functional genomics** Renato Carvalho, Lori B Huberman Plant Pathology and Plant-Microbe Biology, Cornell University

Fungi are responsible for diseases that result in the deaths of over a million individuals each year and devastating crop infestations that threaten global food supplies. Unfortunately, spraying of fungicides on crops and overuse of antifungal drugs in hospitals has resulted in the emergence of strains of fungi that are resistant to commonly used antifungal drugs. To improve our ability to control fungal infections and infestations, we must understand what cellular processes the fungicides target. We used massively parallel screens in combination with transcriptional profiling to investigate the genetic mode of action of a novel fungicide developed by VM Agritech called Curezin™. Using a library of over 300,000 barcoded *Rhodosporidium toruloides* mutants, we identified genes that are involved in responding to fungicide exposure. Genes that, when mutated, caused a significant growth phenotype in massively parallel screens were often not differentially expressed in transcriptional profiling data. Similarly, genes differentially expressed during exposure to fungicides frequently did not cause growth defects when mutated in massively parallel screens. Molecular genetic analysis of genes identified for their role in responding to Curezin™ in both massively parallel screens and transcriptional profiling showed that genes identified via both methods are important in the genetic response to Curezin™. Our work demonstrates the power of using massively parallel screens in concert with transcriptional profiling to identify a more complete set of genes associated with the genetic mode of action of fungicides. We expect that our work in identifying genes involved in responding to fungicides will enable an improved understanding of fungicide target genes to help in designing strategies to control fungal disease going forward.

457B **Characterizing the histone post-translational modification enrichment and genome organization in species of the *Ogataea* clade** Tiffany J Lundberg[1], Nickolas M Lande[1], Sara J Hanson[2], Andrew D Klocko[1] [1]Chemistry & Biochemistry, University of Colorado Colorado Springs, [2]Molecular Biology, Colorado College

The DNA of eukaryotic genomes is packaged in the nucleus as chromatin – an association of DNA and proteins that is necessary for the regulation of the genome, including for the control of gene transcription. Chromatin can either be active and open (euchromatin) or silenced and compact (heterochromatin), the latter typically characterized by a lack of histone acetylation in budding yeasts. Recent advances have shown that chromatin composition is a determining factor controlling genomic DNA organization, including how the aggregation of the heterochromatic regions at the nuclear periphery, which segregates euchromatin to the center of the nucleus, is critical for the compaction of fungal genomes into the nucleus. Specifically, the centromeres aggregate independently of the telomere clusters to form a Rabl conformation. However, it is unknown if chromatin composition and genome organization differ or is conserved in closely related species, including in fungi. To this end, we examined differences in histone post-translational modification deposition by Chromatin Immunoprecipitation-sequencing (ChIP-seq) and genome organization, assessed by chromosome conformation capture coupled with high-throughput sequencing (Hi-C) in two species of yeast in the *Ogataea* clade. We are focusing on *Ogataea polymorpha*, which is typically used for industrial protein production, and the closely related *Ogataea haglerorum*; the *O. haglerorum* isolate used in this study has a translocation between chromosome one and six, relative to *O. polymorpha.* Here, we will present our preliminary analysis of the species-specific differences between *O. polymorpha* and *O. haglerorum*. Using two activating marks, trimethylation of lysine 4 histone H3 (H3K4me3) and the acetylation of lysine 16 on histone H4 (H4K16ac), we will assess differences in euchromatin and heterochromatin formation. Further, we will explore the genome organization changes between these two *Ogataea* species by Hi-C. Preliminarily, *O. polymorpha* and *O. haglerorum* show chromosome-wide euchromatin compaction and the clustering of centromeres that is independent from telomere bundles, suggesting the genomes of both species form a Rabl chromosome conformation. Together, our work should help elucidate differences in chromatin composition and genome organization between Ogataea species.

458B **The High Osmolarity Glycerol transcription factors Atf1 and Srr1 regulate stress response and cellulase production in *Trichoderma reesei*** David B Maués[1], Roberto N Silva[2] [1]Biochemistry and immunology, University of São Paulo, [2]Biochemistry and Immunology, University of São Paulo

Mitogen-Activated Protein Kinase (MAPK) is a conserved signaling pathway and is responsible for the transduction, integration, and amplification of intracellular signals in many cellular processes. During osmotic and oxidative stress, the cellular response is controlled by the High Osmolarity Glycerol (HOG) MAPK pathway. In the cellulolytic fungus *Trichoderma reesei*, the MAPK Tmk3 mediates the HOG response. Tmk3 is not only involved in the response to hyperosmotic, oxidative, and cell wall stresses but also regulates cellulase production in *T. reesei*. Here, the role of the two transcription factors (TFs) from the HOG pathway in *T. reesei* was investigated: Atf1, the homolog of SKO1 in the Sho1 branch, and Srr1, the homolog of SKN7 in the Sln1 branch. A BLASTp search was performed to identify the major homologs of these TFs in *T. reesei*, and deletion of the corresponding genes was performed using the CRISPR-Cas9 system. Deletion of *atf1* increased tolerance to cell wall stress caused by Calcofluor White and Congo Red, as well as to osmotic stress caused by NaCl and sorbitol, while deletion of *srr1* caused only a slight increase in tolerance to cell wall stress. Gene expression analysis showed that Srr1 plays an important role in cellulase expression after 8h of culture on cellulose by acting as a repressor for the expression of *cel6a*, *cel3a*, and *xyn1* and as an activator for the expression of *cel7b*. However, after 48h of cultivation, deletion of *srr1* led to a deregulation of *T. reesei* metabolism, with a decrease in overall cellulase expression and a threefold increase in protein secretion compared to the wild-type (WT) strain. In addition, the specific activities of CMCase, β-glucosidase, xylanase, and β-xylosidase were reduced in the Δ*srr1* strain in the whole cultivation. Atf1 is important for the appropriate induction of cellulase expression at 8h of culture on cellulose, as the expression of *cel7a, cel7b, xyn1,* and *bxl1* was reduced in the Δ*atf1* strain. However, after 48h, the expression of *cel7a* and *cel3a* was higher in the Δ*atf1* strain than in the WT. Interestingly, the specific β-glucosidase and β-xylosidase activities were higher in the Δ*atf1* strain after 24h of culture on cellulose, while the xylanase activity was decreased. These results indicate that both Atf1 and Srr1 have dual and temporal roles in regulating cellulase expression in *T. reesei* and are also important for the stress response in this fungus, providing new aspects of *T. reesei* physiology.

459B **Generation of haplotype phased genomes and nuclei specific expression profiles by long read sequencing in major Australian stripe rust lineages** Rita Tam, Mareike Moeller, Benjamin Schwessinger Australian National University

Rust fungi are a group of pathogens infecting a variety of species including important crops. Stripe rust (*Puccinia striiformis* f.sp. *tritici*) is a fungal pathogen specialized in infecting wheat. Different lineages of the fungus have been introduced to Australia in the last decades and four lineages are responsible for the majority of stripe rust infections.Relative frequency of these lineages and pathotypes causing disease changed over time with more recently introduced lineages replacing older introductions. Recent data suggests that isolates do not only differ in terms of susceptibility to specific plant resistance genes but also in their

"aggressiveness" in causing disease. Stripe rust, as many other basidiomycetes, partitions individual haplotype genome copies into discrete nuclei. We utilized long read sequencing (PacBio HiFi and Oxford Nanopore (ONT) duplex reads) combined with HiC to generate haplotype phased genomes for the four different Australian lineages. To characterize haplotype specific infection programs, we performed long-read ONT cDNA sequencing comparing different time points during wheat infection for each lineage. Analysing both, differences as the infection progresses, as well as differences between the distinct lineages on a genomic and transcriptomic level will improve our fundamental understanding of how the presence of multiple nuclei contributes to disease severity and rapid adaptation to variable and changing agricultural environments.

460B **Evaluating histone acetyltransferases in *Parastagonospora nodorum* as potential fungal targets for alternative disease management** Anjana Sharma, Kar-Chun Tan, Chala Turo, Francisco J. Lopez-Ruiz Centre for Crop and Disease Management (CCDM), School of Molecular and Life Sciences, Curtin University

Sustainable crop disease management requires an integrated approach, which includes the use of fungicides. However, the loss of chemicals to both resistance and regulation, combined with the difficulty finding new fungicide modes of action poses a serious threat to global food security. This study applies genomic approaches to identify new targets for the development of future novel fungal inhibitors. We have undertaken a high-throughput characterisation of putative histone acetyltransferase genes (*HATs*) in the model crop pathogen *Parastagonospora nodorum*. We hypothesized that the underlying proteins regulated by these HATS could be also targeted for fungal inhibition. The targeted deletion of *P. nodorum* HATs; *GCN5*, *NAT9, RTT109 and KAT7* resulted in a significant reduction in growth, sporulation and disease development compared to the wildtype. Attempts to delete *PnEsa1* failed, however, partially gene-silenced mutants exhibited reduced sporulation and pathogenicity. The results suggest an essential function for *PnEsa1*. We are further aimed at determining the processes that are disrupted by the deletion and silencing of *HATs* in *P. nodorum*. This will facilitate the potential identification of novel fungal inhibition pathways that could be exploited for sustainable disease management.

461B **Exploring the Unique Genome of Fusarium solani in Sugarbeet: Insights on its Opportunistic Habits** Abbeah Navasca[1], Jatinder Singh[2], Viviana Rivera-Varas[2], Upinder Gill[2], Gary Secor[2], Thomas Baldwin[1] [1]North Dakota State University, [2]Plant Pathology, North Dakota State University

Fusarium diseases threaten sugarbeet production and can cause significant yield and economic losses. During a field disease survey in Wilkin County, MN, growers found dark galls on sugarbeets. A fungal isolate with morphological characteristics consistent with *Fusarium* was isolated and identified as belonging to *Fusarium solani* FSSC clade 5 using sequence analysis of RNA polymerase II subunit B gene. Inoculation of sugarbeet roots with *F. solani* isolate SB1 resulted in mild vascular discoloration and no external symptoms. We assembled the complete genome of this *F. solani* sugarbeet isolate from combined Oxford Nanopore MinIon genome assembly and Illumina NovaSeq Hi-C sequencing. The resulting genome has 15 chromosomes with predicted 17,792 protein-coding genes with more predictions currently underway. Among the publicly available *F. solani* genomes, the SB1 genome has one of the highest percentage of repeats and transposons, contributing to its large size of 59.33 Mb. *Fusarium* species acquire accessory chromosomes with regions that enable pathogenicity. Compared to *F. vanettenii* 77-13-4, SB1 has inversions and transpositions, including syntenic regions to the accessory chromosome of the reference genome. Understanding the genomic characteristics of *F. solani* SB1 will shed light on possible strategies for its adaptation and role in the unknown dark galls of sugarbeet.

462B **Identification of specific gene expression profiles of two conidia types in *Colletotrichum graminicola*** Disha Rathi[1,2], Karsten Andresen[3], Marco Guerreiro[4,5], Kai Heimel[6], Jim Kronstad[2], Matthias Kretschmer[2], Minou Nowrousian[7], Stefanie Pöggeler[1], Anja Poehlein[8], Lars Voll[9], Daniela Nordzieke[1] [1]Dept for Genetics of Eukar yotic Microorganisms, Georg-August University Göttingen, Institute of Microbiology and Genetics, [2]Dept of Microbiology and Immunology, University of British Columbia, Michael Smith Laboratories, [3]Johannes Gutenberg University Mainz, Institute of Biotechnology and Drug Research (IBWF), [4]Christian-Albrechts University of Kiel, Botanical Institute, [5]Max Planck Institute for Evolutionary Biology, [6]Dept of Molecular Microbiology and Genetics, Georg-August University Göttingen, Institute of Microbiology and Genetics, [7]Dept of Molecular and Cellular Botany, Ruhr University Bochum, Faculty of Biology and Biotechnology, [8]Dept of Genomic and Applied Microbiology, Georg-August University Göttingen, Institute of Microbiology and Genetics, [9]Dept of Biology, Philipps-University Marburg

Colletotrichum graminicola is a hemibiotrophic plant pathogen causing anthracnose on *Zea mays*. This phytopathogenic fungus forms two morphological distinct asexual spores, oval and falcate conidia, in the plant's vascular system and on the surface of infected leaves, respectively. As we showed recently, they differ significantly in their developmental processes, secondary

metabolite profiles and infection strategies on leaves and roots. In 2012, the genome sequence of *C. graminicola* M1.001/CgM2 (wild type strain) was published, based on Sanger and 454 platforms, containing regions of repetitive elements, which were quite difficult to analyze. This is especially true for the minichromosomes of CgM2. Since these might encode for genes essential for plant interaction and virulence, we decided to re-sequence genome of *C. graminicola* wildtype strain using a combination of Nanopore and Illumina sequencing techniques. From the obtained reads we were able to assemble the resulting sequences in 13 gap-free contigs and 15481 genes, which sum up to 57,600,233 bases in total. To get an overview about relevant encoded genes groups, we analyzed all predicted genes for their putative functions during development and pathogenicity of this fungus (CAZymes, secondary metabolite genes clusters, putative conserved developmental genes, effectors, involvement in the secretory pathway, nutrient transport).

In this project we investigate the bases for the specific behavior of oval and falcate conidia using RNAseq analyses. We found that already in freshly harvested oval and falcate conidia >1000 genes are differentially regulated, underpinning the different nature of those conidia types per se and also their difference to vegetative growing hyphae. We have also compared different developmental stages, that is, germination (5h), germling fusion (16h) and leaf infection (1dpi) in both asexual spore types. In each we found a set of several hundred differentially expressed genes, which we are currently analyzing regarding their involvement in distinct biochemical pathways and probable functions in the overall process. We will also verify our RNAseq results by qRT PCR for the most interesting genes. Together, the obtained results will provide us with a broader viewpoint and a sound basis for studying conidia type-specific pathogenicity on *Zea mays* in the future.

463B **The pangenome of human and banana infecting *Fusarium musae* strains** matias pasquali[1], Luca Degradi[1], Valeria Tava[1], Anna Prigitano[2], Maria Carmela Esposto[2], Andrea Kunova[1], Daniela Bulgari[3], Cristina Pizzatti[3], Paolo Cortesi[1], Marco Saracchi[1], Matthias Brock[4], Greetje Vande Velde[5] [1]DEFENS, University of Milan, [2]Dept of Biomedical Sciences for Health, University of Milan, [3]University of Milan, [4]School of Life Sciences, University of Nottingham, [5]Dept of Imaging and Pathology, Biomedical MRI unit, KU Leuven

Fusarium musae was described as a species in 2011. It has been reported as one of the causal agents of crown rot, a postharvest disease of bananas. Originally misclassified as *F. verticillioides*, in the last 10 years it has been reported also as a cause of superficial and, in the case of immunocompromised patients, systemic infections in humans.

We obtained and sequenced a worldwide collection of *F. musae* isolates (n=18) combining illumina and nanopore sequencing. Mitochondrial genomes confirmed the similarity of strains from banana and human patients and traced the possible spread from banana-producing countries to banana-consuming countries. The assembly and annotation allowed us to identify two main groups within the species that have diverse genomic sizes, likely associated with the acquisition of genetic material that carries genes from evolutionary distantly related fungi. The pangenome analysis revealed that genomic differences do not discriminate between the origin of strains from different hosts. A few orthogroups are over-represented in human pathogenic strains and they are currently investigated for their potential role in directing host specificity. Infection studies in both *Galleria mellonella* (used as a human proxy) and banana fruits do not link host origin to virulence confirming that the *F. musae* genome reservoir allows efficient infection of both organisms.

464B **Deciphering the mechanistic basis of tolerance to olorofim in *Aspergillus fumigatus*** Clara Valero, Myles Mcmanus, Jamie Tindale, Ashvatti Anna, Sara Gago, Michael Bromley The University of Manchester

Respiratory infections caused by the mould pathogen *Aspergillus fumigatus* annually kill as many as tuberculosis or malaria. These infections occur in a scenario with limited therapeutic options and resistance to azoles, the main antifungal agents to treat and prevent aspergillosis, is globally increasing. The development of new antifungals with novel mechanisms of action has been proposed as the most promising intervention to stop and contain the emergence of antifungal resistance. Olorofim acts by inhibiting the *de novo* synthesis of pyrimidines in a fungal-specific manner and will reach the clinic in the following years. However, we have recently demonstrated that *A. fumigatus* exhibits tolerance to olorofim that relies on increased survival in the presence of lethal concentrations of the drug. By screening *A. fumigatus* genome-wide mutant libraries for resistance to olorofim killing, we have identified the genetic factors contributing to olorofim tolerance. Understanding the mechanistic basis of *A. fumigatus* tolerance to olorofim will increase our knowledge on its contribution to resistance allowing the development of strategies to suppress it before this promising antifungal reaches clinical implementation.

465B **Genome analysis and molecular detection of *Fusarium solani* f. sp. *phalaenopsis* causing leaf yellows of moth orchids** Wei-Chin Tsao[1], Yi-Hsuan Li[2], Yi-He Tu[1], Yu-Shin Nai[3], Tsung-Chun Lin[4], Chih-Li Wang[5] [1]Plant Pathology, National Chung Hsing University, [2]Doctoral Program in Microbial Genomics, National Chung Hsing University and Academia Sinica, [3]Dept of Entomology, National Chung Hsing University, [4]Plant Pathology Division, Taiwan Agricultural Research Institute, [5]Plant Pathology, National Chung Hsing University, Taiwan

Phalaenopsis, a globally popular ornamental flowers, holds significant economic importance in Taiwan. Leaf yellows of *Phalaenopsis* spp. cause by *Fusarium solani* f. sp. *phalaenopsis* is the major obstacle to orchid production. This disease induces symptoms such as leaf yellowing, sheath rot, and leaf drop, resulting in substantial losses of commercial value. Despite being a significant challenge for growers, our understanding of the disease and its causative pathogen is limited, and effective control methods are currently unavailable. In this study, we sequenced the whole genome of *F. solani* f. sp. *phalaenopsis* and employed a genome comparison approach to design specific primers for the pathogen. The genome of *F. solani* f. sp. *phalaenopsis* FuZ10 was generated through Nanopore and Illumina sequencing. FuZ10s genome was compared with *F. vanettenii* 77-13-4 in synteny analysis. FuZ10s possessed 9 core chromosomes (CCs), 3 fast core chromosomes (FCCs) enriched in virulence-related genes, and a lineage-specific (LS) region containing those contigs that did not align with the *F. vanettenii* core chromosomes. It revealed that the LS region contains a higher percentage of transposon elements than other CCs. Genes encoding effectors and secreted carbohydrate-active enzymes were enriched in the three FCCs, potentially contributing to the characteristic necrotic symptoms of the disease. The study also revealed genes associated with pathogenicity in other pathosystems. For the development of specific primers, whole protein sequences of FuZ10s were compared with those of five other *Fusarium* species using the OrthoVenn2. Further comparisons were made with the NCBI database to identify specific genes unique to the pathogen. Two unique genes were screened and selected to design specific primers, allowing for amplification bands of 662 bp and 557 bp, respectively. The specificity of the primers was validated with non-target isolates of *F. solani* species complex. In the future, the primers will be employed to investigate the ecology of the pathogen, aiding in the establishment of effective control strategies.

466B **Comparative Analysis of Carbohydrate Active Enzymes in *Rhizopus* spp. and *Aspergillus* spp.: A Bioinformatics Approach** Tomás Vellozo Echevarría[1], Kristian Barret[2], Marlene Vuillemin[2], Anne S. Meyer[2] [1]DTU Bioengineering, Danmarks Tekniske Universitet, [2]Danmarks Tekniske Universitet

Solid-state, fungally fermented plant foods, notably variations of tempeh using alternative raw materials, are gaining increased attention as new alternative proteinaceous plant foods. The different fungal fermentation strategies applied to plant material exhibit considerable diversity, drawing attention to *Rhizopus* species due to their possession of a distinct set of carbohydrate-active enzymes, as highlighted by current data. The unique enzymatic profile presents a distinct carbohydrate degradation pattern, different from that observed in *Aspergillus* species. Our study conducts a thorough bioinformatic analysis, comparing the putative enzyme profiles of both previously identified and newly annotated *Rhizopus* species with well-characterized *Aspergillus* species. This report examines and compares the outstanding number of chitin-modifying enzymes and the limited number of plant cell wall degrading glycoside hydrolases (cellulases and polygalacturonases) in *Rhizopus* species. A comparison with *Aspergillus* spp. invites to the hypothesis that *Rhizopus* has evolved a fast mycelium cell wall remodeling strategy and rapid-penetrative and storage-carbohydrate consumption approach, emphasizing the necessity for detailed characterization of starch-degrading enzymes within this group and exploration of their catalytic activities. The primary objective is to unveil correlations between the enzyme profiles with a particular focus on understanding *Rhizopus*' characteristic production of non-saccharifying solid-state fermentation abilities.

467B **Exploring domain assortments in NOD-like receptors of *Sordariales* fungi reveals two types of NACHT domains** Lucas Thibault Bonometti[1], Florian Charriat[2], Silvia Miñana Posada[3,4], Pierre Gladieux[2] [1]UMR PHIM, Institut Agro, [2]UMR PHIM, INRAE, [3]INRAE, [4]Institute of Integrative Biology, ETH

Fungi have cytoplasmic immune receptors, known as NOD-Like Receptors (NLRs), which exhibit a wide range of domain assortments. However, the identity of nucleotide-binding domains and genomic localization of fungal NLRs, as well as the factors that impact the composition of fungal NLR repertoires are not completely understood. To gain more insight into the variability of fungal NLR repertoires and its determinants, we conducted a comprehensive analysis of genome data from the *Sordariales* ascomycete order.

Using a combination of Pfam-A and *Sordariales*-specific Hidden Markov Models profiles for canonical N-terminal, nucleotide-binding, or C-terminal domains, we characterized 4532 NLRs in 82 taxa representing five *Sordariales* families. We found that the size of NLR repertoires was highly variable within and among *Sordariales* families, and that fire-associated *Neurospora* species had smaller repertoires. Although a minority of NLRs were organized in clusters, we identified NLR clusters in the majority of taxa, and

a strong correlation between the numbers of NLRs and NLR clusters. By examining the Helical Third section of the nucleotide-binding domains, we substantially improved their annotation and demonstrated that fungi have NACHT domains of both NAIP-like and TLP1-like types, which is similar to animals.

Our work highlights the resemblance between fungal and animal NLRs in their genomic organization and nucleotide-binding domain types. The observed variation in the number of NLRs suggests that repertoire size may be associated with lifestyle. Our findings will aid in the comparative analysis of the patterns and processes of diversification of NLR repertoires within different lineages of fungi and across various kingdoms and domains of life.

468B **Two genomes of *Fusarium verticillioides* from human patients: a comparative genome analysis** Luca Degradi, Valeria Tava, Cristina Pizzatti, Andrea Kunova, Daniela Bulgari, Maria Carmela Esposto, Marco Saracchi, Paolo Cortesi, Anna Prigitano, Matias Pasquali University of Milan

Fusarium verticillioides (FV) is a plant pathogen, but it was detected as the causal agent of human fusariosis. Here, we report the first two genome assemblies of *F. verticillioides* FV_05-0160 and FV_IUM09_1037 obtained from clinical settings (both from human blood). The genomes were 1.5-1.8 Mb bigger than *F. verticillioides* 7600, a maize pathogen used as a reference. Phylogenomic positioning of the strains confirmed species identity and showed close relatedness with Italian, Australian and American strains isolated from maize and sorghum. Comparative genomic analysis against the genome of the reference strain FV_7600 identified unique differences (n=118 genes) between the plant pathogen-derived genome and the two human strains. Further analysis on all FV genomes available in public databases found five unique genes present only in the human infecting strains. These genes are likely involved in the adaptation to the different hosts. This study contributes to explore the FV genomes diversity and opens the way to comparative genomic studies searching for specific genes in host-niche adaptation.

469B **Comparative genomic and transcriptomic analysis to uncover host defense response and fungal virulence factors in the interactions of mango leaf and *Colletotrichum asianum*** Dai-Keng Hsieh[1], Ming-Che Shih[2], Miin-Huey Lee[1] [1]Plant Pathology, National Chung Hsing University, [2]Agricultural Biotechnology Research Center, Academia sinica

Mango is an important fruit product in the world, but its production faces significant challenges due to anthracnose disease caused by *Colletotrichum* spp. *Colletotrichum asianum* is the major mango anthracnose pathogen in Taiwan. Whole genome sequencing of a *C. asianum* strain TYC-2 revealed that total 16,287 genes are encoded in 58 Mb genome. Microscopic examination and q-RT-PCR assays suggest that a biotrophic stage might not exist in this pathosystem. Transcriptomic analysis of the immature and mature infected Irwin mango leaf showed that gene expression patterns of infected immature leaf were similar to those of the healthy mature leaf, indicating that TYC-2 infection might enhance leaf aging. Ethylene and abscisic acid biosynthesis pathway related genes were upregulated in immature leaf after infection, but no differentially expressed genes (DEG) were detected in the biosynthesis pathway of salicylic acid which is known to be associated with host defense against biotrophic pathogens. Defense genes , such as WRKY1/2, VLCFA related genes, and genes required for lignification, were also upregulated post-infection. In addition, DEG was not found in mature leaf after infection. Transcriptomic analysis of fungal gene expression during mango leaf infection, one effector gene CaEF-1, and one transcription factor gene CaTF66, which were specifically expressed during the infection, were functionally analyzed. The absence of CaEF-1 did not affect the infection of TYC-2 on the leaf of Irwin mango but resulted in reduced virulence of TYC-2 on the leaf of two other mango cultivars, Four Season and Glory. CaTF66 gene deletion mutants had lower virulence than the wild-type on Irwin mango leaf.

470B **Transcriptomic and metabolic changes caused by mutation in xylanase regulator 1 (*xyr1*) in *Trichoderma reesei*** Emmi Sveholm, Hans Mattila, Nina Aro, Mari Valkonen, Tanja Paasela, Tiina Pakula VTT Technical Research Centre of Finland

Trichoderma reesei is known for its protein secretion abilities and is one of the most important industrially used filamentous fungi. Xyr1 as the master regulator is responsible for the activation of cellulase gene expression, normally under inducing conditions. It has been reported that mutations in certain areas of *xyr1* bypass the carbon catabolite repression, allowing cellulase production even in the presence of glucose (1). These mutations also change the pattern of produced proteins, shifting it more towards xylanase production, and increase the protein production in inducing conditions.

In the present study, the aim was to explore changes caused by the mutation in *xyr1* on transcriptomic and metabolic level to better understand the reasons behind the increased protein production in both repressing and inducing conditions. *Xyr1* mutant and strain with a wild type *xyr1* were cultivated in 250 ml bioreactors first on batch lactose and then on glucose feed using minimal media and multiple parameters, e.g., produced CO_2, dry weight and protein concentration were measured to compare strain

phenotypes. Changes in gene expression were assessed through mRNA-sequencing, while metabolite level alterations were investigated via GC-MS-based metabolomics, together providing a more comprehensive view of the molecular responses.

In general, we observed more differences between the strains on lactose, where the *xyr1* mutant strain built more biomass and produced more proteins than the wild-type strain and there were also more differentially expressed genes than during glucose feed. The shift from utilizing lactose to using glucose as carbon source was also faster in the mutant strain. Clustering and enrichment analysis showed overrepresentation of mitochondria-related GO terms in clusters where gene expression was higher in the mutant. Metabolomics revealed that the free tyrosine pools were more abundant in the *xyr1* mutant strain in all measured timepoints, whereas multiple fatty acids were less abundant in the mutant strain on glucose. The results contribute to more in-depth knowledge on *T. reesei* physiology and aid in finding new targets for improved protein production.

(1) Derntl C (2013) Biotechnology for Biofuels 6:62

471B **Evolutionary playgrounds and how to find them** Jake Elton[1], Ester Gaya[2], Alexandra Dallaire[2,3] [1]Queen Mary University of London, [2]Royal Botanic Gardens Kew, [3]Dept of Biochemistry, University of Cambridge

Fast-evolving genomic regions of filamentous phytopathogens are often enriched in transposable elements (TEs) and *in planta*-induced genes that mediate infection (Dong *et al*., 2015). In at least five lineages of mutualistic fungi, TEs repeatedly associate with symbiosis-related gene innovations, to which they may contribute new *cis*-regulatory elements or epigenetic regulation (Hess *et al*., 2014; Dallaire *et al*., 2021; Looney *et al*., 2022; Wu *et al*., 2022; Plett *et al*., 2023). Evolutionary and functional compartmentalisation of genes appears not limited to species with pathogenic lifestyles, and the roles of TEs in generating variation in genome architecture and regulation are now under investigation across the Fungal Tree of Life. Association between TEs and gene families may point to ecologically relevant loci that encode the basis for lineage-specific adaptations. Using the Fungal Tree of Life as a model system, I investigate how associations between TEs and specific gene families may support fungal evolution. Here, I will present a statistical method to identify TE-associated gene families in fungal genomes, and will discuss how this information can be used to discover fast-evolving molecular functions and lifestyle-associated genes in fungi.

472B **Transcriptional Profiles during Spore Germination in Opportunistic Human Pathogenic Ascomycetes: Evolved Adaptations Mediated by *abaA*** Da-Woon Kim[1], Malaika K Ebert[2], Zheng Wang[3], Anita Sil[4], Jeffrey P Townsend[3], Frances Trail[1,5] [1]Plant Biology, Michigan State University, [2]Plant Pathology, North Dakota State University, [3]Biostatistics, Yale School of Public Health, [4]Microbiology and Immunology, University of California, [5]Plant, Soil and Microbial Sciences, Michigan State University

Gene expression changes are crucial for evolutionary adaptations, occurring from spore germination to pathogenesis in opportunistic human pathogenic ascomycetes. In our study, we investigated the transcriptional dynamics of 3,845 single-copy orthologous genes (SCOGs) across five phylogenetically diverse Ascomycota species during four stages of spore germination. Utilizing RNA sequencing and continuous ancestral character estimation (CACE) analysis of SCOGs expression patterns, contrasted with their most recent common ancestor (MRCA), revealed genes with significantly evolved expression in opportunistic human pathogens, including *Fusarium oxysporum* NRRL 32931, *Aspergillus fumigatus* Af293, and *Aspergillus nidulans* A4. These pathogenic species exhibited distinctive gene expression profiles related to metabolism, conidia-related regulation, conidial-wall integrity, and signal transduction in KEGG pathway enrichment analysis, setting them apart from the nonpathogenic *Neurospora crassa* and the biocontrol strain *Trichoderma asperelloides*. These profiles demonstrate essential adaptive strategies for survival and virulence in opportunistic pathogenic fungi of humans, including adaptation to low oxygen and iron levels, recognition by host immune receptors, response to human cytokines, and survival in nutrient-poor.

The transcriptional regulator *abaA* plays a crucial role in conidiation, and our investigation into evolutionary changes in gene expression has revealed significant differences between expression in *A. fumigatus* (*AFabaA*) versus *F. oxysporum* (*FOabaA)*, especially during the hyphal branching stage. To confirm this finding, we experimentally exchanged promoters and genes between *AFabaA* and *FOabaA* in *F. oxysporum*. This exchange revealed structural alterations occurring post-phialide, which had an impact on offspring production. Further observation is needed to understand changes occurring in the downstream stages of *abaA*. These findings provide valuable insights into interspecies ecological adaptations and morphological differentiation, particularly underscoring the evolutionary importance of gene expression changes in opportunistic human pathogens.

473B **Comparative transcriptomics of spore germination stages in a plant pathogenic and an endophytic fungus** Soumya Moonjely[1], Frances Trail[1,2] [1]Plant Biology, Michigan State University, [2]Dept of Plant, Soil and Microbial Sciences, Michigan State University

Comparative transcriptomics within closely related organisms provides insights into gene expression divergence associated with developmental divergence. In filamentous fungi, spore germination is the most critical step for initiating fungal colonization. We compared similarities and differences in transcriptome dynamics during stages of spore germination of two fungal species with distinct lifestyles: *Fusarium graminearum*, and *Metarhizium anisopliae*. *F. graminearum* is a plant pathogen and the causal agent of Fusarium head blight in cereal crops, whereas *M. anisopliae* is entomopathogenic and endophytic and can form beneficial associations with plant hosts. We profiled transcriptomes of these two species under two different conditions, in culture and *in planta*, across four stages of spore germination: fresh spores, polar growth, doubling of the long axis, and formation of the first hyphal branch. A substantial difference was shown in the transcriptome of spore germination stages between *F. graminearum* and *M. anisopliae* during initial interactions with barley. In *F. graminearum*, upregulated genes were related to host cell degradation, mycotoxin biosynthesis, and host immune modulation, showing a necrotrophic mode of nutrition even at the early phase of host interaction. Conversely, in *M. anisopliae*, conidial germination and hyphal growth are induced in the presence of the host compared to the medium, and an upregulation of genes associated with phytohormones during interaction with the host indicates an adaptation to its symbiotic/endophyte lifestyle.

474B **Understanding the role of somatic hybridisation in global wheat stem rust epidemics through the development of haplotype-phased reference genome assemblies** Rebecca E Spanner[1], Eva C Henningsen[2], Feng Li[3], Oadi Matny[3], David P Hodson[4], Nino Virzì[5], Kim-Phuong Nguyen[6], Matthew Moscou[6], Zacharias A Pretorius[7], Willem Boshoff[7], Jana Sperschneider[8], Peter Dodds[8], Brian Steffenson[3], Melania Figueroa[8] [1]Plant Pathology, University of Minnesota, [2]The Australian National University, [3]University of Minnesota, [4]International Maize and Wheat Improvement Center (CIMMYT), [5]Centro di Ricerca Cerealicoltura e Colture Industralia (CI), [6]Cereal Disease Laboratory, USDA-ARS, [7]University of the Free State, [8]Commonwealth Scientific and Industrial Research Organisation (CSIRO)

Genomic resources for rust fungi are rapidly growing with the recent development of technologies that allow haplotype-resolved genome assemblies. Phasing of the two haploid genomes from the dikaryotic urediniospores (found on cereal grass hosts) allows for evolutionary studies into the origins of important epidemic-causing rust isolates worldwide. It is now known that nuclear exchange plays an important role in generating novel genetic diversity as an alternative to sexual recombination on the alternate host. The Ug99 strain (TTKSK) of wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* caused devastating epidemics across Africa and the Middle East after emerging in Uganda in 1998 and remains a threat to global wheat production. Previously, a phased reference genome of an isolate belonging to an old South African and Australian lineage, the most prominent stem rust race in Australia (Pgt21-0), showed that it donated a single nucleus via somatic hybridisation to give rise to the Ug99 race group. We have now generated telomere-to-telomere phased genome assemblies for Ug99 (TTKSK) and another member of this lineage, UVPgt55 (TTKSF), based on PacBio HiFi and HiC data. These assemblies provide further support for the role of somatic hybridisation in the emergence of Ug99. To continue developing genomic resources to study the stem rust pathosystem we sourced isolates from geographic regions where other significant outbreaks had occurred. These isolates were race-typed and sequenced using Illumina short reads to investigate their genetic relationship with sequences from other isolates in the public domain. We identified two isolates ETH_2013-1 and ITA_2018-1 belonging to pathotypes TKTTF, which caused an epidemic in Ethiopia in 2013, and TTRTF, which caused an epidemic in Sicily in 2016, respectively. Whole genome sequence comparisons show that the TKTTF race group, previously designated Clade IV, is genetically diverse. The phased reference genome of TKTTF is an isolate from Clade IV-B, which is related to the clade IV-C group by somatic hybridisation since k-mer containment analysis shows that these lineages share one of the two nuclei. However, the Clade IV-A, which contains the original TKTTF "type" strain, is unrelated to the reference genome lineage. These haplotype-phased genomes also permit genotype assignment of these strains at characterized avirulence loci (*AvrSr35*, *AvrSr50*, *AvrSr13*, *AvrSr22*, *AvrSr27*) that interact with known R genes in the wheat host. We have identified novel variants in TKTTF and TTRTF for these genes and will characterize them for function.

475B **Diversity and functional characterization of filamentous fungal sugar transportomes** Miia R. Mäkelä[1], Christina Lyra[1], Victor M. Gonzalez Ramos[1], Aino-Elina Kuusimäki[1], Liinu Nummela[1], Robert Mans[2], Jack Pronk[2], Li Xu[3], Ronald P. de Vries[3], Ferry Hagen[3] [1]University of Helsinki, [2]Delft University of Technology, [3]Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University

Filamentous fungi play a crucial role in the degradation and modification of plant biomass, contributing significantly to terrestrial carbon cycling. Their abilities are also widely exploited in biotechnological applications. One of the key aspects of the fungal plant

biomass conversion process is the uptake of the biomass-derived small sugar compounds into the fungal cells where they are metabolized as carbon and energy sources. Surprisingly, the knowledge of filamentous fungal sugar transporters (STs) is limited, despite of their significant biological role and biotechnological potential as targets of genetic engineering to improve fungal biomass conversion.

To shed more light on filamentous fungal sugar transportomes, we analyzed the genomic and transcriptomic diversity of four ascomycete fungi, i.e., *Aspergillus niger*, *Aspergillus nidulans*, *Penicillium subrubescens* and *Trichoderma reesei*. Phylogenetic analysis divided the predicted STs into ten subfamilies to which putative sugar specificities were assigned based on the available functional data. Interestingly, the STs within each of the subfamilies showed diverse expression profiles on a broad set of monosaccharides even for orthologs of different fungal species. This suggests the existence of a sophisticated regulatory mechanism for sugar uptake in filamentous fungi.

To systematically investigate the overall sugar transport ability of a filamentous fungus, we are characterizing the identified 90 candidate STs of *A. niger* both physiologically and biochemically. Determination of the *in vivo* roles of the transporters is facilitated by *A. niger* ST deletion strains, whereas their *in vitro* functions are studied in *Saccharomyces cerevisiae*. The comprehensive data aims not only to provide information of the role of individual STs in plant biomass conversion by *A. niger*, but also identify novel candidate genes for engineering of industrial fungi at the level of sugar transport. Highlights of these studies will be presented.

476B **Development of a method for QTL mapping in *Saccharomyces* interspecific hybrids** William Yaeger, Artemiza A. Martinez, Gregory I Lang Dept of Biological Sciences, Lehigh University

Hybrids of the yeast *Saccharomyces cerevisiae* and its sister species *S. paradoxus* display a unique range of phenotypes for complex traits such as temperature tolerance. The relative contributions of genes from each parent species to these hybrid phenotypes is not well understood, and the use of genetic mapping approaches such as Quantitative Trait Loci (QTL) mapping to answer this question is impractical due to extremely low (<1%) F1 hybrid spore viability rates that limit statistical power. By suppressing mismatch repair genes during meiosis, we increased spore viability enough to obtain 20 recombinant F1 hybrids, which show substantially increased spore viability when backcrossed to *S. cerevisiae*. Through backcrossing followed by bulk sporulation and mating type-based Fluorescence Activated Cell Sorting, we have generated asexual populations of millions of unique haploid hybrids, large enough for effective QTL mapping. We are now using these mapping populations to determine the genes that contribute to growth rate at various temperatures in hybrids, and this approach can be extended to map other complex traits of interest in the future.

477B **The lifestyle of Mucoromycotina Fine Root Endophytes through the genomic lens** Alan Wanke[1], Alex Williams[2], Victor Rodriguez Morelos[3], Alexandra Dallaire[4], Scarlet Au[1], Silvia Pressel[3], Katie Field[2], Sebastian Schornack[1] [1]Sainsbury Laboratory, University of Cambridge, [2]Plants, Photosynthesis and Soil, University of Sheffield, [3]Natural History Museum, [4]Dept of Biochemistry, University of Cambridge

Fine Root Endophytes belonging to the Mucoromycotina clade (MFRE) play a key role in terrestrial nutrient cycling through their dual saprotrophic and mycorrhizal traits. Over the past decade, microscopic and physiological studies have yielded valuable insights into the mycorrhizal relationship between MFRE and plants, demonstrating intracellular colonisation and nutrient-for-carbon exchange. However, our understanding of the molecular mechanisms underpinning the saprotrophic and mycorrhizal lifestyles of MFRE remains limited. In this study, we apply genomic and molecular genetic approaches to two MFRE isolates to investigate symbiotic and saprotrophic strategies in this mycorrhizal lineage. We generated the first genome assemblies corresponding to MFRE by combining complementary short-read (Illumina) and long-read (Nanopore) sequencing technologies. Leveraging comparative genomic and transcriptomic analyses, we have identified distinct signatures for transporters and secreted proteins (hydrolases, effector candidates) during in vitro and in planta conditions. Collectively, our findings underscore a unique molecular blueprint characterising the saprotrophic and mycorrhizal lifestyles of MFRE, setting them apart from other mycorrhizal fungi.

478B **A telomere-to-telomere *Coprinopsis cinerea* Amut1Bmut1 genome assembly and gene model annotation** Botond Hegedüs[1], Balázs Bálint[1], Zsolt Merényi[1], Viktória Bense[1], Michael Freitag[2], Igor V. Grigoriev[3,4], László G. Nagy[1] [1]Synthetic and Systems Biology Unit, Institute of Biochemistry, HUN-REN Biological Research Center, [2]Dept of Biochemistry and Biophysics, Oregon State University, [3]U.S. Dept of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, [4]Dept of Plant and Microbial Biology, University of California Berkeley

The number of sequenced genomes has dramatically increased in the last decade. In the Agaricomycetes (mushroom forming fungi), which contains important degraders of plant lignocellulosic biomass and strains that are highly valuable to the food industry,

more than 500 genomes are currently available in various repositories, such as MycoCosm. However, most of these are draft genomes with various levels of fragmentation and incorrect gene models, which greatly affects their usability. Here we present a chromosomal level genome assembly and manually curated protein coding gene model prediction of the *Coprinopsis cinerea* Amut1Bmut1 strain, which has become one of the most important model species for the study of fungal multicellularity. By using HiFi PacBio reads, it was possible to assemble the genome of the *C. cinerea* Amut1Bmut1 strain at the chromosome level, the quality of which far surpasses previous Illumina- and ONT-based assemblies. For gene model prediction, we used NanoPore and PacBio IsoSeq cDNA-seq datasets obtained from libraries enriched for full-length transcripts which we combined with information from the two available annotations of *C. cinerea* Amut1Bmut1 #326 genomes and manually corrected at multiple levels, including misidentified splice sites, transcript flanking regions and CDSs. For further validation of the gene models the transcription termination sites (TTS) were checked with Quantseq reads. Our study provides a high-quality genome assembly with reliable gene model prediction based on longread transcripts covering the splice sites across the entire gene model. A large proportion of the genes have UTR annotation, that is frequently missing from published fungal draft genomes. In addition, several internal microexons were identified, which are mostly ignored by current methods, yet have considerable importance for the definition of CDSs. Furthermore, conserved genomic elements surrounding the typical fungal gene model could be identified like the TATA-box, Inr, Kozak motif, splice site motifs, or polyadenylation sites. This study makes *C. cinerea* Amut1Bmut1 possibly the best-annotated Agaricomycetes model species, which will contribute to multiple rapidly expanding fields, including research on lignocellulose degradation and multicellular development.

479C **Functional *in vitro* and physiological *in vivo* characterization of five new xylose transporters of *Aspergillus niger*** Christina Lyra[1], Aino-Elina Kuusimäki[1], Liinu Nummela[1], Robert Mans[2], Jack Pronk[2], Miia Mäkelä[1] [1]University of Helsinki, [2]Delft University of Technology

Plant biomass degrading fungi are important organisms for bio-based economy. Fungi can convert a pentose sugar D-xylose, abundantly present in plant biomass, through the pentose catabolic pathway (PCP). Recently, an extensive *in silico* identification showed that *Aspergillus niger* has a wide array of putative xylose transporters[1] of which only two, XltA and XltB, have been functionally characterized in *Saccharomyces cerevisiae*[2].

To characterize the *in vitro* functional properties of five of the *in silico* identified *A. niger* xylose transporter candidates, we heterologously expressed them in the yeast *S. cerevisiae* devoid of all hexose and disaccharide transporters, and disaccharide hydrolases[3]. *A. niger* XltA was included as a control. The growth of the recombinant yeast strains was analysed on different sugars both by spot assays on agar medium and in liquid cultures. All five transporters and XltA were able to uptake xylose when endogenous xylose pathway in *S. cerevisiae* was induced with a low glucose concentration. Two out of the five new transporter candidates were also able to uptake hexoses.

In addition, we characterized the xylose transporters *in vitro* in *A. niger* using deletion mutant strains generated by CRISPR/Cas9 method. The deletion strains were analyzed for their ability to uptake xylose through growth and sugar consumption experiments. To determine whether the xylose transporter deletions induce the expression of additional putative xylose transporter genes in *A. niger*, the deletion mutants were also assayed with qPCR. Highlights of these experiments will be presented. Our ultimate aim is to connect the xylose uptake from the exogeneous environment to endogenous processes, to provide a more comprehensive view of plant biomass conversion by *A. niger* and thus advance the transition from our current fossil-based economy to a bio-based economy.

[1]Xu *et al. Bioresource Technology* 391: 130006 (2024)

[2] Sloothaak *et al. Biotechnology for Biofuels and Bioproducts* 9: 148 (2016)

[3]de Valk SC *et al. Biotechnoogy for Biofuels and Bioproducts* 15: 47 (2022)

480C **Regulatory rewiring of mating and environmental responses underlying homothallism in filamentous fungi is revealed with a systems maximally informative laboratory experiment (SMILE)** Zheng Wang[1], Wonyong Kim[2], Dawoon Kim[3], Oded Yarden[4], Frances Trail[3], Jeffrey P. Townsend[5] [1]Yale School of Public Health, [2]Sunchon National University, [3]Michigan State University, [4]The Hebrew University of Jerusalem, [5]Biostatistics, Yale School of Public Health

Diverse genetic mechanisms have been discovered that result in self-fertility in fungi, many by alteration of the roles of mating loci. However, little is known about the fungal genetics networks that promote divergence in fertilization lifestyle. Furthermore, it

is challenging to study such a complex regulatory developmental apparatus using traditional gene-by-gene molecular genetics. To address this challenge, we developed a Systems Maximally Informative Laboratory Experiment (SMILE) criterion to help identify key regulatory genes in sexual development with reference species exhibiting separately homothallic (*Fusarium graminearum*) and heterothallic (*Neurospora crassa*) lifestyles. We identified informative gene knockouts to further dissect the molecular genetics of initiation of the fungal sexual reproduction. Phenotypes of identified knockouts suggested that evolution of the two lifestyles included regulatory rewiring of the developmental roles of *fmf-1* ortholog and *mating loci* between the two lifestyles. Namely, *fmf-1* plays upstream roles and regulates mating loci expression in heterothallic *N. crassa*, while *mating loci* play upstream roles and regulate *fmf-1* ortholog in homothallic *F. graminearum*. To further validate and explore the rewiring of the roles of genes in sexual development between the two lifestyles we performed a proteomic analysis of wild-type and knockouts in the two representative fungal models. Based on comparative genomics and transcriptomics during sexual development among additional species, divergence in the coordination of mating and meiotic regulation of and by *fmf-1* orthologs could play a key role in promoting homothallic lifestyle among Sordariomycetes. Highly harmonious regulation of mating loci coding for MAT 1-1-1 and MAT 1-2-1 between opposite mating types were observed for homothallic lifestyle. In the heterothallic species, expression of the mating locus from the spermatia donor was upregulated after crossing, whereas the mating locus of the maternal strain generally maintained a low and consistent expression. Coordinated elevation of the pheromone MAPK signaling pathway manifested for the homothallic species during the meiotic sporulation process, indicating regulatory and developmental activities of mating and meiotic sporulation machinery are highly coupled. In conclusion, our study employing the Systems Maximally Informative Laboratory Experiment (SMILE) criterion, Bayesian networks, and comprehensive proteomic and transcriptomic analyses sheds light on the intricate regulatory rewiring of genes, such as *fmf-1* and mating loci, governing sexual development in homothallic and heterothallic fungi. The observed divergence in the coordination of mating and meiotic regulation, particularly involving *fmf-1* orthologs, suggests a pivotal role in the evolution of the homothallic lifestyle among.

481C **Investigating the novel role of post-translational modifications of Rra1 in *Cryptococcus neoformans*** Siobhan Duffy[1], Hannah Harding[2], Connie Nichols[3], Andrew Alspaugh[4] [1]Molecular Genetics and Microbiology, Duke University, [2]Harvard Medical School, [3]Duke Univeristy, [4]Duke University

Cryptococcus neoformans (Cn) is an opportunistic basidiomycete yeast found in the environment, which causes severe fungal disease in immunocompromised individuals. To thrive and proliferate, *Cn* must sense and adapt to different biotic and abiotic stresses. Among these stresses is the change from a more acidic pH found in environmental growth niches to a more neutral to alkaline pH often encountered within the human host. To survive in a human host, *Cn* must be able to sense and adapt to pH changes as it disseminates from the primary site of infection in the lungs. The Rim signal transduction pathway is required for growth during alkaline pH stress. Upon shift to alkaline pH, the Rra1 plasma membrane sensor undergoes a conformational change in the C-terminal tail which leads to the endocytosis of Rra1 as well as the downstream activation of the pH-responsive transcription factor Rim101. Previous work demonstrates that pH-dependent phosphorylation occurs at the C-terminal tail and through targeted mutagenesis we have identified a phosphorylation site, T317, as a potential regulator of Rra1 localization and function. My studies aim to investigate how phosphorylation of Rra1 contributes to its localization and function. I will be examining potential regulators of the T317 site and how phosphorylation of this site impacts Rra1 plasma membrane localization and endocytosis. This work will contribute to understanding how Rra1 function and localization is regulated and will help unravel how Rra1 interacts with downstream components of the Rim101 pathway. This will ultimately lead to a better understanding of how *Cn* senses and adapts to the host pH environment.

482C **The Rsp5 ubiquitin ligase contributes to stress response and pathogenesis in the fungal pathogen *Cryptococcus neoformans*** Marnus du Plooy, Calla L Telzrow, Andrew Alspaugh Dept of Medicine, Dept of Molecular Genetics and Microbiology, Duke University

Cellular response to external stress allows microbial cells to grow in a vast array of different environmental conditions, including to colonize another organism and potentially cause infections. The molecular mechanism of stress responses is frequently studied to gain insight into microbial pathogenesis and to identify potential new targets for anti-microbial therapy. Here we explore a novel role for arrestin-mediated ubiquitination in stress response and pathogenesis in the pathogenic fungus *Cryptococcus neoformans*. In a previous study, we identified four arrestin-like proteins in *C. neoformans* and found a role for one of these proteins in fatty acid synthesis during cell division. We subsequently show that a second arrestin-protein serves as an adaptor to facilitate the binding of the Rsp5 E3 ubiquitin ligase to target proteins for ubiquitination. We found that Rsp5 is required for *C. neoformans* pathogenesis and survival in the presence of cell surface stressors. A differential ubiquitination screen revealed that several known proteins involved in cell wall synthesis and nutrient import are ubiquitinated by Rsp5, altering the function, stability, and the localization of these proteins. These findings support a model in which arrestin-like proteins guide Rsp5 to ubiquitinate specific target proteins in a rapid response to environmental changes. A loss of Rsp5 and arrestin-mediated ubiquitination result

in membrane and cell wall defects that increase susceptibility to external stresses. Furthermore, this work demonstrates the importance of ubiquitination in pathogenesis and adds to the growing literature on ubiquitination as a versatile regulator of protein function.

483C **Rapid pooled CRISPR/Cas9-directed insertional mutagenesis screens in *Cryptococcus neoformans* illuminate the biology of a deadly human fungal pathogen** Manning Y Huang, Michael J Boucher, Angela L Wei, Hiten D. Madhani Biochemistry and Biophysics, University of California, San Francisco

Cryptococcus neoformans sits atop the WHO fungal priority pathogens list, causing over 100,000 deaths due to meningitis annually. Still, efficient, facile methods for global mutagenesis and gene overexpression have yet to be developed. We now describe the development and optimization of powerful new genome-wide approaches for gene disruption and overexpression. These methods rely on the programmed insertion of a marker-containing cassette at double-strand breaks produced by Cas9 loaded with a sgRNA. Insertion occurs through non-homologous end-joining (NHEJ), requiring the NHEJ components Ku70 and Ku80. We used junction sequencing to analyze the genomic insertion site produced by each guide of a ~70,000 sgRNA library targeting all annotated genes with 10 guides/gene. This analysis identified ~1100 likely essential genes, namely those genes that are intolerant to insertion. We have also developed degron methods to validate these results. We have generated a highly optimized, second-generation compact sgRNA library carrying ~21,000 guides targeting ~5500 genes that permit extremely rapid (~1 week) genetic screens. To enable genome-wide overexpression, we have generated transformant pools using a library carrying ~35,000 guides that insert outwardly pointing strong promoters upstream of all coding sequences. We have demonstrated that sequencing of sgRNA abundance allows sensitive and reproducible measurement of mutant fitness in pooled assay conditions. We will describe the use of global gene insertions to identify the global genetic interactions (suppressing or exacerbating) of knockouts of genes involved in chromatin biology (Polycomb, HP1, and DNA methylation) and fitness in the mammalian host, as well as the use of our overexpression to illuminate potential targets of antifungal compounds. We anticipate that these rapid genome-wide genetic tools will accelerate progress in our understanding of this deadly human fungal pathogen

484C **Transposable element (TE)-driven genome expansion in giant Entomophthoraceae genomes** Xueyan Xu[1], Carolyn Elya[2], Emily Lee[2], Brian Lovett[3], Ann E Hajek[4], Matt T Kasson[5], Andrii Gryganskyi[6], Jason E Stajich[1] [1]University of California-Riverside, [2]Harvard University, [3]USDA, [4]Cornell University, [5]West Virginia University, [6]UES, Inc.

Fungal species in the family Entomophthoraceae (phylum Zoopagomycota) are primarily insect-obligate pathogens. These zygomycete fungi are largely understudied due to their complex, obligate lifecycle. Recently sequenced genomes of these fungi revealed they have huge genomes ranging from 650-Megabases to 1.5-Gigabases. The genomes of these fungi, including Massospora cicadina, Entomophaga maimaiga, Entomophthora muscae, and Zoophthora radicans are replete with transposable elements with 70~90% occupied by repeat sequences. TE-driven genome expansion and TE silencing can be examined through the lens of an arms race between the TE and its host. In this study, we used the Extensive de novo TE Annotator (EDTA) to discover and annotate TEs in these genomes to study the history of their genome expansion. We found TEs vastly expanded these genomes without significant increase in gene copy numbers compared to their close relatives. 50~60% of all four genomes are occupied by long-terminal repeat (LTR) Class I retroelements, but diverse classes of DNA transposons that were not found in their close relatives are also abundant in these genomes. LTR/Ty3 elements have been expanding these genomes constantly since ancient times, but each genome had its unique DNA transposon expansion at different time points. Family-level divergence and transcriptome analysis suggest these genomes at least possess thousands of recent and still active elements. Furthermore,a majority of the biggest TE families by size in these genomes are LTR/Gypsy, and subclasses of these have become some of the biggest families in a single fungal lineage, indicating these families were smaller in the last common ancestor and have expanded in copy number in a species-specific manner.

485C **Developmental and metabolic gene regulatory network rewiring of a GATA-type multifunctional regulator in two distantly related *Aspergillus* species** Heungyun Moon[1], Mi-Kyung Lee[2], Junha Shin[3], Sung Chul Park[3], Julio C Rivera Vazquez[3], Daniel Amador-Noguez[3], Nancy Keller[3], Kap-Hoon Han[4], Jae-Hyuk Yu[3] [1]Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, [2]Korea Research Institute of Bioscience and Biotechnology, [3]University of Wisconsin-Madison, [4]Woosuk University

The GATA-type transcription factor NsdD regulates sexual/asexual development as well as primary/secondary metabolism in the genus *Aspergillus*. While NsdD's diverse functions have been extensively studied, the genome-wide gene regulatory network (GRN) underlying developmental and metabolic regulations has yet to be explored. Here, we unravel the NsdD-mediated GRNs by performing a network-based multi-omics study in two distantly related fungal species *Aspergillus nidulans* and *Aspergillus flavus*. Within these networks, NsdD directly or indirectly regulates the expression of various genes associated with development, metabolism, signal transduction, and transcriptional regulation. We identified 502 and 674 potential NsdD direct target genes,

respectively, including well-known regulators of development and metabolismin *A, nidulans* (*veA*, *flbD*, *laeA*, *kapA*, *rosA*, and *steA*) and in *A. flavus* (*veA*, *flbA·C·D*, *vosA*, *brlA*, and *rosA*). By analyzing DNA-binding sites, we revealed that 5'-GATCT-3' is the predicted consensus NsdD response element (NRE) in the two species. Of note, we proposed the central regulatory mechanisms of NsdD-mediated GRNs by elucidating components of their core sections. Furthermore, we provided evidence of GRN rewiring in two fungal species by comparing their NsdD-mediated GRNs. In conclusion, NsdD governs fungal development and metabolism via a species-specific NsdD-mediated GRN. Within the network, NsdD directly regulates not only crucial upstream developmental regulators, but also key genes associated with development and metabolism, which subsequently impacts the expression of downstream genes, resulting in distinct cellular and metabolic traits in the two distantly related *Aspergillus* species.

486C **Investigation of Potential CFEM Proteins that Contribute to Host Recognition in *Fusarium oxysporum* 47** Gengtan Li[1], Houlin Yu[2], Domingo Martinez Soto[3], Ryan Lai[4], Li-Jun Ma[1] [1]Molecular and Cellular Biology, University of Massachusetts Amherst, [2]Broad Institute of MIT and Harvard, [3]Experimental microbiology, Center for Scientific Research and Higher Education of Ensenada, [4]Biochemistry and Molecular Biology, University of Massachusetts Amherst

The species complex *Fusarium oxysporum* (FOSC) contains over 100 host-specific pathogens that colonize the host vascular xylem, cause severe wilting diseases, and lead to enormous agricultural losses annually. Interestingly, FOSC also contains endophytic strains that offer protective advantages to host plants. The different mode of actions between endophytic and pathogenic FOSC strains remain elusive. Similar to other filamentous fungi, *F. oxysporum* strains utilize small secreted fungal effector proteins to interact with their hosts. In this presentation, we study an effector protein family encoding the Common in Fungal Extracellular Membrane (CFEM) domain, using 15 FOSC genomes that include both endophytic or pathogenic strains, 9 genomes of other *Fusaria* species, 4 genomes of non-*Fusarium* filamentous fungi, and 2 yeast genomes. Among these 30 fungal genomes, 263 effector-like CFEM proteins—clustered into 17 unique groups—were identified, ranging from 2 to 29 per genome. Interestingly, the genome of the endophytic FOSC strain Fo47 contains 10 which is the average number of CFEM-domain containing candidate effectors among FOSC. RNA-seq experiments supported that 7 of the Fo47 CFEM-containing proteins were induced when interacting with both *A. thaliana* and tomato. For a functional characterization, we transiently expressed an upregulated Fo47 CFEM protein using *Nicotiana tabacum* heterologous expression and observed the accumulation of reactive oxygen species and induction of cell death. The CFEM-domain containing proteins were reported to be involved in fungal pathogenicity. This is the first study documenting the contribution of this group protein in endophytic interactions. Its potential mechanism will be discussed.

487C**Differential codon usage patterns in endophytic and non-endophytic xylarialean fungi** Roxanne Bantay[1], Mario E. E. Franco[2], Jana M. U'Ren[3] [1]Biosystems Engineering, University of Arizona, [2]Sustainable Plant Protection Program, Institute of Agrifood Research and Technology (IRTA), [3]Plant Pathology, Washington State University

Xylariales (Sordariomycetes) is one of the largest and most ecologically diverse Pezizomycotina orders. Xylarialean fungi commonly occur as saprotrophs, pathogens, and endophytes in tropical, temperate, and boreal forests. Previous comparative analyses of >90 xylarialean genomes revealed no clear differences in gene content associated with different ecological modes, although pairwise comparisons of closely related foliar endophytes and non-endophytes revealed that symbiont genomes contain fewer genes involved in secondary metabolism and plant cell wall degradation, yet differences were not consistent across all clades. Here, we examined whether genomes of endophytic and non-endophytic taxa differ in their preferential use of specific codon synonyms (i.e., codon usage bias) and the degree of codon optimization for the genomic tRNA complement. Codon optimization can increase gene translational speed and efficiency, generating phenotypic differences and fitness outcomes. Optimization has been implicated in differences in ecological niches for bacteria and archaea, as well as yeasts. To assess the relationship of codon optimization to ecological modes in xylarialean fungi, we analyzed gene-level codon usage optimization in genomes of 30 species that represent pairs of endophytic and non-endophytic sister taxa across two major clades of Xylariales: Hypoxylaceae and Xylariaceae. Using the species-specific tRNA adaptation index (stAI)—a metric of translational efficiency that accounts for both genomic tRNA availability and species-specific efficiencies of codon-anticodon pairings—we compared optimization for single-copy orthologs shared by each pair of sister taxa using paired t-tests. We observed significant differences in mean stAI values between fungi with different ecological modes for 12 of 15 pairs (3/5 Hypoxylaceae and 9/10 Xylariaceae pairs). Among pairs with significant differences, non-endophytic isolates had higher mean stAI values in 2 of 3 Hypoxylaceae and 5 of 9 Xylariaceae pairs. Our ongoing analyses will examine the relationship between (i) specific functional categories of genes and ecological mode-specific differences in stAI and (ii) stAI variance in shared multi-copy gene families. Overall, our results suggest that closely related xylarialean fungi with different ecological modes vary in their levels of codon optimization, which may contri bute to ecological differences between endophytes and non-endophytes despite similar genomic repertoires.

488C **Using CAZyme secretome relatedness for elucidating *Fusarium* evolution and speciation** Lene Lange[1], Kristian Barrett[2], Anne S Meyer[2], Jens C Frisvad[2] [1]LL-BioEconomy, [2]DTU BioEngineering, Technical University of Denmark

The *Fusarium sensu lato* concept has recently been challenged. In order to contribute to elucidating evolution and speciation within *Fusarium s.l.*, we here take a new approach: Comparing *Fusarium* Enzyme Profile Relatedness to *Fusarium* organismal phylogeny. The phylogenetic tree of *Fusarium s.l.* used here, is based on concatenation of 227 single copy orthologs, each presenting 99.8% of the genome assemblies, resulting in a 82.480 aa multiple alignment as basis for construction of a tree by fasttree, displayed using itol. The CAZyme-EPR tree is defined by the relatedness in composition of the CAZyme secretome of the *Fusarium* species analysed. Constructing the CAZyme-EPR tree: 1. Peptide-based functional annotation, by CUPP analysis, giving a robust prediction of function directly from sequence. 2. Comparative analysis of secretome composition across all species, enabled by Enzyme Profile Relatedness analysis; EPR annotates CAZyme secretome to integrated "Function;Family" observations. 3. Finally, the dendrogram is calculated, using Yule similarity, based on whether or not individual "function;family"observations were identified or absent in the genome assemblies. *Notably*, EPR analysis has previously been used to test the hypothesis, that the digestive CAZyme secretome is an integrated part of fungal evolutionary speciation (case: *Aspergillus* and *Penicillium).* For these two genera, comparing CAZyme-EPR tree to the phylo-tree resulted in a stunning congruence between the organismal phylogenetic tree and the EPR-based tree. Results for *Fusarium s.l.*: The *Fusarium* Enzyme profile relatedness clustering gave a stunning congruence to the *Fusarium s.l.* organismal phylogeny calculated for this study! In both EPR-dendrogram and Phylo-tree all the *Fusarium s.c.* species complexes cluster together, e.g., *F. oxysporum s.c, F. fujikoroi s.c. & F. sambucinum s.c.;* and for *Neocosmospora,* all the many species analyzed cluster together. Similarly, for *Albonectria, Bisifusarium, Rectofusarium, Luteonectria,* and *Setofusarium;* however, this result is less robust, due to few genomes analyzed. Interestingly, a discrepancy was found for *Geejayessia, Nothofusarium and Cyanonectria*; in the phylogenetic tree these genera are grouped together with the rest of *Fusarium,* while the CAZyme-EPR tree places these genera (plus a number of *Fusarium* species) in a sister clade to *Neocosmospora;* possibly suggesting an evolutionary drift away from *Fusarium s.s.*

489C **Functional redundancy among members of the *TLO* expanded gene family in *Candida albicans*** Emily Simonton[1], Nayeli Cangelosi[2], Matt Anderson[2,3] [1]Microbiology Doctoral Training Program, University of Wisconsin-Madison, [2]Medical Genetics, University of Wisconsin-Madison, [3]Center for Genomic Science Innovation, University of Wisconsin-Madison

Gene duplicates with redundant functions are often culled from the population, leaving paralogs that improve fitness by increasing expression, retaining a specialized function, or acquiring a new function to expand in a population. Repeated duplication can lead to the formation of paralogous gene families that contain many closely related sequences. The functional space occupied by large gene families may ultimately restrict emergence of new functions and lead to overlapping functions of individual family members, yet relatively little systematic investigation of individual paralog function has occurred for large gene families. Here, we constructed a panel of single deletion mutants for the *Candida albicans* telomere-associated (*TLO*) gene family to test for mutant phenotypes caused by loss of just one of the 14 paralogs. Tlo proteins function as interchangeable subunits of the major transcriptional regulatory complex Mediator and therefore have the potential to alter a wide range of phenotypes, including those linked to the balance between commensalism and pathogenesis in this important constituent of the human microbiome. Analysis suggests both redundant and non-redundant functions exist among *TLO* paralogs. Single *TLO* mutants produced altered phenotypes under some conditions, such as liquid filamentation at 37°C, carrying capacity in standard YPD medium, and flocculation over time, whereas other assays of single *TLO* mutants were indistinguishable from the wildtype, such as growth rate in nutrient rich medium and fluconazole resistance and tolerance. In addition, construction of a double mutant lacking two *TLO* genes that both contribute individually to the same phenotype was identical to either mutant alone, suggesting that these genes exist within the same pathway or network. Understanding the range of unique and redundant functions for individual *TLO* paralogs will resolve the potential for functional innovation in lineage-specific gene expansions and the importance of *TLO*s in *C. albicans* adaptation.

490C **Comparative genomics of *Basidiobolus* isolated from the herptile gut microbiome** Lluvia B Vargas[1], Daniel Farthing[1], Connor Dooley[1], Andrii P. Gryganskyi[2], Stephen Mondo[3], Igor V. Grigoriev[3], Kerry L. McPhail[4], Donald M. Walker[5], Jason E. Stajich[6], Joseph W. Spatafora[7] [1]Dept of Botany and Plant Pathology, Oregon State University, [2]Division of Biological & Nanoscale Technologies, UES, Inc., [3]Dept of Energy (DOE), Joint Genome Institute (JGI), Lawrence Berkeley National Lab, [4]Dept of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, [5]Dept of Biology, Middle Tennessee State University, [6]Dept of Plant Pathology & Microbiology, University of California, Riverside, [7]Botany and Plant Pathology, Oregon State University

Basidiobolus is a zygomycete fungus that possesses a complex life cycle. It produces multiple spore types adapted to different ecological niches. This fungus is best known for being associated with the gut microbiome of herptile (amphibians and reptiles) species. Two *Basidiobolus* species have been sequenced as part of the 1000 Fungal Genomes project revealing the genus is

characterized by relatively large genomes of ~100 MB with approximately 50% repetitive DNA, and an unusual genome architecture compared to most eukaryotes (e.g. hundreds of chromosomes). Compared to other zygomycete fungi of Mucoromycota and Zoopagomycota, *Basidiobolus* genomes also possess a higher count of genes functioning in secondary or specialized metabolism. Many of these genes are hypothesized to be acquired by horizontal gene transfer (HGT) from bacteria, a finding that supports *Basidiobolus* as an example of animal-associated gut fungi adapting to their environment through HGT. We will present results of the analysis of six new *Basidiobolus* isolates, obtained from different herptile species, focusing on genome organization, comparative genomics of *Basidiobolus* species, the genomic diversity of secondary metabolite gene clusters, and the impact of HGT from co-occurring gut bacteria.

491C ***Macrophomina phaseolina*** **clonal and recombinant genotypes specialized for virulence against strawberry and soybean hosts** Kayla K Pennerman[1], Polly Goldman[1], Christine J Dilla-Ermita[1,2], Gerardo Ramos[1], José H Jaime[1], Javier Lopez[1], Jacqueline Ramos[1], Manuel Aviles[3], Celia Borrero[3], Apollo Gomez[4], Jodi Neal[4], Martin Chilvers[5], Viviana Ortizlondono[5], Eva H Stukenbrock[6,7], Danilo Pereira[6,7], Gustavo H Goldman[8], Alemu Mengitsu[1], Steven Wallace[1], Jason Deffenbaugh[1], Zach Weatherford[1], J Philipp Benz[9], Alexandre R Machado[10], Teresa Seijo[11], Natalia A Peres[11], Jenny Broome[12], Kelly Ivors[12], Glenn Cole[2], Steven Knapp[2], Dylan McFarlane[13,14], Scott Mattner[13], Marina Gambardella[15], Peter M Henry[1] [1]USDA-ARS, [2]University of California, Davis, [3]Universidad de Sevilla, [4]Queensland Dept of Agriculture and Fisheries, [5]Michigan State University, [6]Christian-Albrechts University of Kiel, [7]Max Planck Institute for Evolutionary Biology, [8]University of São Paulo, [9]Technical University of Munich, [10]Universidade Federal de Pernambuco, [11]University of Florida - Gulf Coast Research and Extension Center, [12]Driscoll's, [13]VSICA Research, [14]La Trope University, [15]Pontificia Universidad Católica de Chile

Macrophomina phaseolina has a broad host range, but individual isolates may be pathogenic to a limited number of hosts as previous work has shown. However, a comprehensive understanding of host susceptibility by pathogen genotype interactions is lacking. There is also incomplete knowledge of how *Macrophomina* spp. genetic diversity is generated and maintained. Information about host specificity and the potential for recombination would be instrumental to future control efforts of the fungus. A survey of *Macrophomina* spp. was conducted to identify host x pathogen genotype associations, pangenomic structure and mechanisms of genetic exchange. Short-read sequence data were obtained for 427 *Macrophomina* spp. isolates collected from 92 host plant species and soil in 23 countries. High-quality SNPs identified with three genomic references grouped *M*. *phaseolina* into eight lineage clusters, with high admixture in some isolates and equal mating type ratios in some clusters suggesting on-going meiotic recombination. Three of the identified clusters were associated with the isolates' host of origin; 82% of strawberry-derived isolates were in a single clonal lineage, whereas 89% of soybean isolates resolved into two admixed lineages. This pattern suggested that host specialization may have occurred among isolates in specific clusters. Pathogenicity tests of select isolates from each cluster showed only isolates from the strawberry-associated cluster were highly aggressive against the host. Isolates from other clusters did not yield disease symptoms. However, we did not find genes or genomic regions that were exclusive and universal to strawberry cluster isolates, suggesting that strawberry specialization within *M*. *phaseolina* is governed by polygenes, gene regulation and/or epigenetic effects. The SNP and gene sequence data grouped three *Macrophomina* spp. into two clusters that were distinct from *M*. *phaseolina.* Two of these species, *M*. *euphorbiicola* and *M*. *pseudophaseolina,* had fewer SNP and genetic differences between them than did clusters of *M*. *phaseolina* isolates. This suggests that *M*. *euphorbiicola* and *M*. *pseudophaseolina* should be considered a single species. Our work provides insight into host specialization and evolutionary mechanisms within this economically-important phytopathogenic genus.

492C **Genome assemblies of microbes isolated from soil post wildfire as foundation for decoding pyrophilic traits** Ehsan Sari, Dylan J. Enright, Maria Ordenez, Sydney I. Glassman Microbiology and Plant Pathology, University of California Riverside

Wildfires are increasing in frequency, size, and severity across the globe. Post-fire environments favor pyrophilous "fire-loving" microbes whose traits remain uncharacterized along with their associated ecosystem impacts. Comparative genomics is an unprecedented opportunity to decode traits governing the function and abundance of pyrophilous microbes. In this study, we assembled the genomes of 16 bacteria (5 phyla representing 5 orders) and 14 fungi (2 phyla representing 8 orders) from our culture collection of pyrophilous microbes that were isolated from burned soils using PacBio HiFi sequencing. All bacterial assemblies led to a single contig while genome contiguity varied among the fungal genome, with the lowest number of contigs for *Penicillium restrictum* (# contigs = 8, contig N 50 = 3.9 Mb) and highest for *Pyronema omphaloides* (# contigs = 46, contig N 50 = 2.1 Mb). We investigated the genomes for traits associated with post-fire survival, including thermotolerance, fast growth, and post-fire resource acquisition while simultaneously testing these traits with in-situ biophysical assays. The genomes showed significant variation in the presence/absence and copy number variation of genes associated with the degradation of aromatic carbon and nitrogen cycling. The Pezizales genomes were distinct in having rare aromatic carbon degradation genes, while Eurotiales genomes were enriched in heat and extreme pH tolerance genes. Basidiomycetes had relatively low numbers of stress tolerance genes, but higher capacity for complex hydrocarbon degradation. Actinomycete bacteria had lower number of complex hydrocarbon degrading genes than Bacillota and Proteobacteria, but had unique extreme pH, osmotic and oxidative stress tolerance genes. This

study highlights the possession of different traits of pyrophilous microbes, which is in line with patterns of post-fire microbial succession for both bacteria and fungi. The candidate pyrophilous genes found here paves the way for decoding the genetics of pyrophilous traits and their impact on post wild-fire ecosystem.

493C **Ectomycorrhizal *Suillus* fungi represent hot-spots of metabolic diversity, structured by gene presence/absence variation and significant horizontal gene transfer** Lotus Lofgren[1], Steven Ahrendt[2], Sameer Mudbhari[3,4], Paul Abraham[4], Sara Branco[5], Hui-Ling Liao[6], Nhu Nguyen[7], Peter Kennedy[8], Kerrie Barry[2], Alan Kuo[2], Igor Grigoriev[2], Rytas Vilgalys[1] [1]Biology, Duke University, [2]Joint Genome Institute, [3]University of Tennessee, [4]Oak Ridge National Lab, [5]University of Colorado Denver, [6]Soil and Water Science, University of Florida, [7]University of Hawai''i at Manoa, [8]University of Minnesota

The ectomycorrhizal genus *Suillus* is speciose and widespread. *Suillus* fungi display a gradient of partner specificity responses, possess unique traits with high ecological relevance, and are tractable to laboratory manipulation, making the genus an ideal model for studying ectomycorrhizal ecology and evolution. Leveraging 46 whole-genome sequencing projects, considerably more than exist for any other ectomycorrhizal fungal taxa, we conduct a comprehensive analysis of *Suillus* fungi using a combination of phylogenetics, pan-genus comparative genomics, gene ancestry analysis, and machine learning to identify genomic elements associated with important ecological traits. We present evidence for significant gene presence/absence variation across the genus, horizontal gene transfer, and mitogenome diversity. With a particular focus on primary and secondary metabolic capacity, we follow up this in-silico analysis with LC/MS based metabolomics with the goal of linking genome-based predictions to realized metabolic diversity.

494C **Investigating the impact of transposable elements on genome evolution during human infection in *Cryptococcus neoformans*** Anna Mackey, Vesper Fraunfelter, Callan Schroeder, John Perfect, Sue Jinks-Robertson, Asiya Gusa Duke University

Transposable elements (TEs) are mobile genetic elements that have been shown to impact transcriptional networks, virulence traits, genome architecture, and evolution across eukaryotes. Prior work has shown that various stressors, including growth at mammalian body temperature, can increase rates of TE mobilization in yeast (Gusa et al, *PNAS*, 2020, Esnault et al, *Genome Res*., 2019). Using a *Cryptococcus deneoformans* murine model of infection, our group showed that multiple TEs mobilized in isolates recovered from the mouse (Gusa et al, *PNAS*, 2023). We currently do not understand how TEs shape genome evolution during human infection in fungal pathogens. To address this question, we will use clinical isolates of the opportunistic human fungal pathogen *Cryptococcus neoformans*. Cryptococcosis is acquired through inhalation of spores, which establish infection in the lung and by escaping containment by the immune system can disseminate, passing the blood brain barrier and causing deadly cryptococcal meningitis (CM). *C. neoformans* primarily causes disease in immunocompromised individuals, accounting for 15% of annual AIDS-related deaths. Despite receiving antifungal drug treatment, approximately 10% of patients diagnosed with CM suffer from recurrent disease.

The *C. neoformans* genome is known to harbor diverse TEs including both class I retrotransposons and class II DNA transposons capable of mobilization. Using a 'transposon trap assay' we screened serial clinical isolates from patients with recurrent CM collected at the time of diagnosis (incident) and after disease relapse (relapse) for the presence of mobile TEs, leading to the identification of characterized and uncharacterized TEs (Chen et al, *mBio*, 2017). Using long read sequencing we are generating high quality genome assemblies for a subset of clinical isolates, which span the three of the four major *C. neoformans* sub-lineages (VNI, VNII, and VNBII). Using TE annotation tools, we will investigate TE diversity and identify TE-dependent genomic changes that occurred during persistent infection. This work will explore understudied aspects of *C. neoformans* genome diversity and mechanisms for genome evolution during recurrent human infection. Future work will focus on characterizing and elucidating the mechanism of stress-induced TE mobility in *C. neoformans* and whether mobile TEs can provide a mechanism for adaptive evolution in stressful environments.

495C **Loss of RNA interference in *Cryptococcus neoformans* clinical and environmental isolates: a pathway to hypermutation** Jun Huang[1], Shelby J Priest[2], Fred Dietrich[2], Paul Magwene[2], Vikas Yadav[2], Connor Larmore[2], Sheng Sun[2], Joseph Heitman[2] [1]MGM, Duke University, [2]Duke University

To survive and proliferate in changing environments, microbes have evolved multiple mechanisms for rapid adaptation. While an increased mutation rate typically has negative consequences in multicellular organisms, hypermutation can be advantageous for microbes subjected to significant selective pressures. Previously, we identified two hypermutator *Cryptococcus neoformans* clinical isolates, Bt65 and Bt81, that can rapidly overcome antifungal selection by uncontrolled transposition of a specific retrotransposon, Cnl1. These isolates harbor a nonsense mutation in a novel RNAi component, Znf3, and have accumulated a tremendous transposon burden (~150 copies of Cnl1), and loss of RNAi is responsible for hypermutation. To better understand the adaptation

mechanisms in *C. neoformans*, we developed two bioinformatics pipelines to identify additional isolates with RNAi loss-of-function mutations by screening an extensive Strain Diversity Collection. Remarkably, several loss of RNAi isolates were identified but these isolates do not exhibit a hypermutator phenotype and have not undergone transposon amplification. To test if these RNAi loss isolates can become hypermutators, they were crossed with an isolate containing a high Cnl1 burden. F1 hypermutator progeny were identified with distinct mutation spectra. In addition to the Cnl1 insertion, the transposition of a novel gigantic DNA transposon (~11 kb), widely distributed in natural isolates with varying copy numbers, contributed to the hypermutator phenotype of the progeny. Taken together, our results suggest natural isolates with RNAi defects are not uncommon and many lie on a pathway to hypermutation. Additional passage assays and genome editing of the RNAi loss-of-function mutations are being conducted to determine the connection between RNAi loss and transposon burden in contributing to the evolution of hypermutator isolates.

496C **Genomic Architecture of Fungal Metabolism Involved in Host and Ecological Specialization** Rodrigo Olarte[1], Dean K Malvick[2], Kathryn E. Bushley[3] [1]Plant and Microbial Biology, University of Minnesota, [2]Plant Pathology, University of Minnesota, [3]Emerging Pests and Pathogens Unit, USDA-ARS

In fungi, host and ecological specialization is shaped both by the products of secondary metabolism (i.e., host-selective toxins) and those from primary metabolism involved in utilizing specific classes of host carbohydrates or proteins. Secondary metabolite genes synthesizing toxins are among the fastest evolving genes classes in fungi and are often localized to unstable regions of the genome such as subtelomeres and other transposable element (TE) and repeat rich regions. They respond to selective pressures imposed by either the host or the environment, enabling fungi to rapidly adapt to changing conditions. Additionally, secondary metabolite genes, as well as genes involved in virulence, are often found clustered within fungal genomes, which may facilitate efficient epigenetic regulation. Genes involved in ecological adaptation may also be localized to small “accessory” chromosomes, which like bacterial pathogenicity plasmids, may facilitate their horizontal transfer among fungi. Yet the mechanisms by which these genes and clusters evolve remain elusive. Using a dataset of six nearly chromosomal-scale assemblies of the insect pathogenic fungus *Tolypocladium inflatum*, as well as examples from several plant pathogenic fungi, we examine evidence for genetic processes such as transposition, inversions, microdeletions, and homologous recombination or gene conversion on the ends of chromosomes for driving the diversification of metabolite clusters and gene families involved in host and ecological adaptation. In particular, the role of transposable elements in rearrangement or mobilization of clusters within fungal genomes is addressed. We also examine how these types of structural rearrangements impact the expression of metabolite clusters or lead to loss of function through pseudogenization mutations that may also serve as the basis for host and ecological adaptation.

497C ***Fusarium graminearum* as an apple fruit pathogen** Mladen Petres[1], Mila Grahovac[1], Dragana Budakov[1], Marta Loc[1], Tatjana Dudas[1], Li-Jun Ma[2] [1]University of Novi Sad, Faculty of Agriculture, [2]University of Massachusetts

The genus *Fusarium*, comprising diverse filamentous fungi, is well-known for its numerous plant pathogenic species. Among these, *Fusarium graminearum* is known for causing serious problems in wheat and maize crops, like Fusarium head blight in wheat and ear rot and stalk rot in maize. Recently, it has also been found as causal agent of apple rot, alongside *F. avenaceum*, imposing substantial challenges for apple production. This fungal pathogen not only induces significant yield losses, but also deteriorates the quality and increases the risk of mycotoxin contamination among infected crops. To understand this fungus better, the previously assembled genome of *F. graminearum* strain TaB10 from Serbia was compared with a reference strain PH-1 performing whole genome alignment. In TaB10 genome 347 unique sequences longer than 500 bp were found, with 275 sequences longer than 800 bp. In the masked TaB10 genome 305 unique sequences longer than 500 bp and 236 sequences longer than 800 bp were detected. These genetic differences provide insights into how *F. graminearum* varies, helping us understand why it can be pathogenic to different plants. Knowledge on these differences is important to develop effective ways to deal with *Fusarium*-related issues and protect significant crops. It shows that fungi like *Fusarium* can adapt and pose challenges in different environments, emphasizing the need for ongoing research to tackle emerging agricultural problems.

498C **Exploration of secondary metabolite genetic diversity in *Fusarium sambucinum* through comparative genomic approaches** Theodora G. Borland[1], Nicholas C. Cauldron[2], Heidi A. Nunnemacher[1], Cynthia M. Ocamb[1], Niklaus J. Grunwald[3], David H. Gent[4] [1]Botany and Plant Pathology, Oregon State University, [2]Oregon State University, [3]Horticultural Crops Research Unit, USDA-ARS, [4]Forage and Seed Crop Research Unit, USDA-ARS

The genus *Fusarium* includes some of the most impactful plant pathogens confronting agriculture today. In addition to crop damage, *Fusarium* poses a serious risk to human and animal health due to the production of toxic secondary metabolites (SMs), mycotoxins, which are governed by a diverse array of biosynthetic gene clusters (BGCs). Hop (*Humulus lupulus*) is an economically important crop in the Pacific Northwest (PNW) region of the United States, where almost 100% of the US crop is produced and is

the largest hop production region in the world. Fusarium canker is an emerging disease of hop in the PNW that causes cankering and girdling at the base of the bines, and ultimately wilting and dieback of entire bines, leading to yield and crop quality impacts for growers. Fusarium canker is caused primarily by *Fusarium sambucinum*, the type species of the genus *Fusarium* and a causal agent in other diseases such as dry rot of potato. Although *F. sambucinum* is a notable member of the genus, there is little understanding of the genotypic diversity among its populations or variation in SM BGCs. The objective of our research is to investigate what SM BGCs are present in a diverse sample of *F. sambucinum* isolates from different geographic regions and hosts, with a focus on isolates derived from hop, and whether the presence and architecture of BGCs are consistent across populations. We collected *F. sambucinum* isolates from hop yards across Oregon, Washington, and Idaho and obtained isolates derived from additional plant hosts. We initially sequenced two isolates from hop and potato using PacBio Sequel II and assembled the 39 Mb genomes to serve as high quality reference genomes. We re-sequenced a larger collection of isolates from various hosts and geographic locations using Illumina NextSeq and aligned reads to the reference for assembly of individual genomes. SM BGCs were identified among the population using antiSMASH in addition to a database of known *Fusarium* BGC sequences. Preliminary analyses revealed incongruences among identified SM BGCs from the literature and those present in the genomes we sequenced. Follow-up work will include a larger collection of isolates, genome-scale population analyses, and direct measurement of mycotoxin production in *F. sambucinum* isolates. Research into secondary metabolites can help us gain a basic understanding of diversification of *F. sambucinum* across geographic regions and host species.

499C **Genomic Resources for the ARS Entomopathogenic Fungi Collection** Kathryn E Bushley[1], Brian R Lovett[2] [1]Emerging Pests and Pathogens Unit, USDA-ARS, [2]Emerging Pests and Pathogens Unit, USDA/ARS

The USDA-ARS Entomopathogenic Fungi (ARSEF) culture collection is the largest and most comprehensive collection of entomopathogenic fungi in the world. Housing over 14,000 fungi and protistan microorganisms isolated from insects and other invertebrates (i.e. spiders, nematodes, mites), ARSEF serves as a significant resource for the taxonomic identification and phylogenetic placement of insect pathogenic fungi, as well as a germplasm repository for development of microbial biocontrol agents and biopesticides targeting agricultural pests. The associated metadata on fungal pathogen host-associations, host-substrates, and collection location can be utilized for biodiversity inventories as well as guide initial selection of appropriate strains for development as biocontrol agents. However, optimizing use of these fungi for biocontrol requires identification of strains with suitable host specificity and small non-target effects on other insects, wildlife, and humans. Additionally, some entomopathogenic fungi (i.e. Entomophthorales) are also difficult to grow and sporulate in culture, presenting challenges to mass production and deployment for biocontrol. A better understanding of the genetic basis of virulence and host-specificity and a phylogenomic framework for predicting them is required. As ARSEF moves into the genomics era, we are synthesizing publicly available phylogenetic, genomic, and biochemical resources for ARSEF isolates to create a phylogenomic framework for predicting host-specificity and biocontrol phenotypes, as well as developing best practices for the generation and sharing of biological control phenotypic data. Centralizing genomic resources for entomopathogenic fungi will also allow researchers worldwide to access to genetic elements responsible for virulence or other traits from isolates that are not easily cultured for biotechnological development and facilitate genetic modifications of strains for improved virulence, host-specificity, and survival in field settings. Ultimately, we aim to provide an improved resources for taxonomic placement of isolates and prediction of host-specificity, virulence, and biocontrol phenotypes. We envision these resources will enable researchers to more efficiently identify genetic factors underlying key phenotypes and facilitate their genetic manipulation, thus expediting the development of successful biocontrol agents.

500C **Global analysis of circuitry governing *Candida albicans* morphogenesis within host immune cells and identification of inhibitors of morphogenesis** Nicola T Case[1], Johannes Westman[2], Michael T Hallett[3], Jonathan Plumb[2], Aiman Farheen[1], Michelle E Maxson[2], Mami Yoshimura[4], Takeshi Sonoda[4], Toshie Kaizuka[4], Makiko Itou[4], Hiroyuki Hirano[4], Jessie MacAlpine[1], Sean D Liston[1], Bernard Hube[5,6], Yoko Yashiroda[4], Nicole Robbins[1], Luke Whitesell[1], Hiroyuki Osada[4], Minoru Yoshida[4], Charles M Boone[1,4], Sergio Grinstein[1,2,7], Leah E Cowen[1] [1]University of Toronto, [2]The Hospital for Sick Children, [3]Western University, [4]RIKEN Center for Sustainable Resource Science, [5]Hans Knoell Institute, [6]Schiller University, [7]St. Michael's Hospital

The evasion of killing by immune cells is crucial for fungal survival in the host. For the human fungal pathogen *Candida albicans*, the morphogenetic transition from yeast to filament upon internalization by macrophages is a key intracellular survival strategy that occurs through mechanisms that remain unclear. Here, we employed functional genomic screening of conditional expression mutants covering >50% of the *C. albicans* genome to identify genes selectively required for filamentation inside macrophages. Through manual and machine learning-based image analyses, we uncovered a role for the mitochondrial ribosome, respiration, and the SNF1 AMP-activated kinase complex in governing filamentous growth within the phagosome, suggesting that *C. albicans* relies on respiration to evade the antifungal activities of macrophages. We demonstrated that downregulating the

expression of these genes reduces ATP levels and impedes filamentation as well as growth under monoculture conditions in medium lacking glucose. In co-culture with physiological glucose concentration, downregulation of genes involved in mitochondrial function and respiration prevented *C. albicans* from expanding within the phagosome, escaping, and inducing immune cell death. Additionally, we screened ~50,000 compounds for their ability to inhibit *C. albicans* filamentation using a dual-strain screening strategy where a nourseothricin (NAT)-resistance marker was placed downstream of the filament-specific promoter *HWP1p* or downstream of the constitutive promoter *TEF1p*. This enabled us to identify compounds that specifically inhibit filamentation using optical density as a readout. Through this approach, we identified 259 putative inhibitors of *C. albicans* filamentation and prioritized 16 based on potent activity only against the *HWP1p*-NAT strain. We subsequently focused on three compounds with available chemical-genomic profiles and confirmed their ability to inhibit filamentation without substantially affecting growth. Moreover, we determined that these compounds inhibit filamentation across diverse filament-inducing cues and in some cases, inhibit filamentation induced by overexpression of transcription factors that positively regulate filamentation. Together, our work highlights respiration and the SNF1 AMP-activated kinase as key effectors of *C. albicans* metabolic flexibility and filamentation within phagocytes and identifies novel inhibitors of the yeast-to-filament transition.

501C **Comparative Genomics Resource (CGR) at NCBI: new possibilities to advance fungal research** Barbara Robbertse, Nuala O'Leary, Sanjida Rangwala, Terence D Murphy National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health

The NIH Comparative Genomics Resource maximizes the impact of eukaryotic research organisms and their genomic data to biomedical research. CGR facilitates reliable comparative genomics analyses for all eukaryotic organisms through community collaboration and a National Center for Biotechnology Information (NCBI) genomics toolkit. The toolkit includes high-quality data, tools, and interfaces for connecting community-provided resources with NCBI.

CGR is of key interest to the fungal research community, where limited resources and scarcity of quality data from fungal pathogens has been identified in a recent World Health Organization (WHO) report as one of the factors hindering research progress of fungal diseases. The NCBI toolkit allows users to improve data quality before submission, explore and download NCBI sequence data, compare genomic sequences and visualize data. NCBI Datasets provides easy and FAIR access to genomic sequence and metadata from across the tree of life, with user-friendly web and programmatic interfaces well suited for integration into new workflows. The Foreign Contamination Screening (FCS) tool detects contaminants from foreign organisms in genome assemblies. The FCS tool enables a researcher to prepare quality data for submission or reference datasets to evaluate mycobiome data which will ensure accuracy of downstream analyses. The Comparative Genome Viewer (CGV) enables visualization and comparison of genome assemblies while the Genome Data Viewer (GDV) helps to explore and analyze a genomic region's annotations. Whole genome alignments for fungal pathogens are now available in CGV and GDV. Through the identification of conserved regions and genomic rearrangements, these alignments provide a comprehensive view of the genetic similarities and differences that underpin the adaptability and virulence of fungal pathogens. More information about these resources and examples of fungal genomes benefiting from these resources will be presented. Contact cgr@nlm.nih.gov or visit the CGR website to get involved.

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502C **Genome sequencing and analysis provides novel insight into *Septoria glycines*** Kona Swift[1], Ahmad Fakhoury[2], Burt Bluhm[1] [1]Dept of Entomology and Plant Pathology, University of Arkansas, [2]Dept of Plant, Soil, and Agricultural Systems, Southern Illinois University

Septoria brown spot, caused by *Septoria glycines*, is one of the most prevalent foliar diseases of soybean in the U.S. Warm, humid conditions favor disease development, and symptoms are exacerbated by a variety of biotic and abiotic stresses. Although long regarded as a disease of minor economic importance, many factors associated with climate change are predicted to potentially exacerbate Septoria brown spot, and concern about yield loss has increased substantially in recent years. Surprisingly, little is known about pathogenesis in *Septoria glycines*, and very few genomic resources exist for the pathogen. The broad goal of this study is to create a suite of molecular tools to dissect pathogenesis in *Septoria glycines*, with initial emphasis on genomic sequence resources. To this end, a collection of *S. glycines* isolates was obtained from diseased soybean leaves in Arkansas and Illinois. From this collection, a wild-type strain that exhibited stable growth and conidiation in culture was selected for whole-genome sequencing to provide an initial reference genome. Illumina sequencing yielded 24,022,684 paired-end 150 reads, totaling 3,603,402,600 bases. The resulting genome assembly was comprised of 328 contigs, with an average length of 93,379 bases, resulting in a predicted genome size of 30.6 Mb. Contig sizes ranged from 1,009 bp to 1,164,023 bp, with an N50 of 396,433 bp.

Initial analyses of genome annotations identified putative orthologs of known pathogenicity genes, as well as suites of genes predicted to encode effectors and secondary metabolites. To complement sequencing efforts, efforts are underway to optimize transformation systems and establish protocols for targeted gene deletion and genome editing. In summary, this work has provided the first reference genome sequence for *S. glycines*, as well as foundational tools for molecular genetics and functional genomics. These tools will enable molecular investigations into mechanisms of pathogenesis, host resistance, pathogen population dynamics, and the emergence and spread of fungicide resistance, and thus will help provide a more thorough understanding of Septoria brown spot of soybean.

503C **Differences in thermotolerance between ecotypes of *Neurospora discreta* are primarily due to only two genomic regions** Aaron J Robinson[1], Donald O Natvig[2], John W Taylor[3], Igor Grigoriev[4], Julia Kelliher[1], Patrick Chain[1] [1]Genomics and Bioanalytics, Los Alamos National Laboratory, [2]Biology, University of New Mexico, [3]University of California, Berkeley, [4]Joint Genome Institute (JGI)

Differences in maximal growth temperature among *Neurospora discreta* isolates from the western United States correlate with differences in mean annual environmental temperature. Isolates from New Mexico (NM) and Alaska (AK) exhibit comparable growth rates below 35°C, but isolates from New Mexico grow much better near and above 40°C. Progeny generated from a sexual cross between NM and AK parental isolates possessed temperature response phenotypes resembling either one of the two parents or an intermediate phenotype. This confirmed the trait of thermotolerance is heritable and the range of progeny phenotypes suggests the involvement of multiple gene regions. To investigate the genetics underlying thermotolerance in *N. discreta*, a functional genomics approach was applied. A total of 82 (39 thermotolerant and 43 non-thermotolerant) progeny from crosses with parents from NM and AK were selected for genome-wide association studies. With support from the DOE Joint Genome Institute (JGI) Community Science Program (CSP), we obtained complete genome sequences for both parental strains and the 82 selected progeny. High-quality genome assemblies of the parental strains were obtained utilizing sequence data from both Illumina and Oxford Nanopore MinION platforms. Bulked-segregant analysis was also performed using the MinION platform and combined genomic DNA from 22 New Mexico-like progeny and 29 Alaska-like progeny. Comparative analyses demonstrated a strong association between the genotypes in two genomic regions and the thermotolerant phenotype. Genetic differences in a region of linkage group III appear to primarily contribute to thermotolerance in *N. discreta*, while a region on linkage group I appears to play a secondary role. Intriguingly, these identified regions overlap with a region previously identified in *N. crassa* that may be under selection as a result of adaptation to colder environments. This work has provided perspective on how fungi may respond to increasing global temperatures as a result of climate change.

504C **The telomeric-linked helicase genes are highly dynamic members of the *Fusarium oxysporum* subtelomere** Sahar Salimi[1], Mohammad Foad Abdi[2], Mostafa Rahnama[3] [1]Tennessee Tech university, [2]Tennessee Tech University, [3]Biology, Tennessee Tech University

Fusarium oxysporum species complex is a diverse group of filamentous ascomycete fungi with an extensive host range from monocotyledonous and dicotyledonous plants to immunocompromised humans and other mammals. Many phytopathogenic species of *F. oxysporum* complex are responsible for devastating diseases in multiple economically important crop species. However, despite the economic significance of these fungi, little is known about their functional diversity that contributes to their pathogenicity and host specificity. One of the significant factors influencing fungal functional diversity is their chromosomal structure, particularly at the end of the chromosomes or telomeres. Telomeres are crucial features of eukaryotes' linear chromosomes that prevent chromosome ends from exonucleolytic attack, illegitimate recombination, and being recognized as double-strand breaks. In this study, we examined the chromosome end composition of five different strains of *F. oxysporum*. Our results show that nearly all the telomeres of these strains are associated with a telomeric-linked RecQ-like helicase (TLH) gene. The RecQ helicases belong to superfamily II (SFII) of DNA helicases involved in unwinding DNA duplexes. The RecQ helicase family has been described in prokaryotes to eukaryotes and has been highly conserved throughout evolution. RecQ helicases play a significant role in DNA metabolism, including preventing illegitimate recombination, repairing stalled replication forks, and initiating homologous recombination. TLH are a unique family of helicases that reside within ~10 kb of a telomere, contributing to the dynamic nature of these chromosomal ends. In this study, we identified, characterized, and investigated the role of the TLH gene in the *F. oxysporum* chromosome end structure.

505C **Comparative genomics of *Cryptococcus* and *Kwoniella* reveals pathogenesis evolution and contrasting modes of karyotype evolution via chromosome fusion or intercentromeric recombination** Marco Dias Coelho[1], Marcia David-Palma[1], Terrance Shea[2], Minou Nowrousian[3], Sheng Sun[1], Christina A. Cuomo[2], Joseph Heitman[1] [1]Dept of Molecular Genetics and Microbiology, Duke University Medical Center, [2]Broad Institute of MIT and Harvard, [3]3Lehrstuhl für Molekulare und Zelluläre Botanik, Ruhr-Universität Bochum

In this study, we conducted an in-depth comparative genomic analysis of global human fungal pathogens within the *Cryptococcus* genus, non-pathogenic *Cryptococcus* species, and related species from the sister genus *Kwoniella*. Chromosome-level genome assemblies of multiple species of both genera were newly generated, resulting in a dataset that comprehensively encapsulates their known diversity.

Although *Cryptococcus* and *Kwoniella* have comparable genome sizes and similar gene content, hinting at potential pre-adaptive traits for human pathogenicity, our analysis also found evidence in pathogenic *Cryptococcus* species of specific examples of gene gain (via horizontal gene transfer) and losses, which might represent evolutionary signatures of pathogenic development.

Another key finding was significant variation in chromosome number and structure between the two genera. By combining synteny analysis and experimental centromere validation, we found that most *Cryptococcus* species have 14 chromosomes, whereas *Kwoniella* species typically have fewer, ranging from 14 to 11, 8, 5 or even as few as 3. This reduction in chromosome number in *Kwoniella* was the result of formation of giant chromosomes (up to 18 Mb) through repeated chromosome fusion events, each marked by a pericentric inversion and centromere loss. In contrast, *Cryptococcus* species with fewer than 14 chromosomes showed chromosome reductions primarily through rearrangements associated with the loss of repeat-rich centromeres. Additionally, *Cryptococcus* genomes exhibited far more interchromosomal rearrangements potentially related to abundant transposable elements, as evidenced by clear examples of translocations via intercentromeric recombination, facilitated by the presence of shared transposons at centromeres.

Overall, our findings advance our understanding of the possible genomic changes associated with the onset of pathogenicity in *Cryptococcus*, and provide a foundation for elucidating the mechanisms behind centromere loss and chromosome fusion, contributing to remarkably distinct karyotypes in closely related fungal lineages.

506C **Assessing genome assembly and annotation quality in MycoCosm.** Sajeet Haridas[1], Ran Liu[1,2], Asaf A Salamov[1], Igor V Grigoriev[1,2] [1]DOE Joint Genome Institute, [2]University of California, Berkeley

Over the last decade, MycoCosm has grown from <200 genomes to nearly 2500 publicly available genomes today. The quality of assemblies and annotations produced over this time varies, both due to the project requirements (minimal, standard, high-quality draft, etc) and changes in sequencing technologies. While generally used metrics to measure assembly quality such as N50 / L50 is sufficient to compare across assemblies of similar sizes, it becomes meaningless in the face of the >100x variation in genome sizes as seen across the fungal tree of life. Other measures such as CEGMA or BUSCO that estimate the capture of genic space from assemblies or annotated proteomes do not account for fragmented genomes and the lack of some core genes in lineages with reduced genomes due to specialized lifestyles.

Here, we present evidence that single measures to estimate annotation quality such as BUSCO or number of gene models is insufficient. We have generated several metrics including protein length distribution, annotation completeness estimation, presence of particular PFAM domains, estimates of ploidy, etc, each of which assess different facets of annotation quality. We employed machine learning models which revealed that protein length distribution and the percentage of incomplete genes are the most critical factors affecting annotation quality. We have developed methods to summarize these multi-dimensional evaluation metrics into comprehensive assembly and annotation quality scores. This will provide users a guide to selecting genomes of varying qualities across MycoCosm for comparative studies.

507C **Identification of a putative gyromitrin biosynthesis gene cluster in the false morels** Alden Dirks[1], Timothy James[2] [1]Ecology and Evolutionary Biology, University of Michigan, [2]University of Michigan

Gyromitrin (acetaldehyde *N*-methyl-*N*-formylhydrazone) is a mycotoxin produced most infamously by the false morel mushroom *Gyromitra esculenta* (family *Discinaceae*, class *Pezizomycetes*), which is consumed as a delicacy in Scandinavia after its detoxification. At its core, gyromitrin contains an N-N bond that makes it a member of the rare, chemically interesting, and

unusually bioactive class of molecules called N2NPs (N-N bond-containing natural products). Much progress has been made in elucidating the genes responsible for N-N bond formation in prokaryotic N2NPs, but the enzymes that form this bond in fungi remain a mystery. We utilized comparative genomics on various species of false morels with different gyromitrin phenotypes to identify a candidate gyromitrin biosynthesis gene cluster (BGC). The putative gyromitrin BGC is of the ribosomally synthesized post translationally modified peptide (RiPP) class and contains 10-20 genes, including a 2-oxoglutarate/Fe (II)-dependent oxygenase. The cluster is conserved across *Discinaceae* except for the core RiPP genes whose presence and evolutionary history align with gyromitrin production in phylogenetically disparate false morels. The putative gyromitrin genes are otherwise absent from available *Pezizomycetes* genomes and may have been acquired via horizontal gene transfer from *Dothideomycetes*. Future research should confirm the function of this BGC via knockout experiments and identify the biosynthesis pathway of gyromitrin N-N bond formation.

508C **Applied machine learning models for elucidating complex relationships between epigenomic regulatory design rules and gene expression between fungal species across phylogenetic distances.** Laura Weinstock, Cameron Kunstadt, Anna Fisher, Jenna Schambach, Elizabeth Koning, Wittney Mays, Raga Krishnakumar Sandia National Laboratories

Engineered fungi are promising chassis for future sustainable biomanufacturing and bioproduction. Reliable regulation of the functionality in diverse fungi at scale remains a significant challenge to commercialization. Determining ground rules of gene regulation and their applicability across fungal species is critical for minimizing the number of conditions that need to be tested to achieve optimally engineered fungi across species. Epigenetic modifications play a crucial role in regulating gene expression and having a handle on regulation of epigenetics across fungal species will significantly improve engineering prospects, both in existing and emerging synthetic biology chassis. Recently, there have been efforts to better understand the relationship between gene sequence, epigenetic modifications, and gene expression within fungal species through the use of machine learning and deep learning (ML/DL) methods that are able to ingest the high-dimensional, complex sequencing data. However, there remains limited understanding of how similar epigenetic modifications control gene expression across fungal species. The discovery of conserved epigenetic modification design rules that control gene expression would greatly improve the efficiency of engineering across diverse fungal species. In this study, we aimed to predict gene expression levels based on combinatorial epigenetic modification expression within and across fungal species. By predicting the effect of epigenetic modifications on gene expression, we can a) predict more accurately how a given engineered strain might optimally leverage epigenetics and b) determine how to engineer strains to take full advantage of these epigenetic pathways. We trained and tested ML/DL models within and across fungal species to identify the relationship between epigenetic modification features and gene expression. We tested a battery of models, ranging from regression to deep neural networks, on increasingly complex engineered data features to not only predict gene expression based epigenetic modifications, but also to understand the degree of complexity required to make accurate predictions. Overall, there is some conservation of epigenetic mechanisms across related species, though the predictive capacity of our cross-species epigenetic models was limited, which may be due to underlying biological constraints or limited data availability.

509C **Characterization of transcriptional differences during Cercospora beticola disease progression on infected detached and attached sugar beet leaves** Mari Natwick[1], Nathan Wyatt[2], Gary Secor[1], Melvin Bolton[3] [1]North Dakota State University, [2]United States Dept of Agriculture, [3]USDA - ARS

Sugar beet (Beta vulgaris) accounts for 60% of domestic sugar and 20% of global sugar. The most economically important disease of sugar beet is Cercospora leaf spot (CLS) caused by the hemibiotrophic fungal pathogen Cercospora beticola. Detached leaf assays have been used in other plant-pathogen systems as a model for monitoring disease processes and would present as a useful high-throughput phenotyping method for CLS. However, few studies have characterized transcriptional differences between attached and detached leaf assays to understand the validity of detached leaf assays for making inferences regarding infection of whole plants. Our study established a sugar beet detached leaf assay with successful C. beticola infection. We found that while C. beticola is capable of infecting detached sugar beet leaves, there were notable differences in disease progression when compared to attached leaf assays. RNA-seq analysis comparing the transcriptional events of infected detached and attached leaves revealed differentially expressed genes for both host and pathogen. Notably in the later stages of infection, it was observed that C. beticola did not sporulate from detached leaves and necrosis was absent or reduced. Mirroring this observation, the pathogen transcriptional data showed divergence between detached and attached leaves at eleven days post inoculation, strongly suggesting that detached leaf assays are not a proxy for conventional attached leaf assays in this pathosystem. Additional functional annotation of host and pathogen differentially expressed genes will be presented.

510C **Functional genomics of loblolly pine EMF communities revealed by metatranscriptomics** Daniel Rodriguez[1], Keaton Tremble[1], Brian Looney[1], Jake Nash[1], Lotus Lofgren[1], HUI-LING Liao[2], Jennifer Bhatnagar[3], Rytas Vilgalys[1] [1]Duke University, [2]University of Florida, [3]Boston University

Ectomycorrhizal fungi (EMF) form symbiotic associations with roots of most of the world's forests where they help regulate nutrient exchange between plants and soil. Environmental metagenomic approaches show promise for studying molecular-based functions of EMF. Here we utilized metatranscriptomics to identify metabolic roles and modes of interaction among EMF communities collected across the range of loblolly pine in the USA. Loblolly pine (Pinus taeda) is a model species for Pinaceae and the most commercially important species for timber production in the world. RNASeq data were obtained from EMF mycorrhizae samples collected across the range of loblolly pine in the USA. Expressed genes were assembled de novo into contigs, functionally annotated and mapped to fungal genomes produced by the JGI (mycocosm.jgi.doe.gov). After mapping, transcripts were clustered into orthogroups and loci with statistically significant differential expression were identified. Based on RNA expression, EMF composition varied among sites and samples and was largely dominated by a core group of EMF species (Lactarius, Piloderma, Russula, Cenococcum, and Tuber). EMF transcriptomic profiles mostly cluster according to phylogeny, suggesting that closely related EMF inhabit similar ecological niches in the rhizosphere. KEGG mapping of expressed genes highlights several metabolic pathways that are uniquely expressed among subsets of the soil fungal community. For example, rhamnose degradation is exclusively expressed by ascomycete ectomycorrhizae (Oidiodendron, Cenococcum and Tuber), while sucrose metabolism was commonly expressed by all EMF as well as the pine host suggesting this pathway may be fundamental to microbiome function. Several unique roles were identified among EMF involving different metabolic pathways for nitrogen and phosphate cycling, carbon utilization, and phytohormone synthesis. These results support the concept of niche conservatism and microbial exchangeability within the EMF community. Similar application of metatranscriptomic analyses can be used to identify unique metabolic pathways used by fungi in complex soil microbiomes, providing exciting insights into the ecology of cryptic fungal communities.

511C **Development of a CRISPR/Cas9-mediated gene knockout method for functional genomics of the barley spot blotch pathogen *Bipolaris sorokiniana*** Alireza Poursafar, yueqiang Leng, Shaobin Zhong Plant Pathology, North Dakota State University

Bipolaris sorokiniana (=Cochliobolus *sativus*) is an important fungal pathogen with a wide host range infecting wild grasses and economically important cereal crops including barley and wheat. However, the molecular interactions between *B. sorokiniana* and its host plants are poorly understood. Development of a highly efficient approach for gene mutagenesis and knockout is essential for understanding the molecular mechanisms underlying pathogen biology and pathogenicity. In recent years, CRISPR/Cas9 technology has widely been used to facilitate site-directed mutagenesis in different groups of fungi, but its application in *B. sorokiniana* has not been reported. In this study, we aimed to develop a CRISPR/Cas9-mediated genome editing protocol for efficiently knocking out genes of *B. sorokiniana*. We first assessed the efficiency of the CRISPR/Cas9-based genome-editing combined with the split marker system for gene replacement in the fungus. We designed gRNAs for a polyketide synthetase gene (*PKS1*) that is involved in melanin production of the fungus and delivered the preassembled Cas9 ribonucleoproteins (RNPs) along with the split fragments of the hygromycin resistance gene (*HygR*) into the protoplasts of *B. sorokiniana*. We showed that use of RNPs in the split marker system significantly increased the number of transformants with the *PKS1* gene replaced compared to the control without RNPs. We then combined RNPs with PCR-amplified hygromycin resistance gene flanked by 40bp or 60bp sequences homologous to the flanking sequences of the gRNA target site of *PKS1* for fungal transformation. Our results also demonstrated that RNPs significantly enhanced gene disruption efficiency through the short-homology recombination. Finally, we disrupted a new non-ribosomal peptide synthetase gene (*NPS*) in isolate ND90Pr using the RNP-mediated gene knockout approach and showed the *nps* mutants lost virulence on barley Bowman. The high efficiency of the Cas9/sgRNA-mediated gene knockout method will facilitate large-scale functional genomics studies of *B. sorokiniana*.

512C **Truffles population genomic and associated fungal and bacterial communities - who shapes the true truffles aroma?** Tine Grebenc[1], Nejc Suban[1], Nataša Šibanc[1], Aleksander Mahnič[2], Lidija Strojnik[3], Nives Ogrinc[3], Cene Gostinčar[4] [1]Slovenian Forestry Institute, [2]National Laboratory for Health, Environment and Food, [3]Jožef Stefan Institute, [4]4Biotechnical Faculty, University of Ljubljana

Truffles are the fruiting bodies (ascocarps) of fungi belonging to the genus *Tuber* that are fruiting in the soil and are best known for their aromas. Besides truffles ascocarps' produced aromatic volatiles, associated bacteria and yeast are also recognized to contribute significantly to the truffle. Recently we performed a *Tuber aestivum* and *T. magnatum* whole genomes population re-sequencing, aiming to analyze and correlate the outcome of aroma analysis with the bacterial and fungal communities on surface and within ascocarps of the same truffle ascocarps. In addition, an extensive studies of truffles aromas in Europe (Strojnik et al. 2020, Šiškovič et al. 2021) were done on same collections. Both, the bacterial and the fungal associated communities were further

assessed with site and ecological characteristics of each truffle genotype. Results of the preliminary analysis and statistical assessment of truffle genomes diversity and associated bacterial and fungal communities will be presented.

513C **Host specificity of oak-associated foliar endophytes and saprobes associated with enhanced *in vitro* growth on polyphenolic compounds** Jana M U'Ren, Megan N Nickerson Plant Pathology, Washington State University

Foliar fungal endophytes are horizontally transmitted symbionts that inhabit healthy, photosynthetic tissues of all lineages of land plants where they influence plant health and productivity. Endophyte communities often are more similar among closely related hosts, potentially as a result of a preference for particular morphological, ecophysiological, or chemical host traits. However, the various ecological and evolutionary factors that drive these patterns often are difficult to disentangle. We first examined the impact of six polyphenolic compounds on the growth of 15 phylogenetically diverse *Quercus* (oak)-associated fungal species and assessed whether tolerance to phenolics is associated with their degree of specialization to oaks in nature. Despite frequently reported antifungal properties of phenolics, we found that oak-associated fungi grew the same or better than positive controls in 78% of trials with all compounds. Although fungal sensitivity differed as a function of both compound type and concentration, the type of phenolic compounds had a greater impact on fungal growth than compound concentration. On average, species of Dothideomycetes grew significantly better than species of Sordariomycetes on all phenolic compounds, yet fungal species with the highest growth on phenolic compounds were not always closely related. Instead, the degree of fungal host specificity to *Quercus* was the best predictor of phenolic tolerance. Fungal species isolated more frequently on oak leaves vs. other hosts had the greatest *in vitro* tolerance to phenolics, whereas generalists that were isolated in culture from many different hosts were more sensitive. Comparative genomic analysis of these strains will examine the relationship between fungal phenolic growth tolerance and the presence of genes related to the catabolism of phenolic compounds, such as peroxidases (POD), polyphenol oxidases (PPO), and hydrolytic enzymes. In addition, we will examine the evolutionary history of genes putatively involved in phenolic degradation and assess the potential for horizontal gene transfer between phylogenetically diverse oak-associated fungi.

514A **Inter-kingdom and intra-kingdom interactions in the microbiome of fungal fruiting body and associated decaying wood** Wenzi Ren[1], Risto Kasanen[1], Reijo Penttilä[2], Fred O Asiegbu[1] [1]Dept of Forest Sciences, University of Helsinki, [2]Forest Health, Natural Resources Institute of Finland (LUKE)

Microbiome community of decaying wood and associated fungal fruiting body have been extensively studied, but not much is known on the interactions between bacteria and fungi component of the microbiota. In this study, we aim to 1) Unravel the contribution and development of bacteria community and other fungi during the wood decay process (D1 – D4) 2) Uncover how the environmental factors and the white rot fungus (e.g. *Heterobasidion* spp.) drive the microbiome community structure and functional changes. The next generation ITS and 16S amplicon sequencing data were used in this analysis. Wood samples and fruiting body collected from managed and unmanaged forest sites were classified into four decay classes. Our result showed that in natural forest, inter- kingdom (bacteria-fungi) interaction appeared less common than intra- kingdom (bacteria-bacteria; fungi-fungi). Although bacteria have the most abundant interactions, each bacteria species has less connections, while fungi have more. In fruiting body, the keystone network was dominated by fungi at the beginning of the decay (e.g. *Dacrymyces ovisporus*). Whereas in wood, the keystone network was dominated by bacteria at the beginning of the decay (e.g. *Phenylobacterium* sp). The network experienced a process of network fragmentation from the D1 class to the D3 class and reconstruction from D3 to D4 class in both materials. The analysis revealed that the abundance of active bacteria might be related to the instability of the microbial community while the abundance of active fungi could be related to the stability of the community. Among the core microbiome with ecological negative correlation, some were identified to be antagonistic and could serve as potential biocontrol agents. The fungal community and functions were more sensitive to the environmental factors than bacteria. Natural forest management has the most impact on the microbiome community as well as decay, stand age and canopy cover of the forest.

515A ***Seiridium* species causing cypress canker: Insights from South African isolates and historical disease reports** Janneke Aylward[1,2], Francois Roets[2], Brenda D Wingfield[1], Michael J Wingfield[1] [1]FABI, University of Pretoria, [2]Conservation Ecology and Entomology, Stellenbosch University

Fungi in the genus *Seiridium* (Sporocadaceae: Xylariales) are best known as the causal agents of cypress canker on trees in the Cupressaceae. The disease was first reported from Monterey cypress (*Hesperocyparis macrocarpa*) in the San Francisco Bay area in 1928. Subsequently, it has been found in many different regions of the world. Interestingly, the disease is caused by numerous different *Seiridium* species. In South Africa, cypress canker has been known for many years on non-native species of *Cupressus*, and related genera, that are planted as ornamentals. Its recent appearance on a native cedar (*Widdringtonia nodiflora*) has raised concern. This situation has highlighted knowledge gaps, firstly regarding the scarcity of information about *Seiridium* species in

South Africa and secondly, the unresolved identities of several historical cypress canker reports, post a recent taxonomic revision of the genus.

In this study, we collected *Seiridium* species widely in South Africa and also validated the identities of isolates from published disease reports that were not considered in the recent taxonomic revision. South African *Seiridium* isolates were obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. New isolations were made by sampling symptomatic Cupressaceae across South Africa. The most informative gene region, *RPB2*, was sequenced in all isolates, with *EF-1α* and *BT2* sequenced for a subset of isolates. Maximum Likelihood phylogenetic trees were produced using RAxML-NG, incorporating isolates from previous disease reports.

Interestingly, all six species of *Seiridium* that have been associated with cypress canker were found to be present in South Africa. Notably, the species responsible for the outbreak on *W. nodiflora* has not yet been found in South African gardens. Our study also enabled us to update identities of *Seiridium* species from disease reports from the United States and Europe. The results highlight the fact that *Seiridium* species have been moved widely around the world, likely via the nursery trade. Furthermore, they call for a much better understanding of their pathways of movement and the threats that they could pose to native Cupressaceae globally.

516A **Exploring the *Cryptococcus wingfieldii* complex: from African scolytine beetles to novel species discovery** Janneke Aylward[1], Marco A Coelho[2], Marcia David-Palma[2], Anna F Averette[2], Renier J Basson[3], Seonju Marincowitz[4], Nam Pham[4], Francois Roets[3], Sheng Sun[2], Joseph Heitman[2], Brenda D Wingfield[1], Michael J Wingfield[4] [1]University of Pretoria, [2]Duke University, [3]Conservation Ecology and Entomology, Stellenbosch University, [4]FABI, University of Pretoria

Cryptococcus owes its notoriety to species in the *C. neoformans*/*C. gattii* species complex, which cause human cryptococcosis and significant mortality globally. Four non-pathogenic environmental species are closely related to this complex, namely, *C. amylolentus*, *C. depauperatus*, *C. floricola* and *C. wingfieldii*. The latter species is known only from one 1987 isolate obtained from the frass of an unidentified scolytine beetle in South Africa. Its close taxonomic proximity to pathogenic cryptococci has sparked interest in *C. wingfieldii*, especially because non-pathogens could shed light on the evolution of pathogenicity. This prompted an intensive study to identify the origin of *C. wingfieldii*.

Collections were made in the Cederberg Mountain area of the Western Cape Province, South Africa, based on evidence that the original isolate of *C. wingfieldii* could have been collected there. Isolations targeted beetles infesting the native and highly endangered *Widdringtonia wallichii* trees. Yeasts were isolated directly from the beetles, by rolling them across agar plates using a variety of different media, or indirectly by scattering their frass (beetle dust) over the agar surface. Putative *Cryptococcus* colonies were purified and preliminary identifications were made based on ITS sequencing and phylogenetic analysis. Subsequently, 33 isolates underwent Illumina whole-genome sequencing, *de novo* genome assembly and gene prediction.

Phylogenetic analyses using protein sequences from shared single-copy orthologs and single nucleotide variants, combined with average nucleotide identity and mating-type gene examination, led to the discovery of two new *Cryptococcus* species, closely related to *C. wingfieldii*. Both species were isolated from members of an African genus of bark beetles, *Lanurgus* (Coleoptera: Scolytinae). One of the new *Cryptococcus* species could be further resolved into three groups possibly representing different populations. A large collection of *Lanurgus*-associated cryptococci are being isolated to enable the description of new species and to study their relatedness to other species in the genus. *Lanurgus* beetles will also be collected in other parts of southern Africa, following the likelihood that additional species of *Cryptococcus*, and possibly *C. wingfieldii sensu stricto*, will be found. This study unveils a potential environmental reservoir of *Cryptococcus* species and suggests that unexplored native scolytine beetles could further enrich our understanding of these yeasts.

517A **Form follows function in endophyte communitites of carnivorous plants: trap type determines endophyte community.** Brandon Shaw[1], Jon Millett[1], Dave Ryves[1], Helen Glanville[1], Erica Young[2], Michelle Barthet[3], Lexie Carnaggio[3] [1]Geography and Environment, Loughborough University, [2]Biological Sciences, University of Wisconsin Milwaukee, [3]Biology, Coastal Carolina University

Carnivorous plants evolved adaptations to attract, capture and digest animal prey in nutrient poor environments. Plant carnivory has evolved independently 11 times representing striking examples of convergent and divergent evolution. Fungal endophytes are a key part of the plant microbiome, essential to plant health, function, and drive plant evolution. The dynamics of endophyte community assembly are largely unresolved, carnivorous plant endophytes are understudied compared to other plant groups, current methods rely on culture dependent approaches, which often underestimate true endophyte diversity.

For the first time, high throughput sequencing is used on trap leaves of three plant orders (Eriales,Caryophyllales, and Lamiales) covering four carnivorous genera. To study the impact of plant functional trait (trap type) and relatedness on endophyte community, *Pinguicula* and *Drosera* were sampled, which have independently evolved flypaper traps. Additionally, co-occurring *Dionaea muscipula* (Venus flytrap) and *Drosera* were sampled, both of these plants share a common carnivorous ancestor but use different trap types. The pitcher plant *Sarracenia purpurea* and *Drosera* were sampled from three sites in the UK and one in the US to gain insight into endophyte communities across environments and to compare to nearby non-carnivorous plants.

Endophyte communities of carnivorous plants contained primarily saprotrophs, these fungi may aid in prey digestion, *Acrodontium crateriforme* dominated 2/3 of *Drosera* communities, this fungus has been shown to improve prey digestion in *Drosera*. Beta diversity of co-occurring carnivorous and non-carnivorous plants reveal endophyte communities clustering on the basis of plant host trap type. Independently evolved flypaper traps *Pinguicula* and *Drosera*, host similar endophyte communities which differ from nearby non-carnivorous plants. Closely related *Dionaea* and *Drosera*, which use different trap types, host different endophyte communities. Co-occurring *Sarracenia* and *Drosera* (which have independently evolved two different traps) hosted different endophyte communities, which were again different to nearby non-carnivorous plants. These results suggest that plant functional traits (trap type, carnivory vs no carnivory) have a greater impact on endophyte community than plant relatedness.

This study provides the most comprehensive exploration of carnivorous plant endophytes to date and found a higher diversity of endophytes than previous culture-dependent studies, providing new perspectives on the diversity and poten tial roles of endophytes in plant carnivory. In a wider context carnivorous plants have been shown to be a useful case study to test community assembly rules, building on our current limited knowledge of the impacts of host plant function trait (trap type), host plant identity (phylogeny), and environment.

518A **Determining the Impact of Perfluorinated Compounds on Microbial Species Diversity** Halie A Martin, Clayton Hull-Crew, Wendy Haggren, Andrew D Klocko Chemistry & Biochemistry, University of Colorado Colorado Springs

Perfluorinated Compounds (PFCs) are chemicals characterized by multiple extremely strong carbon-fluorine bonds that convey lipophobic and hydrophobic properties to industrial products. PFCs are used in a wide range of products from fire extinguishers and waterproof clothing to food wrappers and non-stick pans. Due to its prevalence within many industries, PFCs have extensively accumulated within the environment. Currently, there is no efficient way to clean PFCs from soil and water sources after sites are contaminated. This poses a health risk to the human population surrounding contaminated areas, as PFCs can bioaccumulate in the body and can cause adverse health effects. In fact, increased cancer rates have been well documented in PFC contaminated regions. In Colorado Springs, Colorado, the release of fire retardants into the Fountain Creek Watershed has caused documented PFC contamination requiring the urgent development of remediation strategies. Thus, there is a critical need for remediating acute PFC contamination from our local environment. Unfortunately, current methods for PFC removal are expensive and energetically costly. However, microbes (fungi or bacteria) found in PFC contaminated environments hypothetically would have developed biochemical pathways that metabolize PFCs into nontoxic byproducts. To identify microbes that potentially could bioremediate PFCs, we are comparing the fungal and bacterial communities of collected soil samples from areas with presumed clean and known PFCs contamination. By examining the microbiome diversity, we may identify a particular microbial genus that thrives in PFC contaminated areas that could be used for bioremediation. We present our characterization of fungal and bacterial community diversity in PFC contaminated soil.

519A **Hawaiian ridge to reef census of Basidiomycete yeasts demonstrates high novel biodiversity in cryptic habitats** Anthony Amend[1], Daniel B Raudabaugh[2], Mary Cathie Aime[2] [1]Univ Hawaii, [2]Purdue University

Basidiomycete yeasts are among the least documented species of the fungal tree of life, with far less than 1% of presumed global diversity accounted for in formal taxonomy. Several innate qualities of these yeasts, including minimal surface to area ratio, pigmentation, and rapid reproductive and metabolic rates contribute to their distributions in unusual and extreme environments long considered better suited to Bacteria or Achaea lifestyles. These same attributes also contribute to uniquely high levels of ecological plasticity and the ability to exist within a variety of hosts and habitats. To better understand the constraints on Basidiomycete yeast distributions, while controlling for long distance dispersal limitation, we leveraged a comprehensive database of more than 4,000 Hawaiian amplicon datasets ranging from the stratosphere to oceanic subsurface sediments, including several near-complete censuses of food webs in marine, stream and terrestrial habitats. Our results indicate that Basidiomycete yeasts from our sampling on Hawai‘i recovered putative species numbering more than 50% of all described global diversity. After accounting for sampling effort, we found that mammals and streams, two habitats comparatively poorly surveyed in fungal biodiversity studies, were the most enriched in Basidiomycete yeasts. Phylogenetic analysis suggests several novel clades at the

Family taxonomic rank, as well as extensive radiations of species from groups previously known from a small number, or even single collections. Many of these showed preferences to habitats with little representation in global collections. Collectively, our study demonstrates how thorough ecological sampling, coupled with informatic analyses of environmental DNA can begin to fill gaps in this enigmatic tree of life.

520A **Fungal diversity associated with grapevine trunk diseases in Northern Italy and development of a qPCR for the detection of Botryosphaeriaceae** Greta Dardani[1,2], Tawanda E. Maguvu[3,4], Rosa J. Frias[3,4], Davide Spadaro[1,2], Florent P. Trouillas[3,4], Vladimiro Guarnaccia[1,2] [1]Dept of Agricultural, Forest and Food Sciences, University of Torino, [2]Agroinnova, University of Torino, [3]Dept of Plant Pathology, University of California, [4]Kearney Agricultural Research and Extension Center

Grapevine Trunk Diseases (GTDs), caused by different fungal pathogens, are major threats to wine grapes in Mediterranean countries, causing severe economic losses. The colonization of woody tissues by those pathogens can occur at the nursery, where plant material collected from mother plants may already be contaminated. Despite significant knowledge about GTDs etiology and epidemiology, no curative methods are yet available, and prevention remain the most effective strategy. Due to limited information on GTD related pathogens in Northern Italy, a survey was conducted during 2021-2022 to investigate fungal species diversity and distribution associated with symptomatic plants. Four species associated with Botryosphaeria dieback, including *Botryosphaeria dothidea*, *Diplodia mutila, Diplodia seriata* and *Neofusicoccum parvum,* were recovered at high frequency. Additionnal pathogens were also isolated, including *Eutypa lata*, *Fomitiporia mediterranea, Phaeomoniella chlamydospora*, *Paraconiothyrium brasiliense*, *Seimatosporium vitis-viniferae* and *Truncatella angustata*. The high frequency of Botryosphaeriaceae isolated from diseased grapevines suggests these pathogens are rising in Italy, as observed in other Mediterranean countries in recent years. A reliable diagnostic tool targeting Botryosphaeriaceae could be used to test propagation material, such as canes from mother vines, at the nursery, and to facilitate the production of healthy cuttings, thus limiting the spread of contaminated plant material. This tool could also be used to facilitate early diagnosis in the field and to help grape farmers to promptly adopt best management strategies. For these reasons, we developed a quantitative method for assessment and quantification of *N. parvum* and *B. dothidea*. A qPCR assay with SYBR Green method was developed to detect and quantify the infection levels of both pathogens directly from grapevine wood.

521A **Fungal diversity in deep-sea sunken plant substrates** Yuriko Nagano, Yoshiyuki Ishitani, Noriyuki Isobe, Ryota Nakajima, Shunichi Ishii, Hiroyuki Kashima, Hidetaka Nomaki JAMSTEC

Fungi are the main decomposers of plants on land, and it is also presumed that they play an important role in the decomposition of sunken plants in deep-sea environments. However, the diversity, distribution, and ecology of deep-sea fungi associated with sunken plant substrates are still largely unknown and only six species of obligate deep-sea fungi that form fruiting bodies on sunken woods have been reported to date.

In this study, 21 samples (19 deep-sea sunken plants, 1 deep-sea sunken seaweed, and 1 shallow water sunken plant) were collected from 5 different sites (shallow water depth: 5m, deep water depths: 720–5,707m) in the Western Pacific Ocean off Islands of Japan, to investigate the diversity and distribution of fungi related to deep-sea sunken plant substrates. Fungal amplicon analysis was performed on the samples by targeting the ITS rRNA gene region.

As a results, fungal fruiting bodies were observed on one of the sunken wood samples which was collected at a water depth of 5,707m, and morphological and phylogenetic analysis suggested this fungus as novel sp. closely related to *Oceanitis scuticella,* one of the obligate deep-sea fungi. No fungal ascomata was observed in other samples, but amplicon analysis detected sequences highly homologous to *O. scuticella* in 8 samples collected from different depths and locations. These results indicated that *O. scuticella* and its relatives commonly exists in sunken plant substrates in deep-sea environments. Furthermore, sequences with highly homologous to *Ceriosporopsis halima,* a cosmopolitan shallow marine fungus, were predominantly detected in the sample collected from the shallow water. Interestingly, sequences which showed homologous to *C. halima* but presumed to be a different species (approximately 86% homology) were detected in 4 samples from deep-sea environments. This suggests that a novel species closely related to *C. halima* inhabit in deep-sea environments.

Our results indicated that there are many undiscovered deep-sea fungi and some obligate deep-sea fungi, such as *O. scuticella*, are distributed in a wide range of water depths, from bathyal to abyssal zones. Further comprehensive investigations on their diversity, distribution, physiology and genomics will provide key insights into the adaptation, evolution and ecology of deep-sea fungi.

522A **Exploring the biogeography of Backusella: Insights into the distribution of early diverging fungi** Andrew S Urquhart[1], Alexander Idnurm[2] [1]Systematic Biology, Uppsala University, [2]University of Melbourne

The global distribution of nearly all micro fungal species, including within the Mucorales, is poorly understood in part because few studies have made direct comparisons between regional species compositions. The issue is compounded by the fact that novel Mucorales species are frequently described from only single isolation events. To address this deficit, we sampled the eastern states of Australia for strains in the genus Backusella (Mucorales; Mucoromycota). Backusella is a genus of saprotrophic fungi typically isolated from leaf litter. Because Backusella can be readily recognised by its recurved juvenile sporangia it can be readily recognised and isolated making it a convenient genus for culture-based study. In total, we have isolated 657 strains from across different regions of Australia including tropical rainforests in the north-east and temperate Eucalyptus forests in the south. Analysis of diagnostic DNA regions revealed that these new strains include at least 20 putative novel species that are so far unique to Australia. We generated genome assemblies for all species isolated and used these to robustly delimit species boundaries through genome-wide comparisons. The geographic distributions of the identified species show that the Australian wet tropics harbor a unique set of Backusella species compared to the temperate south-eastern region.

523A **Diversity and characterization of filamentous fungi isolated from sediments of Basque estuaries** Ainara Otamendi[1], Ziortza Agirrezabala Urkia[2], Carla Perez-Cruz[3], Raquel Liébana[3], Laura Alonso-Sáez[3], Maria Teresa Dueñas[1], Anders Lanzén[4,5], Oier Etxebeste[1] [1]Laboratory of Biology, Dept of Applied Chemistry, Faculty of Chemistry, University of the Basque Country (UPV/EHU), 20018 San Sebastian, [2]Applied Chemistry, Laboratory of Biology, Dept of Applied Chemistry, Faculty of Chemistry, University of the Basque Country (UPV/EHU), 20018 San Sebastian, [3]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Sukarrieta, [4]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Pasaia, [5]IKERBASQUE, Basque Foundation for Science, Bilbao

Fungi and bacteria within marine ecosystems contribute to ecological balance by playing critical roles in nutrient cycles and by shaping food webs. In this context, marine microbes developed genetic mechanisms to adapt and survive in marine environments and stress conditions such as, *e.g.*, high salt concentrations and nutrient scarcity, or to degrade complex polymeric substrates. These features make marine microorganisms a valuable source for the development of new biotechnological tools. However, marine environments and mainly marine fungi are still underexplored. Research on marine microorganisms is mainly focused on bacteria, with a couple of hundreds of fungal species retrieved from marine environments, despite the fact that the kingdom fungi is composed of millions of species. Here, we focused on the isolation of filamentous fungi, using sediment samples collected in estuaries of the Basque Country, Bay of Biscay. Their phenotypic characterization led to the identification of strains potentially able to grow on minimal culture medium supplemented with recalcitrant algal polysaccharides or to produce secondary metabolites. Two isolates belonging to the order Hypocreales were selected for genome sequencing (Illumina and Nanopore technologies) and analysis: 1) *Marquandomyces marquandii* due to its ability to secrete a yellow pigment described in the literature as urea sorbicillin and 2) *Albophoma yamanashiensis* for its apparent ability to grow in minimal culture medium supplemented with commercial fucoidan. Analysis and comparison of their CAZyme and secondary metabolite gene cluster repertoires with those of other species of the order Hypocreales, in combination with RNA-seq results, suggest that these isolates could be used as a source of new enzymatic activities and secondary metabolites.

524A **Accessing Fumonisin risk in corn from Nebraska and insight into the associated *Fusarium spp.* populations** Ram Kumar Shrestha[1], Andreia Bianchini Huebner[1], Heather Hallen-Adams[1], Jayne Stratton[1], Tamra Jackson-Ziems[2] [1]Dept of Food Science and Technology, University of Nebraska-Lincoln, [2]Dept of Plant Pathology, University of Nebraska-Lincoln

Fumonisins (FBs) are among the most toxic mycotoxins, primarily produced by *Fusarium proliferatum* and *F. verticillioides*. They are involved in disrupting sphingolipid metabolism and inhibiting ceramide synthase. Exposure to these mycotoxins can cause equine leukoencephalomalacia and porcine pulmonary edema in animals. FBs have also been associated with esophageal cancer and neural tube defects in humans. Notably, corn remains a crop susceptible to FB contamination. As Nebraska represents one of the significant states for corn production in the U.S., a survey had been designed to quantify the risk of FBs in corn from Nebraska and investigate the relative dominance of toxigenic *Fusarium* species contributing to FB accumulation. This study employed a field survey, sample collection, fluorometer-based fumonisin analysis, and genetic studies for *Fusarium* diversity. A total of 52 corn samples across the major corn-producing counties of Nebraska were collected in 2022. The overall mean FB concentration found in the samples was 2.7 ppm with a median of 1.5 ppm. However, 84.6% of samples were positive for FBs, with 17.3% of the samples above 4 ppm, and the highest concentration recorded was 19 ppm. Diverse *Fusarium* species were associated with corn, where *F. proliferatum* was the dominant FB-producing species. This data suggests a reasonable risk of FBs associated with corn produced in Nebraska, which indicates the need for effective mitigation strategies for affected crops intended for human consumption and

animal feed. An integrated approach, including optimal agronomic practices, better pest management, and improved storage conditions, can effectively reduce contamination risks and improve the overall quality of corn in Nebraska.

525A **Microbiome Profiling of Soybean Roots as Affected by Sudden Death Syndrome (SDS) and Fungicide Applications** Ma. Theresa Jonna Atienza-Parcon[1,2], Halil Polat[1], Leonardo Rocha[1], Febina Mathew[3], Nitha Rafi[3], Jason Bond[1], Ahmad Fakhoury[1] [1]School of Agricultural Sciences, College of Agricultural, Life and Physical Sciences, Southern Illinois University Carbondale, [2]National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, [3]Dept of Plant Pathology, North Dakota State University

Soybean *(Glycine max*) is one of the major crops in the United States. Almost four billion bushels of soybeans were produced in the United States in 2022. One of the most devastating diseases on soybeans is Sudden Death Syndrome (SDS). From 1996 to 2016, nearly $8 billion in economic losses were attributed to SDS. The disease is caused by the plant pathogenic fungus *Fusarium virguliforme*. In this study, two commercial chemical seed treatments that target F. virguliforme, Fluopyram (ILEVO) and Pydiflumetofen (Saltro), were tested. Soybean roots were collected and processed for metagenomic analysis. Two DNA markers targeting distinct microbial groups were used: bacteria (16S V3 and V4) and fungi (ITS2). Amplicons were sequenced using an Illumina MiSeq platform. Sequencing datasets were processed in R using the DADA2 pipeline. Across treatments, bacteria alpha diversity was found to be similar ($F = 4.1158$, $p = 0.0538$), while in the case of the fungal communities, alpha diversity was significantly different ($F = 6.5070$, $p = 0.0179$). For bacterial communities, no treatment effect on beta diversity was observed ($R^2 = 0.3489$, $p = 0.1440$). Similar to the bacterial profiles, the treatment did not affect the fungal beta diversity ($R^2 = 0.3606$, $p = 0.0730$). Ongoing study is being conducted to quantify *F. virguliforme* DNA in soybean roots and correlate it with the microbiome profiles.

526A **Virome characterization of a collection of *Botrytis cinerea* from Australia** Lorena Rodriguez[1,2], Rosalie Sabburg[3], Donald M Gardiner[3], Kim M Plummer[1], Scott Mattner[1,2,4], Anthony Gendall[1,2] [1]Deparment Animal, Plant and, La Trobe University, [2]Australian Research Council Research Hub for Sustainable Crop Protection, [3]The University of Queensland, [4]Victoria Strawberry Industry Certification Authority

Gray mould, caused by *Botrytis cinerea*, is a highly damaging pathogen impacting a wide range of crops, with prevalent fungicide resistance. Products derived from microorganisms offer an ecofriendly alternative to chemical controls that could be incorporated into biocontrol strategies. Among these biologicals, mycoviruses have emerged as potential biological control agents. Mycoviruses are widespread viruses infecting fungi, including *B. cinerea*, that are being investigated for their role in such approaches. Notably, there are currently no reports of the mycoviral diversity in *B. cinerea* in Australia. In this study, we conducted an exploration of the mycovirome of *B. cinerea* isolates from various hosts across different states in Australia. RNASeq analyses was used to investigate mycovirus diversity in 24 Australian *B. cinerea* isolates from diverse hosts and geographic regions. This lead to the identification of sequence contigs that corresponded to either partial or complete genomes of mycoviruses. Most isolates were infected with more than one mycovirus, and some isolates from different hosts shared identical or near-identical mycoviruses suggesting a recent transmission. To examine the impact of the mycovirus on the host, we documented alterations in the *in vitro* and *in planta* phenotype and growth characteristics of isolates with different mycoviromes. This study has enhanced our understanding of mycoviral diversity, and identified mycoviruses that could serve as active ingredients in biological products for the effective control of this devastating fungus. Furthermore, the identification of fungal viruses is a crucial step in initiating an understanding of the dynamic relationship between mycoviruses and the RNA silencing machinery (RNAi) in fungi. This opens up a new field of study where viruses influence or manipulate host gene expression regulation, with implications for both fundamental mycology and potential applications.

527A ***starbase*: A database and toolkit for classification of extremely large mobile genetic elements** Adrian E Forsythe[1], Emile Gluck-Thaler[2], Aaron Vogan[1] [1]Organismal Biology, Uppsala University, [2]Dept of Plant Pathology, University of Wisconson-Madison

Microbial genomes contain a diverse set of mobile genetic elements (MGEs), which can in turn mobilize large collections of host genes, including genes affecting pathogenicity. However, finding existing MGEs in a genome can be difficult, given that MGEs are often present across diverse taxonomic groups and MGE databases generally lack sufficient organization or ontology. This work focuses on the *Starships*, a recently described superfamily of extremely large (~20-700kb) cargo-carrying MGEs, endemic to *Pezizomycotina*. Their prevalence across highly diverse groups, emphasizes the potential of *Starships* for accelerating rates of adaptation in fungal pathogens. Yet, despite their widespread distribution, much remains unknown about the mobilization of host genes by *Starships* and the extent of their horizontal transfer between species. In order to address these questions, we curated a database of existing *Starship* sequences and developed a user-friendly toolkit to enable exploration and classification of these elements within existing genomes, which we call *starbase*. The current version consists of 635 unique *Starship* sequences, which are used to train a classifier for identification of existing elements within a collection of 4,144 fungal reference genomes, providing insight into horizontal transfer of elements between species. We also use *starbase* to survey the repertoire of cargo genes associated with different classes of *Starships*. The specific clusters of virulence/pathogenicity cargo genes mobilized

via *Starships* have the potential to enable niche expansion for ecologically or clinically relevant fungi. The diagnostic tools provided in *starbase* can be applied to future investigations of structural variation, furthering our understanding of how genes involved in pathogenicity can be mobilized by MGEs.

528B **The effects of urbanization on the community composition of amphibian, water, and sediment samples in a Worcester, MA waterway** Sara Wheeler, Manning DelCogliano Biology, Clark University

Urbanization negatively impacts the physical, chemical, and biological characteristics of water quality which in turn impacts the communities inhabiting these systems. As the rate of urbanization increases, it is becoming increasingly critical to study its effects on the health of urban biological communities to determine their responses to urban stressors and environmental degradation.

The Tatnuck Brook waterway in Worcester, Massachusetts presents a semi-continuum of urbanization, starting in a rural, protected area that transitions into increasingly developed areas with high levels of human activity and pollution making this watershed an ideal system to study the impact of urbanization on freshwater ecosystems and the amphibians that inhabit them.

Microorganisms are ubiquitous, abundant, and play vital roles in the nutrient and energy cycling of ecosystems. Because they exhibit fast growth rates and a sensitivity to environmental change, microbial taxa have the potential to serve as biological indicators of overall habitat health.

To gain new insight into urban impacts on microbial communities, we sampled and analyzed planktonic, sediment, and frog gut microbiomes. By studying all three sources of microbiomes, we sought to gain further insight into the associations between, and unique to, each of the different habitats, allowing us to assess and compare the potential changes in communities across these connected habitats in response to urbanization.

We found significant differences in fungal and bacterial species richness across different sources of sampling, and between high and low urbanization levels. Further, bacterial and fungal community compositions differ along the urbanization gradient indicating that both communities are being driven by similar environmental factors. These results indicate that urbanization affects this freshwater ecosystem with potential implications for environmental health due to increases in fungal pathotrophs, disease factors, and microorganisms associated with extensive human activity.

529B **Diversity of cycloheximide-tolerant fungi in South African gold mine substrates** Taygen Fuchs[1], Cobus Visagie[2], Brenda Wingfield[3], Michael Wingfield[2] [1]BGM, University of Pretoria, [2]FABI, University of Pretoria, [3]University of Pretoria

Gold mines provide optimal conditions for the growth of fungi. In a South African context, the significance of these fungal communities can be found in a historical link to outbreaks of sporotrichosis in gold mines. The causal agent of this disease, *Sporothrix schenckii*, is hypothesised to be associated with timber used to support the mine shafts. However, the pathogen has never been isolated from the timber or soil in mines where sporotrichosis is known to have occurred . Furthermore, the diversity of the fungal communities in South African gold mines remains largely unexplored.

The aim of this study was to evaluate the diversity of *Sporothrix* and other cycloheximide tolerant fungi in South African gold mineshafts.

We collected samples from the mining timber supports and soil in two mineshafts. Isolations from these samples were performed using a dilution-to-extinction approach and media containing high concentrations of the fungicide cycloheximide, to which *Sporothrix* are known to be highly tolerant. Fungi isolated from these samples were identified to at least genus level by sequencing the ITS region, with the LSU region sequenced for a subset.

Our approach proved useful in culturing slow-growing species and allowed us to isolate, preserve and identify a diverse group of fungi from each mine. The isolates included more than 30 genera residing in 14 orders and included several undescribed species. Our results showed that the environment in gold mines provides conditions for a diverse community of filamentous fungi to thrive.

530B **ZymoSoups: A high-throughput forward genetics method for rapid identification of virulence genes in *Zymoseptoria tritici*** Haider Ali[1], Megan McDonald[2], Graeme Kettles[2] [1]Bioscience, University of Birmingham, [2]School of Bioscience, University of Birmingham

The wheat disease Septoria tritici blotch is caused by the fungal pathogen *Zymoseptoria tritici* and is a serious threat to global wheat production. The ability of the fungus to overcome host genetic resistance conferred by disease resistance (*R*) genes makes disease management difficult. The mechanisms enabling this fungus to evolve and evade recognition by these *R* genes remains poorly understood. Identification of the avirulence (*Avr*) genes in *Z. tritici* that trigger host resistance would improve understanding of how this fungus escapes disease control. Conventional methods for *Avr* gene discovery in *Z. tritici* are time consuming and resource intensive. We therefore sought to develop an *in planta*-based forward genetic screen that would allow for rapid *Avr* gene identification.

We used the well-established *Stb6-AvrStb6* gene-for-gene interaction to develop this protocol. Co-inoculation of virulent and avirulent strains of *Z. tritici* led to observable disease symptoms, even when virulent strains were present in minimal abundance. This indicated that inoculation of strain mixtures (soups) onto leaves was a viable strategy. We then subjected the avirulent strain IPO323 to UV mutagenesis and evaluated ~92,000 mutants *in planta* on wheat containing *Stb6*. Mutants were combined in soups ranging from 200 to 500 mutants per soup. In total, we recovered 12 gain of virulence (GoV) mutants from 5 soups across two screens performed using the Stb6-containing wheat cultivar Cadenza. Subsequent whole-genome sequencing (WGS) of the GoV mutants (and avirulent controls) revealed different mutations associated with the *AvrStb6* locus in the GoV strains. One mutant had a single nucleotide polymorphism (SNP) in the AvrStb6 coding region. Notably, the other 11 virulent mutants exhibited significant genomic changes, specifically, large deletions at the end of chromosome 5, including the AvrStb6 locus.

These results would have allowed rapid identification of the *AvrStb6* gene as the virulence determinant on *Stb6*-containing wheat. This method could therefore be useful for identifying currently unknown *Avr* genes in this important phytopathogen.

531B **Elucidating the role of lipid flippase in host-*Cryptococcus neoformans* interactions during pulmonary cryptococcosis** Siddhi Pawar[1], Yina Wang[2], Varsha Gadiyar[1], Raymond Birge[1], Chaoyang Xue[1] [1]Dept of Microbiology, Biochemistry, and Molecular Genetics, Rutgers University, [2]Dept of Microbiology, Biochemistry, and Molecular Genetics Public Health Research Institute

Cryptococcus neoformans (*Cn*) is a facultative intracellular pathogen that infects the lung and disseminates to the central nervous system in immunocompromised patients. Alveolar macrophages are the first line of defense against *C. neoformans* infection, but the molecular basis of macrophage recognition and interaction with this yeast pathogen remains incompletely understood. Our previous studies have identified that Cdc50, a regulatory subunit of lipid translocase (flippase), is essential for virulence in *C. neoformans* and the *cdc50Δ* mutant cells were quickly cleared in the lung during infection. *In vitro Cryptococcus*-macrophage interaction assays revealed that the *cdc50*Δ mutant had an increased phagocytosis and macrophage killing than wild type, indicating macrophages in the lung may play an important role in its clearance during infection. Interestingly, the *cdc50*Δ strain has increased exocytoplasmic phosphatidylserine (PS) accumulation. We hypothesize that the accumulation of PS on the *cdc50Δ* cell surface may function as a phagocytic signal to promote macrophage recognition and phagocytosis, aiding to its clearance during the lung infection. Our recent findings also showed *cdc50*Δ strain produced more extracellular vesicles (EV) and expressed more PS on its surface compared to the wild type H99. Furthermore, *Cn* H99 when co-cultured with *cdc50*Δ EVs showed significant increase in phagocytosis, indicating EVs with increased PS level can stimulate macrophage function and enhance their fungicidal activity. To further understand how macrophages sense PS on fungal surface, we are testing the TAM PS receptors (Tyro3, Axl, and MertK) on their role in the early interactions between macrophages and *C. neoformans.* Our preliminary data suggest that one TAM PS receptor MertK is dispensable, while the potential contribution of other PS receptors remains to be determined. In addition, we are also investigating the potential host genes involved in *cdc50*Δ-macrophage interaction using RNA-Seq analysis. This study may lead to a better mechanistic understanding of early host-*Cryptococcus* interaction during pulmonary cryptococcosis and develop fungal flippase function as a potential therapeutic target.

532B **Characterization of *Candida auris* and other fungal pathogens in the dog oral mycobiome** Theodore C. White[1], Brooke D. Esquivel[1], Elisa M Rouse Salcido[1], Brandon L. Holder[1], Allison M. Schweiker[1], Erin Petro[2], Butch KuKanich[3], Kate S. Kukanich[4] [1]Biological and Biomedical Systems, University of Missouri-Kansas City, [2]Bureau of Epidemiology and Public Health Informatics, Kansas Dept of Health and Environment, [3]Dept of Anatomy and Physiology, Kansas State University, [4]Deptartment of Clinical Sciences, Kansas State University

The purpose of this study is to characterize the oral **mycobiome** of domestic dogs, to identify the commensal and potentially pathogenic fungi present, and to determine the level of drug susceptibility of these isolates to commonly-used antifungal drugs. 251 buccal swabs from dogs were obtained and struck onto ChromAgar medium that distinguishes fungal species based on colony color and morphology. Genomic DNA was extracted, PCR was used to amplify a fungal-specific variable rDNA region, and the

fragment was sequenced. The BLAST database was used to identify the fungal species. Of the 251 dogs swabbed, 73 had culturable fungi, and 10 dogs had multiple fungal species. Although the dogs did not show signs of oral infections, we did find fungal species that cause pathogenicity in animals and in humans. Among fungal isolates, *Malassezia pachydermatis* and *Candida* species were predominant. Drug susceptibility tests were performed on each isolate against medically-important antifungal drugs including fluconazole, ketoconazole, and terbinafine. A large number of isolates had high MIC values for all three drugs. Exploring the oral mycobiome of dogs, as well as the corresponding drug susceptibility profiles, has important implications for canine dental hygiene, health, and medical treatment. Identifying the microorganisms within the canine mouth can illustrate a common pathway for fungal pathogens of One Health concern to spread from dogs to humans.

While analyzing the dog mycobiome, we detected a strain of *Candida auris,* an emerging human fungal pathogen first detected in the United States in 2016. This isolate represents the first description of *C. auris*colonizing a human pet, the first identification of *C. auris* in a non-human mammal in the U.S. and the first *C. auris* isolate described from the state of Kansas. The isolate is a member of Clade IV, which has been found in patients in Chicago and Florida, while Clades I and III are the most prevalent in the United States. The isolate is resistant to fluconazole, terbinafine, and amphotericin B, but susceptible to caspofungin, consistent with many human *C. auris* isolates. The source of *C. auris* transient colonization in this dog is unknown, and there is no evidence that it was further transmitted to humans, other dogs in the shelter, or pets in its adopted household. **Isolation of C. auris from a dog in Kansas has public health implications as a potential emerging source for the zoonotic spread of this pathogenic fungus.**

533B **Unveiling an Underground War: Exploring the Interactive Dynamics Between Soybean Root Microbial Communities and the Incidence of SDS** Halil Polat[1], Ma. Theresa Jonna Atienza-Parcon[1,2], Leonarda Rocha[1], Jason Bond[1], Ahmad Fakhoury[1] [1]School of Agricultural Sciences, College of Agricultural, Life and Physical Sciences, Southern Illinois University Carbondale, [2]National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos

Soybean Sudden Death Syndrome (SDS) is a devastating plant disease that significantly affects soybean yields worldwide. Soybean yield losses from SDS in soybean-producing areas in the United States were estimated at nineteen million bushels in 2022. Remarkably, the 2022 estimated percent losses from SDS in the United States were the lowest observed out of 27 years of available data.

The role of microbial communities in plant health has been increasingly recognized. However, the intricate relationship between these communities and the development of SDS remains poorly understood. Root samples from different soybean lines were collected from experimental fields in Valmeyer, Illinois, in 2021 and 2022. High-throughput DNA sequencing was used to determine and analyze the composition and diversity of soybean root microbial communities with varying degrees of SDS incidence. Two DNA markers, 16S V3 and V4 for bacteria and ITS for fungi, were used. The DADA2 pipeline and R were used to analyze the generated sequencing datasets.

The alpha diversity of the bacterial communities at the species level was highly significant ($F = 4.7729$, $p = 0.0027$). In contrast, the alpha diversity of the fungal communities at the species level was not significantly different ($F = 1.4925$, $p = 0.2262$) across the tested soybean lines. Differences in the beta diversity were highly significant among soybean lines in terms of fungal and bacterial species profiles ($R2 = 0.4973$, $p<0.001$ and $R2 = 0.6073$, $p<0.001$, respectively).

This research contributes to a better understanding of the underlying mechanisms driving SDS in soybeans, with potential implications for sustainable management practices.

534B **Testing the role of a subtilase family in spherule formation and virulence of *Coccidioides*** Elena Ochoa, Christina Homer, Anita Sil UCSF

Coccidioides is a dimorphic fungal pathogen with the capacity to cause life-threatening illness in immunocompetent people. *Coccidioides* grows in the soil as a multicellular hyphal form. Once the soil is disturbed, spores can be aerosolized and cause infection if inhaled. Potential infection is a significant hazard to farm and construction workers within the Southwest United States and Central and South America where *Coccidioides* is endemic. Once inside the host, *Coccidioides* spores germinate and, rather than growing as hyphae, form a unique structure called the spherule. Little is known about the molecules that govern spherule formation and function, and it is imperative that more research is conducted since the spherule is the virulent form of *Coccidioides*. As spherules mature, they become filled with internal cells called endospores. Upon spherule rupture, endospores are released and disperse, going on to form new spherules and continue the life cycle. *Coccidioides* genomes encode two families of proteases, subtilases and deuterolysins. We have shown that spherule formation can be inhibited by treating *Coccidioides* with a protease inhibitor that blocks subtilase activity, thereby suggesting that subtilase function is important during spherulation. We have been

exploring the role subtilases play in spherule development, endospore release, and virulence. To accomplish this, we are creating 6 different subtilase knockout mutants to assess their ability to form spherules, release endospores, and cause disease in the mouse model of infection. If we identify individual proteases that are critical for any of these processes, they will be excellent targets for drug discovery, since protease inhibitors have been successful therapeutics for diseases from HIV to cancer. Thus this work is poised to identify key players in fungal development that will be valuable targets for future antifungal drug development.

535B
Genetic transformation of the frog-killing chytrid fungus *Batrachochytrium dendrobatidis* Stephanie M. Brody, Erik Kalinka, Andrew J. M. Swafford, Edgar M. Medina, Lillian K. Fritz-Laylin University of Massachusetts Amherst

Batrachochytrium dendrobatidis (*Bd*) is an etiological agent of chytridiomycosis, an infectious disease which is devastating amphibian population around the globe. *Bd* is a chytrid fungus, a group of early diverging fungi that alternate between two distinct life stages: motile zoospores that lack cell walls and can swim and crawl through the environment and stationary sporangia that are encased in a chitinous cell wall. Studies to understand the molecular mechanisms driving *Bd* biology, development and pathogenesis have been hindered by the lack of genetic tools. Here we describe the development of genetic transformation of *Bd*. We engineered plasmids encoding hygromycin resistance cassettes, as well as luciferase or fluorescent proteins. Using electroporation to deliver these plasmids into *Bd* zoospores, we can detect transgene expression for multiple generations. We can use this system to express fusion proteins under both heterologous and native promoters and have validated transformed strains by fluorescence microscopy and immunoblotting. As a proof of concept, we expressed a genetically encoded fluorescent probe for the actin cytoskeleton, which we imaged in live cells to visualize the distribution and dynamics of polymerized actin through the *Bd* life cycle and during key developmental transitions. Stable transformation mediated by homologous recombination would stimulate testing of key hypotheses using knockout strains, endogenous tagging and multi-generational studies. We are currently extending our work towards stable genetic transformations by using gene targeting vectors that utilize the native homologous recombination pathway. These transformation tools pave the way for answering fundamental questions about *Bd* biology and pathogenesis.

536B
Biosystematics and temperature adaptation in the enigmatic *Lulworthiales* Teppo Rämä[1], Ole Christian Hagestad[2] [1]UiT The Arctic University of Norway, [2]Institute of Marine Research

The *Lulworthiales* (*Sordariomycetes*, *Ascomycota*) is one of the most iconic taxa of marine fungi with more than sixty described species distributed across approximately twenty genera. This fungal order has been studied for more than a century and species have been documented to occur in tropical to cold waters around the globe, yet the systematics and ecological importance of the order remain poorly understood.

Lulworthiales fungi have diverse ecological roles in the marine environment with the majority of species being endophytic or saprotrophic, yet pathogens and parasites can also be found. The sexual morphs develop perithecial ascomata that bear deliquescent asci and typically hyaline, filamentous spores. Morphological characteristics, such as spore dimensions, are largely overlapping and have posed major challenges to the systematics of the order. Overlapping morphology together with poor type material has increased the taxonomic confusion and has resulted in many *nomen dubium* existing in the order. Molecular studies have contributed to several new species and genera being described the recent years, whereas a more comprehensive revision of the *Lulworthiales* based on broad gene and taxon sampling remains to be done. Moreover, only one genome has been published.

Here, we present the cryptic species diversity in the order *Lulworthiales* based on our and publicly available molecular data. We present evidence for multiple new taxa existing in the cold Arctic waters where *Lulworthiales* fungi seem to be abundant and are likely to play major ecological roles. We also present a project studying temperature adaptation in *Lulworthiales* using culturing experiments and comparative omics data.

537B **Genomic insights into recurrent vulvovaginal candidiasis** Abdul-Rahman Adamu Bukari[1], Javier San Juan[1], Yana Syvolos[1], Rebekah Kukurudz[1], Vanessa Poliquin[2], Aleeza Gerstein[1,3] [1]Microbiology, University of Manitoba, [2]Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, [3]Statistics, University of Manitoba
Vulvovaginal candidiasis is one of the most common vaginal and fungal infections. The majority of symptoms are successfully treated with antifungal drugs, but in ~9% of cases, symptoms return even with treatment. Although there are some known risk factors for recurrence, many cases are idiopathic. We sought to examine the genotypic diversity of yeast populations that are present during a symptomatic infection to gain insight into the evolutionary processes that function during these chronic infections.

We collected a total of 116 yeast isolates from vaginal swabs of 12 participants (6-12 isolates each) with a history of symptomatic recurrent vulvovaginal candidiasis. Ten of the participants had a *Candida albicans* infection while two had *Nakaseomyces glabrata*. To precisely quantify the standing genetic variation and genetic relatedness of the isolates colonizing each participant, we conducted phylogenetic analyses placing the *C. albicans* and *N. glabrata isolates* into global phylogenetic trees, consisting of 413 and 526 isolates respectively. Our phylogenetic analyses revealed that all isolates from an individual are highly clonal. The average nucleotide diversity among isolates from the same participant was < 0.003, with between 596 to 5,977 single nucleotide polymorphisms differentiating contemporary isolates. *C. albicans* isolates from seven (of 10) participants clustered closely within clade 1. Examining the entire tree, clade 1 was statistically overrepresented for vaginal isolates compared to other clades. To determine whether individuals were colonized by a unique genotype in the vagina than other body parts, rectal isolates from four participants and an oral isolate from one participant were compared with the vaginal isolates. In all cases, the other-site isolates were phylogenetically overlapping with vaginal isolates, indicative of frequent migration between sites. Isolates from the same participant exhibited a consistent loss of heterozygosity (LOH) profile, but one individual had isolates with two different LOH profiles on the left arm of chromosome 1. Specifically, 11 out of 24 isolates had a ~1Mb LOH region containing 461 genes, most of whose functions are unknown. Notably, this LOH was present in both rectal and vaginal isolates, but it was more prevalent in the vaginal isolates. This study highlights the within-host isolate variation during a yeast infection and shows the similarity between isolates within individuals with recurrent vulvovaginal candidiasis.

538B **Unraveling the complexity of chronic cryptococcosis: mixed cryptococcal infections and in-host evolution** Marhiah C Montoya[1], Kayla Wilhoit[2], John R Perfect[1], Paul M Magwene[3] [1]Infectious Diseases, Duke University, [2]Duke University, [3]Biology, Duke University

Cryptococcus neoformans is an opportunistic human fungal pathogen that is ubiquitous in the environment and is an important cause of disease in immunosuppressed patients. Although incidents of *Cryptococcus* persistence, relapse, and reinfection have been reported since the 1950s, little is known about the genetic and phenotypic changes of *Cryptococcus* that occur within the human host beyond one year. Here we present six patients with chronic cryptococcal infections with serial isolates collected from one year to over 20 years apart. Clinical specimens contained multiple genotypically and phenotypically distinct *Cryptococcus* strains, representing phylogenetically distinct lineages within *C. neoformans*, or in some cases multiple *Cryptococcus* species. Within this small patient cohort, that had no international travel, we identified VNI, VNII, VNIV, VGI, serotype AD hybrids (VNI/VNIV and VNBII/VNIV), and *C. neoformans* molecular type hybrids (VNII/VNBII). In conjunction with observing mixed infections within a single specimen, we also observed time-dependent introduction of new strains and species as well as persistence of multiple co-infecting lineages over time. Among persistent co-infecting lineages obtained from the CSF of multiple patients, genotypic analysis revealed a large amount of ploidy variation which coincided with phenotypic changes including the development of pseudohyphal cell morphology, reduction in capsule size, increase in melanin formation, and reduction in antifungal susceptibility to both Amphotericin B and Fluconazole. From this cohort of chronic cryptococcosis patients and their serial yeast isolates collected over extended periods of time, it is apparent that some infections occur with multiple cryptococcal strains and species wherein the diversity and plasticity of genotypes and phenotypes provide a range of strategies in the production of disease.

539B **Plastic-associated fungi of agricultural polyethylene-mulch in Western Oregon and their bioremediation mechanisms.** Leon Rogers, Gerald Presley Wood Science, Oregon State University

The application of polyethylene plastic to agricultural lands now amount to over 1 million pounds annually in the United States. Globally there are over 12 million tons of plastic applied annually in agriculture and only a fraction of this plastic is ever recovered. Soil-fungi are burdened with this massive influx of un-cyclable carbon and impacts to microbial communities are unknown. Despite several studies identifying fungal activity against various plastics there is no consistent means of biodegradation for synthetic polymers. Explanatory mechanisms such as laccase and peroxidase have little support, despite frequently being assumed to play a key part in plastic degradation and understanding the mechanisms involved will requires a broader approach than single fungi growth trials. This study is part of a larger suite of experiments meant to identify relevant factors in fungal bioremediation of plastics being carried out on a 12-acre (4.85 hectare) field site on Santiam Valley Ranch in Western Oregon (US). In the field-site a leased plot had polyethylene mulch applied then improperly removed and abandoned, resulting in 12” (30 cm) of topsoil being inundated with shredded plastic sheeting which has been weathered and degraded for the past six years. We are using this as a source of plastic enrichment to observe impacts in fungal communities.

We have performed community analysis from Illumina-sequenced ITS regions to compare soil-cores with and without plastic inclusions, recovered two unique fungal isolates that consistently survive surface sterilization of recovered plastic, and performed

mass-loss, FTIR, and microscopic assessment of controlled fungi-on-plastic exposures to replicate prior studies claiming successful bioremediation.

We are also in the process of comparing RNA expression measured by dPCR with primers for conserved regions of *cutinase, laccase, manganese-peroxidase, lignin-peroxidase,* and *versatile-peroxidase* that will compare community-level RNA expression relative to distance from plastic inclusions. This expression analysis will also be performed on single fungi grown in minimal-media with different polymers: LDPE, PET, and degraded field-site polyethylene, as well as so-called "biodegradable plastic mulch." In minimal-media trials LDPE was the most likely plastic to be modified, and "grey-rot" fungi including *Schizophyllum commune*, *Xylaria sp.*, and *Hypoxolon sp.* were more active than other white-rot and brown-rot fungi.

540C **Culture-Based estimation of Mucoromycota Communities: Insight into Plants as Biotic Drivers in Shaping Community Structure** Alicia Kock[1], Mmanoko Napo[2], Kazeem Alayande[3], Jessie Uehling[4], Teresa E Pawlowska[5], Rasheed Adeleke[3] [1]Microbiology, North-West University, Potchefstroom, [2]Microbiology, North-West University, [3]North-West University, [4]Oregon State University, [5]Cornell University

Mucoromycota fungi represent a diverse and ecologically significant group within the fungal kingdom, with members found in a wide range of environments, including soil, plant roots, and in various symbiotic associations. Our study aimed at isolating members of Mucoromycota fungal sub-phylum species from rhizosphere soil of two different plant species; a dominant species from Asteraceae family and a co-evolving plant species from another family. The study area focussed on the desert (Nama Karoo) and coastal regions (Fynbos) of South Africa. In each eco-region, two sampling sites were selected and sampling was done along three 40m long transects separated by 300m, at each site. Our lab-based methods started with culturing the soil on wheat germ agar and once pure cultures were obtained DNA extractions were performed on each single isolate. We then focused on amplifying the 28S region of the pure single isolate DNA and sequenced the 28S region to determine diversity. Our methods have allowed us to isolate multiple representatives of Mucoromycotina and Mortierellomycotina. According to our results Mucoromycotina proves to be the dominant subphylum isolated from the desert eco-region, with the majority of isolated species being *Rhizopus* spp. and *Cunninghamella* spp. It was also found that the desert eco-regions have a higher fungal diversity compared to the coastal eco-region. Through our study we expect to compare fungal communities isolated from two different eco regions to compare their community structure and to investigate the drivers affecting their community assembly. In conclusion, our research represents a significant step forward in understanding the community structure of Mucoromycota fungi, shedding light on their diverse evolutionary strategies and functional roles in different ecosystems.

541C **Fusarium Head Blight poses a new threat to Eastern Africa: examining the pathogen genomics and mycotoxin profiles of the 2022 FHB outbreak** Liza M DeGenring[1], Milton Drott[2], Karen Broz[2], Ashenafi Gemechu[3], Yanhong Dong[4] [1]University of Minnesota, [2]Cereal Disease Laboratory, USDA-ARS, [3]Ethiopian Institute of Agricultural Research, [4]Dept of Plant Pathology, University of Minnesota

Fusarium Head Blight (FHB) is caused by several species of fungi in the *Fusarium graminearum* species complex (FGSC) and is one of the most important diseases of cereal crops worldwide. FHB can cause yield reductions and contamination of the cereal grain with the harmful trichothecene mycotoxins. Over the last several years, there has been an increase in outbreaks of FHB reported in Ethiopia with it reaching epidemic levels in 2022. Ethiopia is the largest wheat producer in Sub-Saharan Africa with over 1.8 million hectares grown primarily by small-holder farmers. While wheat production in the region is increasing, questions remain about the casual species associated with the outbreak and the overarching threat to human health. ITS sequencing on 64 strains isolated from diseased wheat heads demonstrated that while 18% of isolates are from FGSC, the mycotoxin producer, *Epicoccum sorghinum*, is commonly found on diseased heads. Chemical analysis of 27 wheat flour samples found several with deoxynivalonol concentrations above the FDA limit of 1 ppm for human consumption, with evidence that Durum wheat was particularly vulnerable to disease. Additionally, we find a mix of NIV, 3ADON, and 15ADON trichothecenes, emphasizing that the outbreak comprises diverse mycotoxin-producing fungi and is not an epidemic expansion of a single lineage. Comparing whole-genome sequences of eight FGSG isolates to publicly available data clarifies the etiology of this outbreak and emphasizes concerns about the potential contamination with other mycotoxins.

542C **Diversification and Conservation of Fungal Inhibitor of Apoptosis Proteins** Miette Hennessy[1], Meareg Amare[1], Grant Nickles[1], Mehdi Kabbage[1], Nancy Keller[1], Neta Schlezinger[2] [1]Plant Pathology, University of Wisconsin - Madison, [2]Hebrew University of Jerusalem

Inhibitors of Apoptosis Proteins, or IAPs, are one of the only elements of the apoptotic pathway with sequence conservation in fungi and provide a unique window into the regulation of fungal programmed cell death, which is relatively poorly understood.

Fungi have long been thought to have exactly one IAP in their genomes, which is essential in some species and not in others, and these IAPs are highly conserved across the fungal kingdom. We have interrogated the function of AnBIR1, the IAP of the model organism A. nidulans. We find that AnBIR1 is essential for survival and inhibits caspase-like activity, and its precise regulation is required for functional asexual and sexual reproduction, regulation of secondary metabolism, and cell wall stability. Remarkably, our expanded bioinformatic analyses revealed that many fungi have multiple IAPs and have further identified several species with high numbers of IAPs as candidates for further study. These organisms include lichen-forming fungi, which are regularly exposed to long-term desiccation and UV exposure among other abiotic stressors, and an extremely halotolerant black yeast, Hortaea werneckii. This suggests that duplication, radiation, and/or specialization of IAPs may help fungi tolerate unique environmental stressors through distinct mechanisms of cell death regulation. Based on these results, we are investigating the specific functions of the IAPs in these unique organisms, especially as they relate to stress tolerance. We are also interested in parsing the differences between individual IAPs within a species to determine whether they may have more specialized functions based on their unique C-terminal domains. Overall, this work assesses the functional conservation and diversification within and across fungi and continues the genomic and bioinformatic effort to refine our understanding of the evolution of IAPs.

543C **Toward a global understanding of fungal mitochondrial genomics** Steven Ahrendt[1], Sajeet Haridas[1], Asaf Salamov[1], Kurt LaButti[1], Robert Riley[1], Igor V Grigoriev[1,2], Stephen J Mondo[1] [1]DOE Joint Genome Institute, [2]Plant and Microbial Biology, University of California, Berkeley

Mitochondria are specialized organelles found within the cells of nearly all eukaryotes. It is generally accepted that mitochondria are derived from an ancient endosymbiotic event, and therefore possess their own genomes (mitogenomes). Fungal mitogenomes retain a set of genes related to electron transport and oxidative phosphorylation, translation, and tRNA processing, although this gene set is substantially reduced relative to mitogenomes from other Eukaryotes. Fungal mitogenome annotation is challenging due in part to the large number of self-splicing introns, often containing homing endonuclease genes. The existing fungal mitogenome annotations are largely from the Dikarya; as such, comprehensive, kingdom-wide surveys have remained elusive. Here, we test a new annotation pipeline developed at the Joint Genome Institute for automatically annotating fungal mitogenomes, utilizing ab initio predictions as well as HMM-based predictions from a core set of conserved genes. We present a broad overview of around 400 mitogenome annotations from across the Fungi. Single-contig circular fungal mitogenome assemblies range from 12-13 kb (Cryptomycota) to over 1 Mb (Pezizaceae). Genes encoding subunits from Complex III and IV are conserved across our dataset. We see noticeable losses of Complex V genes atp8 and atp9 in related clades of Dothideomycetes, and losses from Complex I in the Cryptomycota. A phylogenetic comparison between mitochondrial and nuclear genome trees reveals that they broadly share the same topology. We investigated tRNA distribution and codon usage related to alternative translation strategies, revealing patterns of codon losses in specific clades and shifts in tRNA utilization. We also investigated the distribution pattern of introns and homing endonuclease genes (HEGs) across the kingdom. Additionally we have used our approach to uncover thousands of fungal mitochondria in public metagenomic datasets from IMG, and continue to assess its advantages in exploring eukaryotic diversity in metagenomic studies. Going forward, MycoCosm will have new mitogenome analysis tabs for individual genome portals, as well as a utility for making kingdom-wide comparisons. Overall, this effort will provide the foundation for a more comprehensive understanding of fungal mitochondrial genome structure and evolution.

544C **The virulence factor Ave1 of the fungal plant pathogen *Verticillium dahliae* displays antimicrobial activity by targeting bacterial membranes and cell walls** Gabriella Petti[1], Nick Snelders[1], Biwen Wang[2], Edgar Chavarro-Carrero[1], Ashok Rout[3], Daniel Friedrich[1], Alvaro Mallagaray[3], Leendert Hamoen[2], Bart Thomma[1] [1]University of Cologne, [2]University of Amsterdam, [3]University of Lübeck

Verticillium dahliae is a soil-borne fungal plant pathogen that causes disease in a broad range of plant species. During host colonization, *V. dahliae* not only interacts with its host but also with the host-associated microorganisms, collectively forming the host microbiota. To promote host colonization, *V. dahliae* secretes molecules called effectors that can target the host or its associated microbiota. The *V. dahliae* effector protein Ave1 contributes to virulence by inhibiting the growth of microbial competitors in the host microbiota. VdAve1 is a small, positively charged secreted protein that lacks structural or sequence homology with known antimicrobial proteins, suggesting that it deploys a novel mode of action. Here, we report the three-dimensional structure of VdAve1 solved by NMR spectroscopy. VdAve1 folds in a globular structure primarily consisting of β-sheets. We show that VdAve1 suppresses bacteria by inducing membrane disruption, mediated by a positively charged stretch of the protein which forms a surface-exposed β-sheet on the VdAve1 structure. To further investigate the mechanism of action of VdAve1 we performed transcriptome profiling and forward genetic analyses on a VdAve1-sensitive bacterium. These analyses pointed to the involvement of the cell wall component lipoteichoic acid in the bacterial response to VdAve1. Additionally, we observed binding of VdAve1 to lipoteichoic acid by NMR spectroscopy. Collectively, our findings indicate that VdAve1 not only interacts with the bacterial plasma membrane but also with its cell wall.

545C **Spatial variability in bacterial and fungal communities of apples (*Malus domestica*): unexpected patterns of nestedness and co-occurrence from individual fruits to the orchard scale** Justin P Shaffer[1], Rob Knight[2,3], Susan R Whitehead[4] [1]Biology, California State University, Fresno, [2]Dept of Pediatrics, School of Medicine, University of California, San Diego, [3]Dept of Computer Science and Engineering, University of California, San Diego, [4]Biological Sciences, Virginia Tech

Advances in the biological sciences have expanded our understanding of how we define an organism. Similar to the human microbiome contributions to human health and sustainability, terrestrial plants are now best understood as just one component of the **phytobiome** – the plant and its associated microbiome, herbivores, pollinators, and other symbionts. Nearly all plant traits can be influenced by the phytobiome, including growth rate, nutrient uptake and retention, and responses to environmental stress and disease. Looking forward, it is of utmost importance that current management practices incorporate our understanding of the phytobiome and its effects on plant health, including the links between management practices, phytobiomes, and food quality and yield. Here, we use apples as a model system to improve our fundamental understanding of the fruit microbiome and overall agroecosystem of an apple orchard, including variables that shape and that are impacted by the fruit microbiome. Most studies of the apple phytobiome have focused on broad patterns of microbial community composition across regions, or at the consumer point-of-purchase. However, a critical first step in developing microbially-based management practices is to understand the spatial variability of apple microbial communities and associated changes in fruit quality at scales relevant for local management. We conducted a spatially-explicit survey of microbial communities at multiple scales within a single orchard: across 17 trees within a single orchard block, across 16 fruits within a single tree, and across 22 microsites within a single fruit. For each fruit, we sampled both epiphytes present on fruit surfaces, and endophytes colonizing internal fruit tissues (i.e., pulp). We performed a spatial analysis of bacterial+archaeal and fungal communities, and volatile small molecules from these samples, including analyses of nestedness of microbes and samples across space, as well as co-occurrence of different microbial taxa. Our results highlight unexpected patterns of nestedness and co-occurrence of bacteria+archaea and fungi, and inform best practices for profiling microbes and metabolites for monitoring rapid changes in community composition and metabolic profiles across space. The long-term outcomes of this work include improving human health and increasing the economic sustainability of farms, in part by optimizing pest management via biotechnological applications with the phytobiome.

546C **A Secondary Account of the North American Species of *Rhizopogon*** Alija Mujic[1], Thelmalyn Montenegro[2], Matt Gordon[3], Emeline Pano[1], Kelli Van Norman[4], Darci Rivers-Pankratz[4] [1]California State University, Fresno, [2]Biology, California State University, Fresno, [3]Molecular Solutions, LLC, [4]USDA Forest Service, Region 6

Rhizopogon is a genus of truffle-forming fungi that forms mutualistic ectomycorrhizal (ECM) relationships with Pinaceae trees, the family of pine trees, which are critical to the healthy function of coniferous forests. ECM relationships are important because fungi protect plant roots from pathogens, directly exchange nutrients with plants, and facilitate environmental nutrient cycling. *Rhizopogon* species possess reduced morphology, or loss of distinguishing morphological features over evolutionary time, compared with other fungi, and traditional identification methods based upon morphology have failed to accurately describe the true species diversity of the genus. The purpose of this study is to refine and clarify the species concepts of *Rhizopogon* species in North America, with a particular focus on the Pacific Northwest geographic region. Alexander Smith and Sanford Zeller defined most of the North American species in their 1966 monograph, "A preliminary account of the North American species of *Rhizopogon*", using morphological characters to assess evolutionary relationships and species boundaries. The 1966 monograph generated many species-level classifications that proved to be an overestimation of true species diversity in the genus. More recent molecular phylogenetic analyses of *Rhizopogon* have established 5 subgeneric levels in genus and synonymized some of the species defined by Smith and Zeller. The current study seeks to further refine species boundaries of North American *Rhizopogon* by generating new sequence data from recent collections as well as Smith's type specimens deposited the University of Michigan Herbarium (MICH). We have generated new sequence data for 95 of the 146 *Rhizopogon* holotype specimens deposited in MICH as well as 81 type and non-type *Rhizopogon* specimens collected from the Western United States. Sequencing older type specimens is difficult, and a modified DNA extraction protocol designed to extract DNA from spores and modern enzyme technology contributed to our success. We inferred multigene phylogenies of *Rhizopogon* sequence data using maximum likelihood and Bayesean analyses and summarize these finding in our "secondary account" of North American *Rhizopogon* species. Our results significantly revise species hypotheses and systematic relationships using the phylogenetic species concept and provide valuable information for federal land managers where sensitive species of *Rhizopogon* are found.

547C **Fungi in the Foothills: *Cortinarius* Species Diversity in the Sierra Nevada Oak Woodlands** Danielle C. Sublett, Alija Mujic California State University Fresno

Cortinarius is one of the most diverse genera among the Basidiomycota and ectomycorrhizal fungi, with an estimated 5,000 species worldwide. Fungi serve integral roles in forest ecosystems, facilitating nutrient uptake via ectomycorrhizal (EcM) associations. Despite these beneficial symbiotic relationships, fungi remain understudied in Central California. *Quercus* species occupy much of the California landscape, serving as ectomycorrhizal tree hosts, and creating critical habitats for native plant and vertebrate species. The once prominent *Quercus* savannah habitat is now an endangered ecosystem in California, with many of these lands privately owned and subject to development. In this study, we performed a biodiversity survey of *Cortinarius* in the Sierra Nevada foothill regions using sporocarp and soil sample collections. We amplified the internal transcribed spacer (ITS) region for Sanger sequencing of 69 *Cortinarius* specimens to determine the systematic placement of local species using maximum likelihood phylogenetic analyses. We collected 42 rhizosphere soil samples of *Quercus douglasii, Quercus lobata*, and *Quercus wislizeni* across five sites spanning an elevation gradient in the Sierra Nevada foothills. We utilized Illumina metabarcoding of the ITS 1 region for soil fungal communities associated with these *Quercus* species.

A total of 28 operational taxonomic units (OTUs) were observed in soil samples, the majority of which differed from *Cortinarius* species observed fruiting. A total of 64 unique *Cortinarius* species were observed between sporocarp and soil samples in my phylogenetic analysis. ANOVA and ordination analyses were conducted to evaluate *Cortinarius* species diversity with respect to elevation and *Quercus* association. Our results indicate a significant difference in EcM species diversity communities across tree host, with greatest EcM species diversity in association with *Q. douglasii*. *Cortinarius* species are diverse in the Sierra Nevada foothill habitats, and appear to be generalists in association with their oak tree hosts.

548C **Genome evolution and diversification in the genus *Cercospora*: Integrative insights from phylogenomics and comparative genomics** Pedro H D Santos[1], Vinson Doyle[2], Jonathan Richards[2] [1]Plant Pathology and Crop Physiology, Louisiana State University, [2]Louisiana State University

Characterizing the evolutionary forces that have shaped the genomes of fungal pathogens is crucial for elucidating their ecological roles and understanding their adaptation and diversification. However, major gaps remain in our understanding of the diversity and evolution of important genera of fungal pathogens, like *Cercospora*, due to a lack of genomic resources for most species. A robust understanding of genome evolution within *Cercospora* requires both a reliable estimate of the species tree and well-annotated genome assemblies. In order to begin addressing the existing knowledge gaps, we generated high-quality assemblies from ex-types and North American field collections of several species of *Cercospora* and combined these with previously published genomic resources to understand genome evolution and diversification in the genus. We used both Nanopore and Illumina sequencing to generate high-quality assemblies and generated evidence-supported gene annotations using RNAseq data from cultures. Additionally, transposable element families were identified and annotated in each genome. We then compared the species tree estimates from ultraconserved elements (UCEs) and single-copy orthologs to evaluate the utility of reduced representation sequencing for expanding diversity studies in *Cercospora* and leveraged the highly contiguous genome assemblies to understand the evolution of genomic architecture. Preliminary analyses suggest the topologies inferred from UCEs and single-copy orthologs are largely concordant. Genome size in *Cercospora* has decreased through the course of evolution and is correlated with a reduction in transposable element content. Based on current sampling and phylogenomic results, most of this genome reduction appears to be the result of a single evolutionary event rather than a stepwise reduction in TEs. This study lays the foundation for expanding genome-scale evolutionary studies in the genus *Cercospora* so that we can better understand host-pathogen interactions, niche adaptation, and genome evolution.

549C **Core set of genes in *Ashbya gossypii* and *Saccharomyces cerevisiae*** Fred S Dietrich Molecular Genetics and Microbiology, Duke University

With next generation sequencing and the ability to sequence large numbers of genomes of diverse strains of a single species, it is possible to ask the question “What is the set of genes in the species”. This is a significantly different question than “What is the set of genes in the reference strain.

Using a set of 94 *S. cerevisiae* strains where the genome sequences have been completely sequenced, except for some repetitive subtelomeric regions, we have been able to identify a preliminary view of the species as having 5873 core genes, where in this case “core gene” was arbitrarily defined as genes present in 95% of these strains at the same location and orientation. Variable genes are present in less than 95% of these strains, or at variable locations. These core genes include both protein coding genes, and

genes not encoding a protein. The analysis of the carefully edited *S. cerevisiae* genomes also revealed previously unrecognized transposons, an unusual tRNA gene, gene remnants, and pseudogenes. More details on the S. cerevisiae core set of genes is available at https://www.biorxiv.org/content/10.1101/2023.09.07.545205v1

Using these sets of core and variable *S. cerevisiae* genes we can thus re-evaluate the set of genes in strains of the genus *Ashbya* to identify the overlap in gene set with the core set of *S. cerevisiae* genes, the variable genes, and the extent to which core genes versus variable genes are conserved between species. This is a preliminary analysis, as a much larger set of fully sequenced, edited, annotated genomes for each species will be required to begin to identify the majority of the variable genes, refine our view of the core genes, and give a more complete view of the genomes of these species.

550C **Phylogenetic diversity of phyllosphere yeasts in *Populus trichocarpa*** Maria-Jose Romero-Jimenez[1], Devin Leopold[2], Posy Busby[1] [1]Oregon State University, [2]Jonah Ventures
Yeasts and yeast-like fungi are abundant colonizers of leaf surfaces that produce secondary metabolites, form biofilms, and modify plant disease severity. Phyllosphere yeasts have been studied using cultured based methods and metabarcoding across several plant hosts. In these studies, the rDNA internal transcribed spacer (ITS) has been used to assign taxonomy and describe diversity. However, its sole use limits taxonomic resolution and our understanding of the phylogenetic diversity of leaf-associated yeasts. The aim of this study is to characterize the phylogenetic diversity of culturable yeasts of *Populus trichocarpa* leaves using a multi-gene phylogeny. A total of 250 yeasts were isolated from leaves of wild trees. Yeasts were morphotyped, and DNA was extracted from 70 representative isolates. The ITS and the partial ribosomal large subunit (LSU) regions were PCR-amplified, sequenced, and aligned to identify taxonomic groups. Yeasts from 20 different genera were represented in the collection, with a high abundance of *Aureobasidium*, *Filobasidium*, and yeasts from the Tremellales order. Uncharacterized clades of Ustilaginales and Pezizomycotina yeasts were also present in the collection. Additional genes (cytochrome b, the translation elongation factor 1-alpha, the largest subunit of RNA polymerase II, and the second largest subunit of RNA polymerase II) are now being amplified and sequenced to expand and improve taxonomic resolution and phylogenetic relationships for the 70 representative isolates.

551C **Leveraging strain heterogeneity within the nonpathogenic fungus *Aspergillus fischeri* to highlight factors associated with virulence** David C Rinker[1], Karin Steffen[1], Thomas Sauters[1], Manuel Rangel-Grimaldo[2], Huzefa Raja[2], Adiyantara Gumilang[3], Thalia Reis[4], Camila Pinzan[5], Patrícia Alves de Castro[5], Gustavo E Goldman[5], Nicholas Oberlies[2], Antonis Rokas[1] [1]Biological Sciences, Vanderbilt University, [2]UNC Greensboro, [3]Vanderbilt University, [4]Universidade Federal do Triângulo Mineiro, [5]Universidade de São Paulo

Strain heterogeneity within the human fungal pathogen *Aspergillus fumigatus* is appreciated to be a complicating factor when addressing questions related to the detection, control, treatment, and prognosis of acute aspergillosis cases. The variable pathogenic potentials exhibited between conspecific fungal isolates hints at the myriad and dynamic evolutionary processes which underlie this phenotype. This range in pathogenicity also suggests the surprising hypothesis that even strains of an *Aspergillus* species considered as being “nonpathogenic” may show similar heterogeneity in their pathogenic potential.

To explore this possibility, we comprehensively characterized the genomics, metabolomics, and pathogenic phenotypes of 16 strains of a widely distributed, “nonpathogenic” sister species to *A. fumigatus, A. fischeri. In vitro* and *in vivo* assays measuring the pathogenic potential of these strains demonstrated there to be a wide range of variation across these *A. fischeri* isolates. Genomic, transcriptomic and metabolomic profiling suggested several pathways and metabolites that may contribute to the observed intraspecific variation in virulence. Notably, pangenome analysis showed that our strains likely did not capture the complete breadth of genomic diversity within *A. fischeri*, holding open the possibility that the range of phenotypic variation may be even greater than we observed.

In summary, we employed a multidisciplinary research strategy to provide a novel perspective on some of the factors underlying the evolution of virulence within *Aspergillus* section Fumigati. Importantly, our results reinforce the contribution of strain heterogeneity to phenotypes, particularly within rapidly evolving species.

552C **Comparative mitogenomic analysis of *Rhizoctonia* spp. anastomosis groups** Irene Blanco-Casallas[1], Juanita Gil[2], Alejandro Rojas[3] [1]Biological Sciences, Universidad de los Andes, [2]Plant, Soil and Microbial Sciences, Michigan State University, [3]Michigan State University

The genus *Rhizoctonia* is comprised of soilborne fungal plant pathogens that can affect agronomic crops, ornamental plants, and forest trees worldwide. Species from this genus are classified into multinucleate *R. solani* and binucleate *Ceratobasidium* spp., and

each of these groups are divided into anastomosis groups (AGs) based on the ability of hyphae to fuse. Mitochondrial genomes (mitogenomes) have become of interest because of their conserved genes and uniparental inheritance, which facilitates evolutionary and taxonomy studies. *Rhizoctonia solani* has one of the largest fungal mitogenomes and it is known that it has gone through expansion processes in time, such as the multiplication of novel repetitive elements and the gain of LAGLIDADG/GIY-YIG homing endonucleases. In this study, we attempted to assemble 19 mitogenomes from 14 multinucleate isolates and five from binucleate isolates belonging to different AGs, of which only one multinucleate isolate corresponding to *R. zeae* and all the binucleate isolates resulted in complete mitochondrial genomes that ranged from 120,557 bp to 169,413 bp. Mitogenomes were annotated and gene order was established for each isolate. Particular focus was given to the synteny of the mitochondrial genes and gene order conservation across groups. We found 14 conserved protein-coding genes, 23 tRNAs, and three ribosomal genes, as well as conserved gene blocks for the rps3-nad2-nad3 and nad4L-nad5 genes in all the genomes, which have been reported for *Rhizoctonia* before.

Moreover, we observed variability in the number of introns ranging from 10 to 30 and homing endonucleases ranging from 14 to 24 in the genomes. This variation most likely explains mitogenome expansion in *Rhizoctonia* species and differences in mitogenome lengths. To identify informative genes, a phylogeny for multi- and binucleate isolates based on *nad*2 mitogene sequences was constructed. From this phylogenetic tree, we obtained a similar result compared to what has been reported with the nuclear ITS marker, but it is still not specific enough to cluster the anastomosis groups together, particularly those in the *Ceratobasidium* spp. group. The main purpose of this study was to better understand the composition and variability of the mitogenomes of different multi- and binucleate anastomosis groups of *Rhizoctonia*, using gene order and genetic diversity to study the taxonomy and ecology of this diverse genus.

553C **Microbiome of North American Ash for Biocontrol of Emerald Ash Borer** Claire C Yager[1], Judith Mogouong[1], Kathryn Bushley[2] [1]Plant Pathology, Cornell University, [2]USDA ARSEF

Ash (Fraxinus) are economically and culturally important deciduous trees in North America, and host to numerous native wood boring beetles and their parasitoids. However, little is known about their microbiota,specifically endophytic or pathogenic fungi that may grow in the living phloem and leaves. The Emerald Ash Borer (EAB; Agrilus planipennis), an invasive phloem feeding beetle from Asia, now threatens all native North American ash species. It is unknown whether EAB, like bark beetles, can carry and introduce fungi into their galleries in the ash phloem, and how communities in ash phloem and galleries may change as EAB infestation increases. To investigate these questions, we collected infested gallery phloem tissue, uninfested phloem tissue, insect frass, and adult insects from public lands in two regions of New York, sampling trees of different sizes and levels of EAB infestation across two years. Samples were surface sterilized, and fungi were cultured on three media types, potato dextrose agar, a Basidiomycete specific agar, and strasser's entomopathogenic fungal media. Paired samples to those cultured were also sequenced at the ITS barcode region using Illumina MiSeq to provide a global view of fungi in this system, including non-culturable fungi. Cultured fungal isolates included several potential insect-pathogenic species from families Cordycipitaceae, Ophiocordycipitaceae, and Clavicipitaceae, as well as other insect-associated fungi that may be either plant pathogens (Ophiostomataceae) or saprophytic wood rot (Peniophoraceae) fungi. We then sequenced multiple loci of the putative entomopathogens and built a phylogenetic tree including other members of Hypocreales to assess species level ID. Several entomopathogenic taxa were isolated not only from adult insects, but also from galleries, frass, and even uninfested ash phloem, suggesting they may grow within healthy phloem as entomopathogenic endophytes. We also observed differences in composition and an overall decrease diversity of fungi found in trees with high versus low EAB infestation. Our findings elucidate the breadth of diversity of fungi in this system, identify fungi with potential roles in ash-decline, and potential entomopathogenic endophytes, which could offer novel options for biocontrol of this invasive insect.

554A **Deciphering binding specificities of transcription factors of the oomycete *Phytophthora infestans* uncovers conserved and divergent evolutionary patterns and helps predict function** Nguyen NT Vo[1], Ally Yang[2], Wiphawee Leesutthiphonchai[1], Timothy R Hughes[2], Howard S Judelson[1] [1]Dept of Microbiology and Plant Pathology, University of California, Riverside, [2]Dept of Molecular Genetics and Donnelly Center, University of Toronto

Identifying the DNA-binding specificities of transcription factors (TF) is central to understanding gene networks that regulate growth and development. Such knowledge is lacking in oomycetes, a group of microbes that includes many important plant and animal pathogens. Here we describe the use of protein-binding oligonucleotide microarrays (PBMs) to define the DNA-binding preferences of 14 families of TFs from the oomycete *Phytophthora infestans,* which causes late blight of potato and tomato. DNA motifs obtained from the PBMs for representatives of each major TF family were validated by electrophoretic mobility shift assays (EMSA) or ChIP-seq. Consistent with the large evolutionary distance of oomycetes from traditional models, only some of the *P. infestans* DNA-binding preferences resembled those of TFs from human and plants. Some families from *P. infestans* included

clusters with canonical targets and others with novel targets. Paralogs having similar binding preferences often displayed distinct patterns of expression, suggesting functional divergence. Many TFs were predicted to either drive stage-specific expression or serve as general activators based on the representation of their binding sites within developmentally-regulated promoters. One such prediction was confirmed using a reporter gene assay. Our data thus provide a basis for understanding transcriptional regulation in *P. infestans* by linking TFs with their targets on a genome-wide level.

555A **Rho-GDP dissociation inhibitor affects growth and aflatoxin production in *Aspergillus flavus*** Nicholas A Jones[1], Reagan Smucker[1], Sean Bassham[2], Michael S Price[3,4] [1]Biology and Chemistry, Liberty University, [2]Liberty University College of Osteopathic Medicine, [3]Molecular and Cellular Sciences, Liberty University College of Osteopathic Medicine, [4]Medicine, Duke University

Aspergillus flavus affects human health in two main ways: mycotoxicosis and invasive disease. The major mycotoxin produced by *A. flavus*, aflatoxin (AF), produces hepatotoxicity, nephrotoxicity, and immune suppression when ingested at high doses. Regulation of aflatoxin (AF) production is complex, involving transcriptional and post-transcriptional regulatory mechanisms focused mainly through the pathway specific transcriptional regulator *aflR*. An investigation into the nature of the transcriptional regulation of AF production by comparing conducive and non-conducive culture conditions revealed a clade of genes with a similar transcription profile to that of *aflR*. One of these genes, a putative Rho-GDP dissociation inhibitor (Rho-GDI; GeneID G4B84_005214), was characterized by gene deletion and shown to regulate AF production in *Aspergillus flavus*. The protein encoded by this gene, *rdiA*, showed 45% identity to Rdi1p in *S. cerevisiae*. Deletion of *rdiA* resulted in increased septation interval and branching as well as severely decreased AF production. The *ΔrdiA* mutant phenotypes are more like the *rdi1Δ* mutant of *C. neoformans* and the *bem4Δ* mutant in *S. cerevisiae* and exhibits a severe growth defect on minimal medium, a moderate growth defect on complete medium, and a cold temperature sensitive phenotype. Interestingly, the *rdiA* gene appears at least partially to rescue the cold-sensitive phenotype of the *S. cerevisiae bem4Δ* mutant providing a potential genetic link with RasA that is known to regulate AF production in *Aspergillus*. Because of the shared phenotypes with *C. neoformans*, we will complement the *rdiA* mutant in *A. flavus* and use the *rdiA* gene to complement the *rdi1Δ* mutant of *C. neoformans* to positively identify the *rdiA* gene.

556A **Deacetylation by sirtuin E is important for *Aspergillus fumigatus* pathogenesis and virulence** Natália S Wassano[1], Jaqueline Gerhardt[2], Everton P Antoniel[1], Gabriela B da Silva[3], Daniel Akiyama[1], Leandro Xavier Neves[4], Elton Vasconcelos[5], Patrícia Alves de Castro[6], Camila Figueiredo Pinzan[6], Gustavo H. Goldman[6], Adriana F. P. Leme[4], Taicia Pacheco Fill[1], Nilmar Moretti[3], Andre R. L. Damasio[1] [1]Universidade Estadual de Campinas (UNICAMP), [2]Biologia Molecular e Morfofuncional, Universidade Estadual de Campinas (UNICAMP), [3]Universidade Federal de Sao Paulo, [4]Laboratorio Nacional de Bioenergia (CNPEM), [5]University of Leeds, [6]Universidade de Sao Paulo

Protein acetylation is a crucial post-translational modification that controls gene expression and a variety of biological processes. Sirtuins, a prominent class of NAD+-dependent lysine deacetylases, serve as key regulators of protein acetylation and gene expression in eukaryotes. In this study, six single knockout strains of fungal pathogen *Aspergillus fumigatus* were constructed, in addition to a strain lacking all predicted sirtuins (SIRTKO). Phenotypic assays suggest that sirtuins are involved in cell wall integrity, secondary metabolite production, thermotolerance, and virulence. AfsirE deletion resulted in attenuation of virulence, as demonstrated in murine and *Galleria* infection models. The absence of AfSirE leads to altered acetylation status of proteins, including histones and non-histones, resulting in significant changes in the expression of genes associated with secondary metabolism, cell wall biosynthesis, and virulence factors. These findings encourage testing sirtuin inhibitors as potential therapeutic strategies to combat *A. fumigatus* infections or in combination therapy with available antifungals.

557A **Transcriptional rewiring of sulfur metabolism in *Candida albicans*** Anagha Menon Chepppanakozhummal Thazhathidam[1,2], Faiza Tebbji[2], Antony Vincent[3], Adnane Sellam[1,2] [1]Dept of Microbiology, Infectiology and Immunology, Université de Montréal, [2]Institut de cardiologie de Montréal, [3]Dept of Animal Sciences, Université Laval

Candida albicans is an opportunistic human fungal pathogen and a leading cause of nosocomial infections in immunocompromised patients. A crucial aspect of *C. albicans*' pathogenicity is metabolic flexibility, which allows the yeast to colonize a variety of host habitats with contrasting nutritional contents. While, sulfur is essential for all biological systems, sulfur metabolism remains only partially characterized in human fungal pathogens such as *C. albicans*. Our study is focussed on abundant sulfur sources in the niches of *C. albicans* such as taurine and dietary amino acids (Met/Cys). Through transcriptomic analysis, we characterized the sulfur starvation and utilization signatures in *C. albicans*. Irrespective of the sulfur sources being supplemented, upregulation of genes involved in sulfur amino acid biosynthesis and utilization of alternative sulfur sources was observed. A total of five orthologs of Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase involved in sulfonate catabolism in *S. cerevisiae* and bacteria, were transcriptionally activated and their contribution to sulfonate utilization in *C. albicans* was demonstrated. Furthermore, we carried

a genetic screen of 300 strains having deletion/mutation in genes of transcription regulators and identified three transcription factors (Cbf1, Met32 and Met4) that were required to utilize different sulfonates such as taurine. While Met32 and Met4 are known as master transcription regulator of MET genes and methionine biosynthesis in *S. cerevisiae* and other fungi, our study suggests a divergence in function in *C. albicans*towards alternate sulfur utilization and metabolism. Insights into the genetic connectivity of the 3 transcription factors Cbf1, Met32 and Met4 as well as the contribution of Met32 in t-RNA transcriptional control and glutathione utilization will be presented. *In vivo* fitness tests employing various infection models revealed attenuated virulence of different sulfur utilization mutants. Since human sulfur metabolism differs significantly from that of fungi, our research aims to shed light on possible targets for the development of novel treatment approaches.

558A **Codon usage variation, selection, and evolution in a fungal subphylum** Bryan Zavala Martinez[1,2], Colin Speer[1], Stevie Clemens[1], Dana Opulente[3], Chris Todd Hittinger[4], Antonis Rokas[5], Abigail LaBella[6] [1]UNC Charlotte, [2]FDA, [3]Villanova, [4]University of Wisconsin-Madison, [5]Vanderbilt University, [6]Bioinformatics and Genomics, University of North Carolina at Charlotte

The genomes of the Saccharomycotina, commonly referred to as yeasts, are highly diverse; levels of gene sequence divergence across yeasts are comparable to levels observed across plants and animals. This includes vast diversity in the usage of synonymous codons. While changes in synonymous codon usage have been traditionally considered silent, emerging work suggests that synonymous codon usage plays an active regulatory role in gene expression. We have used the Saccharomycotina as a model system to explore the evolution of codon usage biases and the associated changes in tRNAs. We are leveraging machine learning, evolutionary, and experimental studies to capture the critical role of codon usage in metabolism, pathogenicity, horizontal gene transfer, and gene regulation. Codon usage bias is a treasure trove of genetic information that has been broadly overlooked and may have broad applications to fungal genetics studies.

559A **Upc2-mediated mechanisms of azole resistance in *Candida auris*** Jizhou Li[1], Lola Aubry[2], Danielle Brandalise[1], Alix T. Coste[1], Dominique Sanglard[3], Frederic Lamoth[3,4] [1]Institut de Microbiologie, Centre hospitalier universitaire vaudois, [2]Institut de Microbiolgie, Centre hospitalier universitaire vaudois, [3]Centre hospitalier universitaire vaudois, [4]Service de Maladies infectieuses

Candida auris is an emerging yeast pathogen of major concern because of its ability to cause hospital outbreaks of invasive candidiasis and to develop resistance to antifungal drugs. A majority of *C. auris* isolates are resistant to fluconazole, a first-line treatment of invasive candidiasis. Mechanisms of azole resistance are multiple, including mutations in the target gene *ERG11* and activation of the transcription factors Tac1b and Mrr1, which control the drug transporters Cdr1 and Mdr1, respectively. In this study, we investigated the role the transcription factor Upc2, which is known to regulate the ergosterol biosynthesis pathway and azole resistance in other *Candida* spp.

Genetic deletion and hyperactivation of Upc2 by epitope tagging in *C. auris* resulted in drastic increased and decreased susceptibility to azoles, respectively. This effect was conserved in strains with genetic hyperactivation of Tac1b or Mrr1. Reverse transcription PCR analyses showed that Upc2 regulates *ERG11* expression and also activates the Mrr1/Mdr1 pathway. We showed that upregulation of *MDR1* by Upc2 could occur independently from Mrr1. The impact of *UPC2* deletion on *MDR1* expression and azole susceptibility in a hyperactive Mrr1 background was stronger than that of *MRR1* deletion in a hyperactive Upc2 background. While Upc2 hyperactivation resulted in a significant increase of expression of *TAC1b*, *CDR1* expression remained unchanged.

Taken together, our results showed that Upc2 is crucial for azole resistance in *C. auris*, via regulation of the ergosterol biosynthesis pathway and activation of the Mrr1/Mdr1 pathway. Notably, Upc2 is a very potent and direct activator of Mdr1.

560A **RNA editing in three members of the *Microbotryum violaceum* fungal complex and characterization of ADAR genes of *Microbotryum dianthorum*** Shikhi Baruri, Roxanne K Hayes, Fatima Rubi Fuentes Osorio, Michael H Perlin Biology, University of Louisville

Microbotryum dianthorum (MvDp), *M. intermedium* (MI), and *M. lychnidis-dioicae* are members of the *M. violaceum* fungal complex. MvDp infects plants in the *Dianthus* genus, causing anther smut in their respective host plants. The lifecycle of these basidiomycete fungi includes the haploid, mating, and infection stages. RNA editing is a post-transcriptional process where adenosine (A) is converted to inosine (I) by adenosine deaminase enzymes (ADARs); such modifications to RNAs may lead to synonymous and non-synonymous codon changes, thereby altering protein function. We observed that 64% to 75% of total editing sites created nonsynonymous codon changes in both haploid and mating stages of the three species. Moreover, the a2 haploid strain of MI had fewer editing sites compared to other haploid strains. Among the edited genes, two were edited only at the mating stage in MvDp, undergoing A to I changes within their functional domains. Differential expression analysis revealed that the gene

called Apoptosis-inducing factor-1, was upregulated in MvDp, while another gene for PHB domain-containing protein, responsible for cell proliferation, was downregulated compared to the haploid stage. When examining *in planta* RNA editing for MvDp, we discovered that the RNA for an autophagy-related gene called polyphosphoinositide phosphatase was edited at a specific position encoding a SAC family domain. During all four stages of the MvDp lifecycle, a specific MAPKKK gene was edited in the portion encoding the PKC-like superfamily domain. Also, that gene was edited in a second site during haploid and mating stages but not in the infection stage. For functional analysis, we expressed the three ADAR genes of MvDp in *Saccharomyces cerevisiae*, a yeast that does not possess any ADAR genes. Heterologous expression in yeast increased resistance to stressors related to cell wall, cell membrane damage, and induced endoplasmic reticulum stress. The functional significance of RNA editing and the role of ADAR genes in fungi is not fully understood. Also, research on RNA editing in basidiomycetes is limited and relatively new. RNA editing mechanisms in fungi have been implicated in fungal pathogenesis, although their exact mechanisms and roles remain unclear. Further research is needed to fully understand functional significance of this apparently ubiquitous process in several members of the *Microbotryum* fungal complex, with possible ramifications more generally in fungi.

561A ***Cryptococcus neoformans* Adaptation to the Host is Regulated by the RAM Pathway** Emma E Blackburn[1], Benjamin Chadwick[2], Xiaorong Lin[2] [1]Microbiology, University of Georgia, [2]University of Georgia

Cryptococcus neoformans is an opportunistic fungal pathogen, responsible for cryptococcal meningitis. Despite existing antifungal therapies, this disease kills over 180,000 people annually. There are no vaccines available, making this fungal pathogen deadly to immunocompromised individuals. Adaptation to host physiological conditions for this environmental fungus is a prerequisite for its pathogenesis. *C. neoformans* can adapt to host high temperatures (≥37°C) and CO_2 levels (≥5%), and the latter differs drastically from its normal niche of ~0.04% CO_2 in the atmosphere; however, the molecular basis of such adaptation to the host conditions are poorly characterized. Our previous research into thermotolerance and CO_2 tolerance placed the *Regulator of Ace2 Morphogenesis* (RAM) pathway at the center of the signaling network allowing this fungus to adapt to host conditions. Consequently, disruption of Cbk1, the terminal kinase of the RAM pathway, prevents growth at ≥37°C or ≥5% CO_2. Through a *cbk1*Δ natural suppressor screen, we found that loss of the novel ribonuclease domain-containing protein Psc1, an RNA binding protein Ssd1, and an uncharacterized protein Psc2 partially restored *cbk1*Δ's growth defects in CO_2 and high temperature. Ssd1 is characterized in *Saccharomyces cerevisiae* and is phosphorylated by Cbk1; the phosphorylation state of Ssd1 dictates its subcellular localization and its ability to suppress translation. We hypothesize that in *C. neoformans* Cbk1 interacts with RNA-binding factors Psc1, Psc2, and Ssd1 to regulate subcellular localization and translation of mRNAs required for CO_2 adaptation. We expect that investigation will reveal the underlying mechanism of post-transcriptional control that enables this fungus to adapt to the host environment.

562A **Roles of P-body factors in *C. albicans* filamentation** Melissa A Tosiano, Fredrick Lanni, Gemma E May, C. Joel McManus Biological Sciences, Carnegie Mellon University

Hyphal growth is strongly associated with virulence in the human fungal pathogen *C. albicans*. While the transcriptional networks that regulate filamentation have been extensively studied, relatively little is known about post-transcriptional regulation of filamentation. Previous work reported that deletion of P Body (PB) factors Edc3p and Dhh1p from an auxotrophic *C. albicans* strain disrupted virulence and filamentation, suggesting an essential role for post-transcriptional regulation of these processes. However, the deletion mutants were generated in auxotrophic strains using methods that have been reported to have off-target effects. To further study the role of PB factors in filamentation, we used CRISPR-Cas9 to generate homozygous deletions of both factors in the prototrophic strain SC5314. In contrast to prior reports, we found *EDC3* was not required for PB formation or filamentation. Indeed, we found that homozygous *edc3* deletion showed no effects on hyphal growth in a diverse panel of *C. albicans* clinical isolates. In addition, heterozygous *dhh1* deletion had no effect on filamentation in SC5314, again contrary to published reports from an auxotrophic strain. However, homozygous *dhh1* deletion has strongly impaired growth and filamentation in addition to exhibiting unusual colony morphology in response to stress. We are currently examining changes in gene expression associated with the absence of Dhh1p. Our results show that *DHH1*, but not *EDC3*, functions in stress resistance and filamentous growth. Furthermore, our work suggests historic studies that used 5-FOA selection to generate mutations in auxotrophic strains may not always reflect the biology of prototrophic clinical strains underscoring the importance of using cloning methods with minimal off target effects.

563A **Modification of transcriptional factor ACE3 enhances protein production in Trichoderma reesei in the absence of cellulase gene inducer** Yun Luo[1], Mari Valkonen[2], Jonathan M Palmer[1], Igor Nikolaev[1] [1]IFF, [2]VTT

Trichoderma reesei is one of the best-known cellulolytic organisms, producing large quantities of a complete set of extracellular cellulases and hemicellulases for the degradation of lignocellulosic substances. Hence, *T. reesei* is a biotechnically important host and it is used commercially in enzyme production, of both native and foreign origin. Many strategies for producing enzymes in *T.*

reesei rely on the *cbh1* and other cellulase gene promoters for high-level expression and these promoters require induction by sophorose, lactose or other inducers for high productivity during manufacturing.

Here we described an approach for producing high levels of secreted proteins by overexpression of a transcription factor ACE3 in *T. reesei*. We refined the *ace3* gene structure and identified specific ACE3 variants that enable production of secreted cellulases and hemicellulases on glucose as a sole carbon source (i.e., in the absence of an inducer). These specific ACE3 variants contain a full-length Zn2Cys6 binuclear cluster domain at the N-terminus and a defined length of truncations at the C-terminus. When expressed at a moderate level in the fungal cells, the ACE3 variants can induce high-level expression of cellulases and hemicellulases on glucose (i.e., in the absence of an inducer), and further improve expression on lactose or glucose/sophorose (i.e., in the presence of an inducer). Finally, we demonstrated that this method is applicable to industrial strains and fermentation conditions, improving protein production both in the absence and in the presence of an inducer.

This study demonstrates that overexpression of ACE3 variants enables a high level of protein production in the absence of an inducer, and boosts protein production in the presence of an inducer. It is an efficient approach to increase protein productivity and to reduce manufacturing costs.

564A **Lipid rafts in *Schizophyllum commune* – insights in localization and composition** Berit Frizzy Porsche, Robert Jesse, Katrin Krause, Erika Kothe Friedrich Schiller University

Schizophyllum commune, a filamentous basidiomycete fungus, is noted for unique growth patterns and ecological significance. This white rot fungus breaks down organic matter, contributing to nutrient cycling in diverse ecosystems, and is known to interact with other organisms of the community. Polarized hyphal growth, a key aspect, requires the supply of proteins and lipids to the hyphal tip by vesicle trafficking. Cellular membranes feature the interplay of lipids and proteins and are visualized as a dynamic fluid lipid structure by the fluid mosaic model of Singer and Nicolson. Cell membranes consist of different membrane microdomains as lipid raft domains, enriched in sterols, sphingolipids, and specific proteins, serving as platforms for signal transduction, membrane trafficking, protein sorting, and polarized growth. Crucial in establishing and maintaining hyphal polarity are the actin cytoskeleton and raft domains. The aim of this work is to investigate the composition of raft domains as well as the localization and role of rafts in *S. commune* using two raft-associated proteins, stomatin and striatin. They were reported to play a role in cell signalling and signal transduction, as well as in numerous protein-protein interactions and the assembly of proteins in distinct signalling complexes in mammals, but not much is known for fungi. Sterol-enriched raft domains were visualized by Filipin staining at hyphal tips and septation sites. Further, stomatin was labelled with the fluorescence protein dTomato and visualization by laser scanning microscopy showed a shift from young branch tips to the apex over time. In the dikaryotic stage, stomatin co-localizes with eGFP-labeled actin. Stomatin deletion in *S. commune* and RNA sequencing were performed and demonstrate its impact on morphology and genetics, upregulating MAPKs, GPCRs, and cytoskeleton-related genes. This study highlights raft-associated proteins' crucial role in hyphal growth and cell signaling in *S. commune*, offering potential for therapeutic development through raft-mediated strategies.

565A **Genetic and regulatory complexity in fungal primary carbon metabolism** Ronald P de Vries[1], Astrid Mueller[1], Jiajia LI[1], Li Xu[1], Ferry Hagen[1], Miia R Mäkelä[2] [1]Westerdijk Fungal Biodiversity Institute, [2]University of Helsinki

Fungal primary carbon metabolism has been a topic of study for many decades, but the availability of fungal genome sequences and large omics datasets has provided an unprecedented view on the organization and diversity of this important biological system.

Starting from an *in silico* metabolic model for *Aspergillus niger*, we proceeded with experimental validation using individual and combined gene deletion strains, which resulted in the addition of novel genes to the pathways as well as removal of previously predicted genes. We then explored the reliability of this model when transferred to other species and showed that this can be done within the same phylum (although with reducing confidence when the taxonomic distance got larger), but it becomes questionable when transferred to other phyla.

To further explore the genomic and post-genomic diversity within and between species, we analyzed the presence and regulation of candidate sugar transporters in four ascomycete species and delved deeper into the paralogs of sugar reductases, which are present in several pathways. This demonstrated that the variation at the transcriptomic level is even higher than at the genomic level indicating a highly refined regulatory system that controls primary carbon metabolism, and which involves many transcriptional regulators, of which only the major ones have so far been identified.

Highlights of these studies will be presented.

566A **Role of the *osaA* gene in *Aspergillus fumigatus* development, secondary metabolism and virulence** Apoorva S Dabholkar[1], Sandesh Pandit[1], Ritu Devkota[2], Sourabh Dhingra[2], Sophie Lorber[3], Olivier Puel[3], Ana M Calvo[1] [1]Dept of Biological Sciences, Northern Illinois University, [2]Dept of Biological Sciences and Eukaryotic Pathogen Innovation Center, Clemson University, [3]Toxalim (Research Center in Food Toxicology), Universite de Toulouse

Aspergillus fumigatus is the leading cause of aspergillosis, with high mortality rates, particularly among immunocompromised individuals. In search for novel genetic targets against aspergillosis infections, we studied the WOPR transcription factor OsaA in *A. fumigatus*. Deletion of *osaA* results in colony growth reduction with respect to the wild type. Our results revealed that conidiation is also influenced by *osaA*; both deletion and overexpression of this gene resulted in a decrease in conidial production, particularly in the former. In addition, expression of the conidiation regulator genes *brlA*, *abaA* and *wetA* was decrease or not present in the absence of *osaA* or when this gene was overexpressed. This study also indicates that *osaA* is necessary for normal cell wall integrity. Furthermore, deletion of *osaA* resulted in a reduction in the ability of this fungus to adhere to surfaces, thermotolerance, and caused increased sensitivity to oxidative stress. Metabolomics analysis indicated that *A. fumigatus osaA* deletion or overexpression resulted in alterations of numerous secondary metabolites, including gliotoxin production. This was concomitant with alteration in the expression of genes in secondary metabolite gene clusters genes involved in the synthesis of these compounds. Importantly, *osaA* is indispensable for virulence in both, the neutropenic and corticosteroid-immunodepressed mouse models.

567A **Feedback-dictated discovery of non-specific binding transcription factors in *Trichoderma reesei*** Xianhua Sun[1], Lina Qin[2], Li Xu[3], Xiaoyun Su[3] [1]Chinese Academy of Agricultural Sciences, [2]Fujian Normal University, [3]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences

Feedback regulation is one basic rule governing gene expression. However, the knowledge about the underlying mechanisms in filamentous fungi is still poorly understood. From publicly available transcriptomic data, we deduced that there could be unidentified transcription factors involved in negative feedback regulation of cellulase expression in *Trichoderma reese*. Based on this hypothesis, we designed an artificial gene network with a positive feedback loop to improve cellulase expression in *T. reesei*, which could simultaneously fortify the potentially concurrent negative feedback and ease the identification of associated transcription factors. With this design, from the up-regulated genes, four transcription factors (*Tr121121*, *Tr70351*, *Tr55274*, and *Tr28781*) were discovered to indeed negatively impact cellulase expression. The former three belong to zinc binuclear finger proteins and Tr28781 is a NTD80-like protein. In vitro EMSA analysis indicated that all of them bind to the cellulase promoter via a non-specific way. Intriguingly, the zinc binuclear proteins rely on an N-terminal alkaline stretch to bind DNA. Deleting the homologs of *tre121121* and *tre28781* in *Aspergillus nidulans* (AN13001 and AN6015, respectively) improved cellulase expression. Moreover, a novel transcription factor (Mycth_2312847) in *Myceliophthora thermophile* with the same domain organization and similar expression pattern but almost no sequence similarity was unveiled to be a negative regulator of cellulase expression. However, in yeast one-hybrid assay, Tr55274 and Tr28781 proved to be transcription activators. Hence, these transcription factors represent a novel regulatory mechanism in gene expression. Interestingly, zinc fingers transcription factors with an N-terminal alkaline region are more abundant in yeasts and filamentous fungi than animals, suggesting that such non-specific binding has been largely discarded during evolution to higher eukaryotes. The study also highlights the power of using a systematic strategy to identify cryptic transcription factors in regulating gene expression.

568A **Phytochromes in *Aspergillus fumigatus*: Light, stress and virulence** Reinhard Fischer[1], Kai Leister[2], Yinyang Ma[3], Ling Lu[4], Zhenzhong Yu[3] [1]Microbiology, Karlsruhe Institute of Technology (KIT), [2]Karlsruhe Institute of Technology, [3]University of Nanjing, [4]Nanjing University

Phytochrome serves as red-light sensor in fungi and regulates light-dependent gene expression. In *Aspergillus nidulans* and *Alternaria alternata*, phytochrome also acts as a temperature sensor and performs functions in the absence of light. This study explores the role of two phytochromes, FphA and FphB, in the opportunistic fungal pathogen *Aspergillus fumigatus*. Both proteins were expressed in *E. coli*. FphA, behaved like the *A. nidulans* orthologue and was photoconvertible, while FphB showed no photoactivity. AfFphA complemented *A. nidulans fphA*-deletion strain phenotypes, unlike AfFphB. Co-overexpression of both proteins in *A. nidulans* stimulated asexual development and induced putative pathogenicity-related genes. AfFphA influenced various stress responses in *A. fumigatus*. *fphA* deletion in *A. fumigatus* had no impact on virulence in *Galleria mallonella*, while *fphB* deletion and *fphA/B* double-gene deletion increased virulence. Similar effects were observed after *fphB* overexpression. RNAseq analyses revealed regulation of mycotoxin genes by FphB, which probably explains its role in

pathogenicity. In *A. nidulans*, AfFphA and AfFphB localized exclusively in nuclei, forming a heterodimer. Our results suggest that *A. fumigatus* FphA responds to red light and plays a role in stress responses, while the photoinactive FphB protein controls virulence.

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569A **A multidisciplinary, cross-species approach to understanding woody plant declines: similarities between Kiwifruit Vine Decline Syndrome (KVDS) and Apple Replant Disease (ARD)** Micol Guaschino[1,2], Tracey S Somera[3], Davide Spadaro[2,4] [1]1Dept of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, [2]AGROINNOVA, University of Torino, [3]Agricultural Research Service, USDA, [4]Dept of Agricultural, Forest and Food Sciences (DISAFA), University of Torino

Kiwifruit Vine Decline Syndrome (KVDS) and Apple Replant Disease (ARD) are both soil-borne diseases affecting fruit trees in perennial cropping systems. Both pathosystems are characterized by a similar complex of soilborne fungi and oomycetes which impact root development and soil health. ARD occurs in apple-producing regions worldwide. In the last decade, KVDS has severely compromised kiwifruit production in Italy and has also been reported in Turkey, Japan, and China. The contribution of similar biotic and abiotic factors to both pathosystems underlines the need for multidisciplinary and cross-species approaches to the management of woody plant diseases. This is of particular relevance in the framework of climate change where novel conditions may lead to shifts in microbial community composition and function. Root anoxia/flooding of the radical system is a key abiotic stress factor required for development of KVDS symptoms, together with infection by *Phytopythium vexans*. Preliminary gene expression analysis on kiwifruit roots revealed upregulation of ROS scavenging pathways and hormonal stress at specific time points in response to this dual stressor combination. Additional transcriptomic studies on inoculated *Actinidia* roots, with and without flooding, are currently underway. Results are expected to improve our understanding of how pathways associated with abiotic vs. biotic stress factors promote KVDS onset. In combination with this analysis, genes previously identified as exhibiting altered expression in apple roots in response to *Pythium ultimum* infection will serve as a foundation to explore potential similarities in KVDS and ARD pathosystems. Results are expected to provide new insights into oomycete pathogenesis in woody plants and improve disease management in both systems.

570A **Screening system based on growth defects due to unscheduled *brlA* expression to identify genes involved in the functional regulation of transcription factors in *Aspergilli*** Katsuya Gomi, Tomoko Shintani, Da-Min Jeong, Jikian
Tokashiki Tohoku University

Pathway-specific transcription factors are involved in regulating the production of polysaccharide-degrading enzymes in filamentous fungi including *Aspergillus*, and to date a number of the Zn_2Cys_6 binuclear cluster-type transcription factors unique to fungi have been identified by many studies. However, much remains to be elucidated about the transporters/sensors for inducing substrates, subsequent signal transduction, and regulation of transcription factor activation.

The aim of this study is to isolate unidentified genes involved in the functional regulation of transcription factors in *Aspergilli*. To this end, we first attempted to find the novel genes involved in the functional regulation of AmyR essential for amylolytic enzyme production as a model. We constructed an *Aspergillus nidulans* strain that overexpressed *brlA*, which is involved in conidiation, under the control of the α-amylase gene promoter, and this strain showed a significantly restricted growth in the presence of isomaltose, an inducer of amylase production. By using this strain as a parent, spontaneous mutant strains that recovered growth were isolated on isomaltose-containing agar medium. Consequently, we successfully identified a putative sugar transporter gene involved in isomaltose transport/sensing through next-generation sequencing of the spontaneous mutants (see Jeong's presentation for details). Further, we are currently applying the screening system to find unidentified genes involved in the functional regulation of the transcription factor XlnR essential for xylolytic enzyme production.

571A **Chromatin Assembly Factor 1 is Required for Normal Gene Repression and Chromatin Structure at PRC2-targeted Genes** Eduardo V Torres[1], Felicia Ebot-Ojong[2], Aileen Ferraro[2], Vivian Ng[3], Igor V Grigoriev[3], Zachary A Lewis[1] [1]Genetics, University of Georgia, [2]University of Georgia, [3]US Dept of Energy Joint Genome Institute

Organismal development, regulation of gene expression, and various other biological processes are heavily influenced by alterations in the chromatin environment. Histone deposition and post translational modification (PTM) are two mechanisms involved in building and maintaining this environment. Our lab studies heterochromatin, which is characterized by tightly packed regions of DNA that serve many biological functions. More specifically, we are interested in facultative heterochromatin (FH), which can become more or less compact in response to various environmental or developmental stimuli. Formation of FH regions is facilitated by Polycomb Repressive Complex 2 (PRC2), a highly conserved protein complex that catalyzes Histone H3 Lysine 27 tri-methylation (H3K27me3) at its target regions and helps stably maintain the epigenetic mark over mitotic divisions. Chromatin Assembly Factor 1 (CAF-1) is a heterotrimeric protein complex whose primary function is to deposit histones on replicating DNA behind the replication fork. An RNA-seq screen performed by our lab revealed that a subset of PRC2 target genes is significantly upregulated in mutants lacking each of the CAF-1 subunits, while expression of Non-PRC2 target genes is widely unaffected. My project seeks to elucidate the direct impact that CAF-1 has on the chromatin environment by observing changes in histone methylation patterns in CAF-1 deficient mutants. These experiments are done using the model fungus *Neurospora crassa (N. crassa)*, which is easy to genetically manipulate and has a fully conserved CAF-1 complex. We found that overall H3K27me3 levels are decreased in CAF-1 mutant strains, while other histone marks (H3K9me, H3K36me, H4K20me) are seemingly unaffected, suggesting that the complex may play a role in facultative heterochromatin formation. Further experimentation is needed to determine the potential mechanism behind this defect.

572A **Altered histone acetylation, genome organization, and facultative heterochromatin in histone deacetylase mutants of *Neurospora crassa*** Farh Kaddar, Ashley W Scadden, Clayton Hull-Crew, Alayne S Graybill, Tiffany J Lundberg, Nickolas M Lande, Andrew D Klocko Chemistry & Biochemistry, University of Colorado Colorado Springs

Chromosomes must correctly fold in eukaryotic nuclei for proper genome function. Eukaryotic organisms hierarchically organize their genomes, including in the fungus *Neurospora crassa*, where chromatin fiber loops compact into Topologically Associated Domain (TAD)-like structures formed by heterochromatic region aggregation. However, insufficient data exists on how histone modifying complexes, including histone deacetylases, affect genome organization and heterochromatin composition. In Neurospora, the HCHC complex (comprised of the proteins HDA-1, CDP-2, HP1, and CHAP) deacetylates heterochromatic nucleosomes, as loss of individual HCHC members increases centromeric acetylation and alters the methylation of cytosines in DNA. Here, we assess if the HCHC complex affects genome organization and the deposition of histone post-translational modifications by performing chromosome conformation capture with high-throughput sequencing (Hi-C) and Chromatin Immunoprecipitation-sequencing (ChIP-seq) in a strain deleted of the *cdp-2* gene. We found that CDP-2 loss increases intra- and inter-chromosomal heterochromatic region interactions and causes gains in heterochromatic H4K16 acetylation is increased while smaller heterochromatic regions lose H3K9 trimethylation and gain inter-heterochromatic region interactions. In addition, we performed ChIP-seq of H3K27 di- or trimethylation, which marks facultative heterochromatin, to address whether another repressive histone mark could be altered in strains lacking heterochromatic histone deacetylation. Here, we present our current results for how the loss of HCHC HDAC activity affects the acetylation and methylation of heterochromatic nucleosomes and the organization of the Neurospora genome.

573A ***Verticillium dahliae* Vta3 promotes *ELV1* virulence factor gene expression in xylem sap, but tames Mtf1-mediated late stages of fungus-plant interactions and microsclerotia formation** Isabel Maurus[1], Rebekka Harting[1], Cornelia Herrfurth[2], Jessica Starke[1], Alexandra Nagel[1], Lennart Mohnike[2], Ying-Yu Chen[1], Kerstin Schmitt[1], Emmanouil Bastakis[1], Marian T. Süß[1], Miriam Leonard[1], Kai Heimel[1], Oliver Valerius[1], Ivo Feussner[2], James W. Kronstad[3], Gerhard H. Braus[1] [1]Dept of Molecular Microbiology and Genetics, University of Goettingen, [2]Dept of Plant Biochemistry and Service Unit for Metabolomics and Lipidomics, University of Goettingen, [3]Dept of Microbiology and Immunology, University of British Columbia

Verticillium transcription activator of adhesion 3 (Vta3) is required for plant root colonization and pathogenicity of the soil-borne vascular fungus *Verticillium dahliae*. RNA sequencing identified Vta3-dependent genetic networks required for growth in tomato xylem sap. Vta3 affects the expression of more than 1,000 transcripts, including candidates with predicted functions in virulence and morphogenesis such as Egh16-like virulence factor 1 (Elv1) and Master transcription factor 1 (Mtf1). The genes encoding Elv1 and Mtf1 were deleted and their functions in *V. dahliae* growth and virulence on tomato (*Solanum lycopersicum*) plants were investigated using genetics, plant infection experiments, gene expression studies and phytohormone analyses. Vta3 contributes to virulence by promoting *ELV1* expression, which is dispensable for vegetative growth and conidiation. Vta3 decreases disease

symptoms mediated by Mtf1 in advanced stages of tomato plant colonization, while Mtf1 induces the expression of fungal effector genes and tomato pathogenesis-related protein genes. The levels of pipecolic and salicylic acids functioning in tomato defense signaling against (hemi-) biotrophic pathogens depend on the presence of *MTF1*, which promotes the formation of resting structures at the end of the infection cycle. In summary, the presence of *VTA3* alters gene expression of virulence factors and tames the Mtf1 genetic subnetwork for late stages of plant disease progression and subsequent survival of the fungus in the soil.

574A **Type 2C Protein Phosphatases MoPtc5 and MoPtc7 Are Crucial for Multiple Stress Tolerance, Conidiogenesis and Pathogenesis** Jules Biregeya[1], Anjago Mabeche Wilfred[2], Chen meilian[2], Zonghua Wang[3], Wei Tang[2] [1]Plant pathology, Fujian Agriculture and Forestry University, [2]Plant pathology, Fujian Agriculture and forestry University, [3]Fujian Agriculture and forestry University

Protein kinases and phosphatases catalyze the phosphorylation and dephosphorylation of their protein substrates, respectively, and these are important mechanisms in cellular signal transduction. The rice blast fungus Magnaporthe oryzae possesses 6 protein phosphatases of type 2C class, including MoPtc1, 2, 5, 6, 7 and 8. However, only very little is known about the roles of these phosphatases in filamentous fungi. Here in, we deployed genetics and molecular biology techniques to identify, characterize and establish the roles of MoPtc5 and MoPtc7 in M. oryzae development and pathogenicity. We found that during pathogen-host interaction, *MoPTC7* is differentially expressed. Double deletion of *MoPTC7* and *MoPTC5* suppressed the fungal vegetative growth, altered its cell wall integrity and reduced its virulence. The two genes were found indispensable for stress tolerance in the phytopathogen. We also demonstrated that disruption of any of the two genes highly affected appressorium turgor generation and Mps1 and Osm1 phosphorylation levels. Lastly, we demonstrated that both MoPtc5 and MoPtc7 are localized to mitochondria of different cellular compartments in the blast fungus. Taken together, our study revealed synergistic coordination of *M.oryzae* development and pathogenesis by the type 2C protein phosphatases.

575A **Mutations in core PRC2 components reveal targeting mechanism of H3K27me3 to sub- telomeric chromatin in *Fusarium graminearum*** Allyson A Erlendson[1], Brian Josephson[2], Michael Freitag[1] [1]Oregon State University, [2]Broad Institute of MIT and Harvard

Facultative heterochromatin is necessary for appropriate spacio-temporal gene expression in plants, fungi, and animals, but an understanding of the stimuli and signals that control the formation of repressed chromatin domains is incomplete, especially in fungi. Enriched in nucleosomes modified with histone H3 lysine 27 trimethylation (H3K27me3), facultative heterochromatin domains are initiated by Polycomb Repressive Complex 2 (PRC2). Mutations within its three primary subunits, Kmt6, Eed, and Suz12, result in inherited diseases and sporadic cancers. In the fungus *Fusarium graminearum*, deletion of the gene encoding the catalytic subunit, *kmt6*, results in derepression of ~20% of the genome as well as distinct phenotypes. After selectively mutating the primary sequences of *kmt6*, *eed*, and *suz12* by *in vitro* site-directed mutagenesis, important catalytic functions and PRC2 subunit interactions were disturbed. As predicted, this led to some mutants exhibiting Δ*kmt6*-like phenotypes. However, most mutants exhibited both novel and diverse phenotypes, indicating discrete partial losses of function or differential silencing of the genome compared to both wild type and Δ*kmt6*. Notably, in strains with several PRC2 point mutants, H3K27me3 is absent from PRC2 targets across the genome, except for sub-telomeric regions, suggesting that these regions may harbor important targeting signals for the PRC2 in *Fusarium*.

576B **The influence of light on the bioluminescence-related transcripts from the Neonothopanus gardneri mycelium** Bianca B. Nóbrega[1,2], Bin Wang[1], Douglas M. M. Soares[3], Cassius V. Stevani[2,3], Jay C. Dunlap[1] [1]Dept of Molecular & System Biology, Geisel School of Medicine, Dartmouth, [2]Dept of Biochemistry, Institute of Chemistry, University of São Paulo, [3]Dept of Fundamental Chemistry, Institute of Chemistry, University of São Paulo

Bioluminescent fungi emit green light peaking at 530 nm. The biochemical mechanism and the genes involved in fungal bioluminescence have been recently described. The emission of light is one of the products of the so-called Caffeic Acid Cycle (CAC), wherein caffeic acid is firstly converted into hispidin in a reaction catalyzed by hispidin synthase (HispS), followed by its hydroxylation by hispidin-3-hydroxylase (H3H), giving rise to the fungal luciferin (3-hydroxyhispidin). Then, a luciferase (Luz) catalyzes the addition of molecular oxygen from the luciferin, yielding an endoperoxide as high-energy intermediate, whose decomposition leads to the formation of oxyluciferin (caffeylpyruvate) and light emission. In the last step, oxyluciferin is recycled to caffeic acid by caffeylpyruvate hydroxylase (CPH), restarting the cycle. Despite metabolites and genes of CAC having been characterized, the molecular regulation and ecological functions of the fungal bioluminescence for the mycelium remain poorly understood. Previous work from our group has demonstrated that the bioluminescence of *Neonothopanus gardneri*, a fungus that can be found in Babaçu Forest in Brazil, is controlled by a temperature-compensated circadian clock. Based on the transcriptome

and genome obtained by our group from *N. gardneri* mycelium, we have identified candidate genes for the biological clock and other ones acting as light sensors. We have developed reference genes for RT-qPCR assays that allow us to explore the regulation of light emission at the level of transcription. In this context, we investigate whether the expression of transcripts of CAC genes and other candidates to the biological clock and light sensors is responsive to light in *N. gardneri's* mycelium. With this in mind, the mycelium was cultivated in liquid medium at 25ºC for *i)* 6 days in constant darkness (DD) and *ii)* subjected to 1-hour of light after constant darkness. After 6 days, the globular mycelium was macerated in liquid N_2 and submitted to RNA extraction, and the expression of target transcripts was assessed by RT-qPCR. Preliminary results indicate that some genes involved in fungal bioluminescence show a decrease in transcripts production when the mycelium is transferred to light. This study can contribute new cues and insights on the regulation of transcripts related to the bioluminescence in fungi as well as the molecular aspects of the circadian regulation of the fungal bioluminescence.

577B **The COMPASS complex regulates morphogenesis and virulence through histone crosstalk in the fungal pathogen *Cryptococcus neoformans*** Youbao Zhao Henan Agricultural University

The Complex of Proteins Associated with Set1 (COMPASS) methylates histone H3K4 and is conserved from yeast to human. It plays critical roles in regulating fungal morphogenesis and pathogenesis in fungal pathogens. Here we identified the core subunits of COMPASS complex in the meningitis-causing fungal pathogen *Cryptococcus neoformans*and confirmed their conserved roles in H3K4 methylation. Through AlphaFold modeling, we found that Set1, Bre2, Swd1, and Swd3 form the catalytic core of the COMPASS complex, and regulate the yeast-to-hypha transition, thermal tolerance, and virulence in *C. neoformans*. The COMPASS complex-mediated histone H3K4 methylation requires H2B mono-ubiquitination by Rad6/Bre2 and the Paf1 complex in order to activate the expression of genes specific for the yeast-to-hypha transition in *C. neoformans*. Taken together, our findings demonstrate that putative COMPASS subunits function as a unified complex, contributing to fungal development and virulence in *C. neoformans*.

578B **Physiological role of a phospholipase D-encoding gene *pla-7* in growth and regulation of the lignocellulolytic response in *Neurospora crassa*** Yifan Chen, Xianzhang Jiang, Haowen Sun, Lina Qin College of Life Sciences, Fujian Normal University

Abstract: Phospholipases are lipolytic enzymes that are widespread in nature and play important roles in signal transduction. Phospholipase D hydrolyzes the distal phosphodiester bond of phospholipids to generate phosphatidic acid (PA). In plant and mammalian cells, PA interacts with numerous signaling proteins to affect a large number of cellular signaling pathways. However, the function of phospholipase D is rarely studied in filamentous fungi. Here we analyzed the growth phenotype of *N. crassa* strains with or without phospholipase D encoding genes on different carbon and nitrogen sources. Our results showed that deletion of phospholipase D encoding gene *pla-7* resulted in significant increases in hyphal branching and biomass accumulation under sucrose carbon source. Overexpression of the major cellulases transcriptional factor gene *clr-2* in Δ*pla-7* strain showed higher production levels of cellulases compared to that in wild-type background under sucrose. However, under cellulosic carbon sources, conidia of Δ*pla-7* strain cannot germinate, but this germination defect could be partly rescued by adding organic nitrogen sources or overexpression *clr-2*. In addition, Δ*pla-7* strain failed to form protoperithecia on synthetic cross medium, but ascospores could be produced when Δ*pla-7* strain crossed with other strain as a male gamete, indicating that deletion of *pla-7* gene led to female sterility phenotype in *N. crassa*. Further global transcriptional profiling analysis showed that a group of antioxidant enzymes encoding genes, which could be associated with elimination of reactive oxygen species (ROS) were down-regulated in *pla-7* gene deletion background. Intriguingly, overexpression of a catalase encoding gene *cat-3* in Δ*pla-7* strain could rescue its germination and growth defect phenotype under cellulose. These data suggested that *pla-7* participated in a variety of the physiological activities and played an important role in maintaining low intracellular ROS level in *N. crassa.*

579B **Localization and functional domain analysis of AmyR in the black *koji*-mold *Aspergillus luchuensis* and its closely related species** Jikian Tokashiki, Taichi Morise, Wataru Hashimoto, Shoki Fujita, Takashiro Shintani, Katsuya Gomi Grad. Sch., Agric. Sci., Tohoku Univ.

Regulatory mechanisms for amylase production have been mainly studied on *Aspergillus oryzae* and *Aspergillus nidulans*. However, recent researches have suggested that the expression pattern of the amylase-related genes differs between the black *koji*-mold *Aspergillus luchuensis* extensively used for the Japanese spirits (*shochu* or *awamori*) manufacturing and *A. oryzae* (1). For example, in *A. luchuensis* the gene encoding acid-unstable α-amylase (*amyA*) identical to *A. oryzae* Taka-amylase A was constitutively expressed but other amylolytic genes including acid-stable α-amylase unique to *Aspergillus* section *Nigri* were regulated under the control of the transcription factor AmyR like in *A. oryzae*. In this study, therefore, we are interested in and

examined the localization and function of AmyR in the *Aspergillus* section *Nigri* such as *A. luchuensis*, *Aspergillus niger*, and *Aspergillus tubingensis*.

The *amyR* genes from A. *luchuensis*, *A. niger*, and *A*. *tubingensis* introduced into *A. oryzae* were shown to be constitutively localized in the nucleus. Also surprisingly, the experimental results showed that amylolytic gene expression was observed regardless of maltose/isomaltose induction, indicating that they were constitutively activated. Thus, we examined which part of *A. lucheunsis* AmyR works for the localization and activation of AmyR. Previous work has suggested that the C-terminal region of *A. oryzae* AmyR plays an important role in AmyR nuclear localization. Therefore, we expected that the C-terminal of *A. luchuensis* AmyR has the same function for localization as well. To address this possibility, we constructed chimeric AmyRs, which consist of the N-terminal region of *A. oryzae* AmyR and the C-terminal region of *A. luchuensis* AmyR, or vice versa, and introduced them into *A. luchuensis* to examine their localization. Contrary to expectations, the nuclear localization of *A. luchuensis* AmyR was not due to the C-terminal region. We also introduced chimeric AmyRs in *A. oryzae* and examined their localization. As a result, the localization pattern of chimeric AmyRs was similar to that in *A. luchuensis*.

(1) Hashimoto et al., *J. Biosci. Bioeng.*, **132**, 321−326 (2021).

580B **Light sensing in mushroom-forming fungi: The White Collar regulatory network of *Schizophyllum commune*** Peter Jan Vonk, Zoé Niemeijer, Marieke van der Poel, Robin A. Ohm Biology, Utrecht University

Blue light is an essential signal in the sexual development of many mushroom-forming fungi. It is detected by the White Collar Complex (WCC), composed of WC-1 and WC-2, which promotes transcription in the presence of light. Most of our knowledge on this complex comes from the ascomycete *Neurospora crassa*. However, due to large structural differences in the WCC in basidiomycetes, it is not known if the WCC has the same function in basidiomycetes and what its role is in initiating fruiting.

We used a combination yeast-2-hybrid, ChIP-Seq and RNA-Seq to identify the direct and indirect roles of the WCC on mushroom development in *Schizophyllum commune*. WC-1 and WC-2 interact with each other both in the light and in the dark. However, WC-2 binds to the promoters of more than 500 genes when grown in the light, but only 3 in the dark. This indicates a different mode of post-translational regulation of the WCC compared to ascomycetes, as the WCC in *N. crassa* is also associated with promoters in the absence of light. Furthermore, the expression profile of a Δ*wc-2* mutant in the light resembled that of the WT in the dark, indicating that a Δ*wc-2* mutant is effectively blind. In the light, WC-2 activates genes related to UV protection, but also genes associated with mushroom development like hydrophobins. Moreover, WC-2 directly activates expression of the C_2H_2 zinc-finger transcription factor *zfc7*, which regulates the progression of primordia development.

Together, these results show that WC-2 directly activates the transcription of fruiting-related genes in *S. commune* in a light-dependent manner that is different from that seen in Ascomycota.

581B **Enrichment of *Magnaporthe oryzae* infected barley cells using Fluorescence Activated Cell Sorting (FACS) for transcriptome analysis** Louisa Wirtz[1], Alex Wegner[2], Florencia Casanova[1,1], Ulrich Schaffrath[1] [1]Molecular Plant Physiology, RWTH Aachen, [2]Molecular Plant Physology, RWTH Aachen

After the attack by a pathogen, plants depend on defense mechanisms of individual cells. Conversely, this implies that in order to achieve success, a pathogen must either evade, suppress or manipulate the natural defense responses of each cell that is penetrated. However, even if a pathogen successfully colonizes one cell, nearby cells may still resist invasion. To gain a comprehensive understanding of the genetic and metabolic mechanisms leading to invasion or resistance of plant cells, it is mandatory to analyze individual cells or cell populations.

We therefore developed a strategy to selectively enrich populations of barley cells that have been colonized by the fungus *Magnaporthe oryzae* and separate them from cells that have not yet been infected by the pathogen. To achieve this, we engineered mutants of *M. oryzae* that constitutively express a fluorescent marker. These mutants were inoculated on the primary leaves of barley plants, which were then used to generate protoplasts. Because the pathogen first invades epidermal cells, we next implemented a sophisticated protocol to separate mesophyll from epidermal protoplasts. Thereafter, Fluorescence Activated Cell Sorting (FACS) was used to sort cells containing fluorescent signal-emitting fungal hyphae from uninfected cells. After performing RNA-sequencing of the infected and uninfected cell populations, we will compare the gene expression profiles to identify barley genes that are differentially expressed between both cell populations. Additionally, we aim to identify fungal genes that play key roles during early biotrophic colonization of the plant.

582B **Insight into the adaptation mechanisms of high hydrostatic pressure in physiology and metabolism of hadal fungi from the deepest ocean sediment** Xi Yu, Maosheng Zhong, Yongqi Li, Ludan Deng Shanghai Ocean University

The deep sea is one of the least explored extreme environments on Earth. Most of the existing research on deep-sea fungi focuses on the screening of secondary metabolites for activity and the development of novel drugs. High hydrostatic pressure (HHP) influences the life processes of organisms living at depth in the oceans. While filamentous fungi are one of the essential members of deep-sea microorganisms, few works have explored their piezotolerance to HHP. In this study, 113 deep-sea fungi were successfully isolated from seawater and sediment samples of the Mariana Trench through a combination of traditional plate separation and culture techniques, in-situ culture simulation techniques and high-throughput screening techniques. High hydrostatic pressure affected the phenotypes, gene expression and secondary metabolite activities of hadal fungi. To explore the pressure-adaptive mechanism of hadal filamentous fungi, we obtained three homogeneous *Aspergillus sydowii* from terrestrial, shallow, and hadal areas, respectively, to compare their pressure-resistance. A set of all-around evaluation methods including determination of growth rate, metabolic activity, and microscopic staining observation was established and indicated that *A. sydowii* DM1 from the hadal area displayed significant piezotolerance. Global analysis of transcriptome data under elevated HP revealed that *A. sydowii* DM1 proactively modulated cell membrane permeability, hyphae morphology, and septal quantities for seeking a better livelihood under mild pressure. Besides, differentially expressed genes were mainly enriched in the biosynthesis of amino acids, carbohydrate metabolism, and cell process, etc., implying how the filamentous fungi respond to elevated pressure at the molecular level. We speculated that *A. sydowii* DM1 could acclimatize itself to HHP by adopting several strategies, including environmental response pathway HOG-MAPK, stress proteins, and cellular metabolisms.

583B **Fruiting body specific *sc4* hydrophobin gene plays a role in *Schizophyllum commune* hyphal attachment to structured glass surfaces** Evans Osahon Iyamu[1], Katrin Krause[2], Erika Kothe[2] [1]Microbial Communication, Institute of Microbiology Friedrich Schiller University, [2]Institute of Microbiology Friedrich Schiller University

Genes encoding hydrophobins play distinct roles at different stages of the life cycle of fungi, and they foster hyphal attachment to surfaces. The hydrophobin Sc4 is known to provide a hydrophobic membrane lining of the gas channels within *Schizophyllum commune* fruiting bodies. Here, we cultivated non-fruiting, monokaryotic *S. commune* 12-43 on glass surfaces that could be verified by micrography. Differential gene expression profiling of nine hydrophobin genes and the hydrophobin-like *sc15* gene by quantitative PCR showed significant up-regulation of *sc4* when *S. commune* was attaching to glass surfaces, confirmed also with RNA-Seq data analysis. Another silicate, namely quartz sand, was investigated, and induction of *sc4* was seen as well. The up-regulation of the hydrophobin gene *sc4* may indicate involvement in *S. commune* hyphal attachment to glass as well as quartz surfaces. We propose that the covering of hyphae by Sc4 allows for direct interaction with the hydrophobic surfaces of silicates, and that differential functions of specific hydrophobin genes is depending on the surface interface involved. This study could help with the clarification of the biological functions of hydrophobins in natural surroundings, including hydrophobic surface attachment. Therefore, the analysis of growth on glass serves as a basis for understanding *S. commune* interaction with glass surfaces while providing the possibility to visualize the interaction microscopically.

584B **Determine the role of an uncharacterized gene for its potential role in uniparental mitochondrial inheritance in *Cryptococcus neoformans*** Anuja Warrier, Ran Shi, Xiaorong Lin University of Georgia

Cryptococcus neoformans is a basidiomycete fungus, responsible for causing deadly cryptococcal meningoencephalitis in immunocompromised patients. *C. neoformans* exhibits uniparental mitochondrial DNA inheritance during sexual reproduction. However, the fundamental processes underlying this phenomenon are not completely understood. We have identified an uncharacterized gene CNG01800 in *C. neoformans* that may play a role in uniparental inheritance. The homologue of the encoded product in *Saccharomyces cerevisiae* is a mitochondrial protein and is known to improve translation efficiency of proteins encoded by the mitochondrial genome such as cytochrome C oxidase. In order to investigate the role of this gene, we will carry out a targeted gene deletion using TRACE followed by gene overexpression to explore the impact of its mutation on cryptococcal mitochondrial uniparental inheritance. We will also fluorescently tag this protein to track its subcellular localization, particularly during the process of sexual reproduction. We will also explore whether this gene plays a role in virulence, cell fusion, yeast-to hypha morphological transition, and sporulation.

585B **Unveiling GRAsp, An Online Tool for the Exploration of Gene Regulatory Networks in *Aspergillus fumigatus* to Gain Insights into Growth, Development, and Pathogenicity** Cristobal Carrera Carriel[1], Spencer A. Halberg-Spencer[2,3], Saptarshi Pyne[3], Sung Chul Park[4], Hye-Won Seo[4], Aidan Schmidt[4], Dante Calise[4], Sushmita Roy[2], Jean-Michel Ané[5,6], Nancy P. Keller[4,5] [1]Dept of Genetics, University of Wisconsin-Madison, [2]Dept of Biostatistics and Medical Informatics, University of Wisconsin-

Madison, [3]Wisconsin Institute for Discovery, [4]Medical Microbiology and Immunology, University of Wisconsin-Madison, [5]Dept of Bacteriology, University of Wisconsin-Madison, [6]Dept of Agronomy, University of Wisconsin-Madison

The notorious mold, *Aspergillus fumigatus*, is responsible for harmful and occasionally lethal respiratory conditions collectively known as aspergillosis. Understanding how genes fit into a regulatory pathway offers us valuable insight into the genetic determinants of this pathogen's growth and development. By leveraging expression datasets of *A. fumigatus*, we developed a comprehensive gene regulatory network we call GRAsp (**G**ene **R**egulation of ***Asp****ergillus fumigatus*). GRAsp successfully recapitulated previously characterized regulatory pathways related to hypoxia response, iron acquisition, and secondary metabolite synthesis. We also experimentally validated one of GRAsp's predictions that the transcription factor AtfA is required for the fungus' response to lipo-chitooligosaccharides, a chitin-based signaling molecule. We further unveil GRAsp as an online and user-friendly resource (grasp.wid.wisc.edu), enabling users to explore regulatory pathways of interest.

586B **Unveiling Novel Players in Polycomb-Mediated Gene Repression using Neurospora crassa** Rochelle Yap, Aileen Ferraro, Abigail Ameri, Zachary Lewis University of Georgia Athens

Post-translational modifications of histones can alter chromatin structure to regulate gene expression. In this context, Polycomb Repressive Complex 2 (PRC2) tri-methylates histone H3K27me3 to establish and maintain stable gene repression in plants, animals, and most fungi. Defective function of PRC2 can lead to aberrant transcriptional profiles, disorganized chromatin, and disease. However, how PRC2 is controlled and maintains silencing of genomic loci is incompletely understood. The goal of my project is to identify genes and proteins that regulate PRC2 or work in concert with this epigenetic regulator to assemble repressive chromatin domains using a simple model fungus. I am combining classical genetics and multi-omics approaches to identify new members of the Polycomb Repression Network. Recent genomic analyses revealed chromodomain protein, eaf3, and a histone acetyltransferase, rtt109, as new components of the Polycomb Repression Pathway in N. crassa. Each candidate knockout exhibits unique gene expression profiles of PRC2 target genes and reduced H3K27me3 levels. I will present the latest experiments of how I am characterizing these candidates' roles in their regulatory mechanisms underlying Polycomb-mediated gene silencing. Overall, these findings will contribute to a more comprehensive picture of the PRC2 repression pathway in a fungal model organism.

587B **On the mechanism of RNAi-mediated silencing of repetitive DNA in *Cryptococcus neoformans*** Sheng Sun, Vikram Ponnusamy, Vikas Yadav, Francis Fang, Anna Floyd Averette, Joseph Heitman Molecular Genetics and Microbiology, Duke University Medical Center

RNA-induced silencing is ubiquitous throughout eukaryotes, and serves central functions including regulation of gene expression, repression of transposable elements, and maintenance of genome stability. In *Neurospora crassa*, genes involved in DNA repair such as *RAD52* are known to be involved in transgene silencing. Additionally, studies have shown that formation of R-loops/RNA:DNA hybrids can also result in gene silencing. In the human fungal pathogen *Cryptococcus neoformans*, transgene tandem repeats are silenced by two RNAi silencing pathways: sex-induced silencing (SIS) and mitotic-induced silencing (MIS). These two pathways share common components but also show distinct characteristics. How transgene repeats are recognized to trigger silencing is unknown. In this study, we analyzed genes that are involved in DNA-repair (*RAD51*, *RAD52*, and *RAD54*), as well as genes required for R-loop resolution (*RNH1* and *RNH2*), for possible roles in silencing of a *URA5* transgene array in *C. neoformans*. Mutation of the *RAD51*, *RAD52*, and *RAD54* genes conferred hyper-sensitivity to DNA damaging agents and abolished sporulation during bi-lateral crosses, consistent with roles in homologous recombination mediated DNA repair. Notably, none of the three *RAD* genes was required for MIS transgene silencing. On the other hand, deletion of the *RNH1* and *RNH2* genes significantly increased MIS during vegetative growth, suggesting formation of RNA:DNA hybrids may promote transgene array silencing. Furthermore, we discovered significantly increased retention of the two introns of the *URA5* gene in strains in which the transgene array was silenced. Consistent with this, deletion of the *GWC1* gene, which is a component of the SCANR complex that recognizes stalled spliceosomes at unspliced introns, led to complete abolishment of MIS. Taken together, our studies provide evidence that transgene silencing does not involve a *RAD* gene mediated DNA homology search but instead involves: 1) intron retention and activation of the SCANR complex, and 2) RNA-DNA interaction, possibly via RNA from one gene repeat involving a neighboring DNA repeat to form an R-loop.

588B **Ryp transcription factors link temperature sensing and morphogenesis in *Histoplasma*** Anna Morrison, Mark Voorhies, Anita Sil UCSF

Temperature plays a critical role in altering the developmental program and virulence of thermally dimorphic fungal pathogens, including *Histoplasma* species. In the environment, *Histoplasma* grows as a multicellular hyphal form that produces vegetative spores. Upon inhalation by a mammalian host, spores undergo a developmental change to a parasitic yeast form that secretes

virulence factors and causes disease. This morphological transition can be recapitulated in the laboratory, where temperature is a sufficient signal to shift cultures of *Histoplasma* between hyphal and yeast forms, which grow at 25°C and 37°C, respectively. To understand how *Histoplasma* links temperature to changes in morphology and gene expression, we performed RNA-Sequencing on samples grown at 37°C versus those transitioned to 25°C for two hours to capture early changes in transcript levels. Expression of the majority of acutely temperature-responsive transcripts is dependent upon the Ryp (Required for Yeast Phase) transcription factors, which were previously identified in our laboratory to be key regulators of the morphogenesis program in *Histoplasma*. To identify temperature-sensitive changes in Ryp DNA-binding activity that might account for transcriptomic changes, we then performed Ryp Chromatin immunoprecipitation followed by sequencing (ChIP-Seq) on samples grown at 37°C versus those transitioned to 25°C for two hours. The majority of Ryp2 association events were not differential under these conditions. However, we observed Ryp2 binding events at heat shock elements in the promoters of key heat shock protein (HSP) genes, including HSP90 and HSP70, only at 37°C. Strikingly, Ryp2 association at these promoters was lost within two hours at 25°C, indicating that Ryp2 association is temperature-dependent. Loss of Ryp2 association was correlated with a greater than two-fold drop in transcript levels of many HSP genes. This study strongly suggests that Ryp2 is upstream of HSP gene expression, and highlights for the first time a link between the heat shock pathway, which is intrinsically temperature-responsive, and the Ryp transcription factors, which control morphology in *Histoplasma*. This work thus provides insight into how thermally dimorphic fungi sense temperature and transduce this signal into downstream pathways involved in morphogenesis and virulence.

589B **Regulation of sugar metabolism under abiotic stress in various yeasts and filamentous fungi** Elisabeth Tamayo[1], Pedro Tomaz da Silva[2], Julien Gagneur[2], J. Philipp Benz[1] [1]Fungal Biotechnology in Wood Science, Technical University of Munich, [2]Computational Molecular Medicine, Technical University of Munich

Fungi are important for biotechnological and medical reasons. Additionally, fungal biotechnology can offer solutions to ensure food supply for a growing human population with little impact on the environment, since mycoprotein is an interesting meat substitute with a high nutritional value and much lower carbon footprint. Fungi have developed elaborated strategies to cope with abiotic stress, as they often experience both in nature and during biotechnological fermentations. Several studies have been conducted to better understand the regulation underlying this adaptability in certain fungi, and e.g. genes related to glycerol transport were found to be important in acidic pH-tolerant yeast strains or under salt stress conditions. However, less is known regarding these processes from an evolutionary point of view, particularly considering the involvement of sugar metabolism in this regulation.

With the aim to identify both conserved and species-specific routes of sugar regulation in abiotic stress tolerance across Ascomycete fungi, we subjected several distantly related fungal species, including eight yeasts and three species of filamentous fungi of the genus *Neurospora*, to five abiotic stress conditions and analyzed their response by transcriptomics. Taking advantage of our previous study of the sugar transportome of *Neurospora crassa*, we focused on the transcriptomic changes of sugar transporters, sugar metabolism-related enzymes and transcription factors. The influence of the different stress conditions was explored in detail for *Neurospora* sp. candidate genes and compared with that in orthologues in the yeast species, to find out which regulatory features are specific or conserved. Several sugar transporters belonging to the major facilitator superfamily were identified as candidates involved in salt stress tolerance, some carbohydrate-active enzymes were found to be upregulated at acidic pH, and nine uncharacterized transcription factors were identified to have a putative role in carbon starvation, pH or ethanol stress.

Our data will help to better understand the mechanisms of sugar regulation in response to abiotic stress in fungi. This knowledge could then be used to develop biotechnological strategies to improve fungal nutritional values under stress conditions in fungal species that could serve to feed the human population in an environmentally friendly manner. Our new findings in this regard will be presented and discussed.

590B **Identification of Intertwined Catabolic Pathways in an Oleaginous Yeast** Joshua D Kerkaert[1], Brandon Reyes-Chavez[2], Lori Huberman[1] [1]Plant Pathology and Plant-Microbe Biology, Cornell University, [2]Microbiology, Cornell University

The capacity to sense and respond to available nutrients is central to every organism's physiology and ecology. The oleaginous yeast *Rhodosporidium toruloides* is of industrial interest, in part, due to its ability to readily sense and catabolize carbohydrates found in lignocellulose hydrolysate, including the building block of cellulose, cellobiose. However, the mechanisms of cellobiose sensing and metabolic regulation are incompletely defined in *R. toruloides*. We screened for genes involved in cellobiose utilization and identified a transcription factor, which we named *CBR1*. Deletion of the *CBR1* open reading frame resulted in cells that were unable to efficiently utilize cellobiose and, curiously, tricarboxylic acid cycle intermediates. In contrast, loss of *CBR1* yielded wild

type levels of growth on building blocks of hemicellulose and pectin, as well as carbon sources that feed into the tricarboxylic acid cycle. Thus, Cbr1 appears to specifically regulate utilization of cellobiose and tricarboxylic acid cycle intermediates, suggesting an interplay between these nutrients in an oleaginous yeast. Further characterization of *CBR1* and the metabolic intertwining of these nutrients may yield critical insights relevant to the industrial production of biofuels and other bioproducts during growth on lignocellulose hydrolysate and plant-derived feedstocks.

591B **Natural variation in the hyphal/biofilm regulatory network of *Candida albicans*** Eunsoo Do[1], Manning Y Huang[2], Gemma May[2], Joel McManus[2], Aaron P Mitchell[1] [1]Dept of Microbiology, University of Georgia, [2]Dept of Biological Sciences, Carnegie Mellon University

Hypha and biofilm formation are pivotal for virulence of the human fungal pathogen *Candida albicans*. The hyphal/biofilm regulatory network includes several master regulators, including Ume6, the activator that maintains hyphal growth. The Ume6-dependent transcriptome is remarkable because of its extensive variability among *C. albicans* clinical isolates. However, ChIP-seq analysis demonstrates highly uniform Ume6 binding sites in two strains analyzed, SC5314 and P75010. Ume6 is associated with three different binding sites, which align with consensus sites for biofilm master regulators Efg1 and Ndt80, and for hypoxia regulator Upc2. Co-immunoprecipitation shows that Efg1, Ndt80 and Upc2 each physically interact with Ume6. Our hypothesis is that Ume6 activates genes through formation of three heteromeric complexes – Ume6:Efg1, Ume6:Ndt80, and Ume6:Upc2. Extensive natural variation in Ume6-responsive gene expression may be explained by the combined variation in activities of both subunits of each heteromeric complex.

592B **A Case for the Kinases: A Role for CKI in Temperature Compensation of the *Neurospora crassa* Circadian Clock** Elizabeth-Lauren Stevenson[1], Christina M. Kelliher[1,2], Jennifer J Loros[3], Jay C Dunlap[1] [1]Molecular and Systems Biology, Dartmouth College, [2]Biology, University of Massachusetts Boston, [3]Biochemistry and Cell Biology, Dartmouth College

Circadian clocks enable organisms to anticipate the daily environmental cycles that result from the Earth's rotation, so that they may then designate appropriate day to night functions. As such, the clock regulates many physiological processes. The molecular circadian clock in animals and fungi consists of a transcription-translation feedback loop that is regulated post-translationally throughout the circadian day by phosphorylation events. In the classic clock model *Neurospora crassa,* the positive arm of the clock, a heterodimeric complex of transcription factors, activates the transcription of the negative arm of the clock, Frequency (FRQ), which complexes with Casein Kinase I (CKI) to inactivate the positive arm via phosphorylation, thereby inhibiting their own transcription. Several key features define circadian rhythms, including the ability to entrain to external cues, the capacity to continue oscillating in the absence of those cues, and the maintenance of a consistent period across temperatures (known as temperature compensation – TC). TC is an essential clock property which is found in all organisms with circadian clocks, yet its mechanism remains undefined.

We discovered a novel mutation in Casein Kinase I (CKI) that confers a long period and severe undercompensation such that period shortens as temperature increases, suggesting a role for CKI in the TC mechanism of *Neurospora*. We find that reduction in CKI levels or activity causes the clock to be undercompensated, compared to a reduction in Casein Kinase II (CKII) activity conferring overcompensation. Inhibiting CKI in a CKII hypomorph still dose dependently altered its normally overcompensated TC profile, suggesting CKI is downstream of CKII in the TC mechanism. Hypothesizing that TC may be achieved by temperature-dependent differential phosphorylation of clock components, we performed phosphoproteomics in WT and CKI mutant backgrounds across a range of temperatures and identified phosphosites whose phospho-occupancy changes significantly with both temperature and genotype. We find that phosphonull mutations at a subset of these sites on FRQ alter the temperature compensation of the clock. These data provide support for a kinase-based model of temperature compensation.

593B **Post-transcriptional control of fungal cell wall synthesis by Ssd1 and co-operating RNA-binding proteins** Edward W. J. Wallace School of Biological Sciences, The University of Edinburgh

Fungal cell walls are the surface where the fungus meets the world. Yet, there are major gaps in understanding of fungal cell wall synthesis and its regulation, notably at the post-transcriptional level, where the time and place of protein synthesis are controlled. Advances in mRNA regulation have identified several RNA-binding proteins that regulate cell wall synthesis, however their importance in fungal cell biology remains underappreciated[1]. Our recent work focuses on the RNA-binding protein Ssd1/sts5/gul-1, which is required for fungal growth and virulence, and genetically interacts with cell cycle regulators. We characterised the evolution of Ssd1's fungal-specific RNA-binding function[2]. We identified the RNA motif bound by Ssd1 and showed that it is

enriched in the 5'UTRs of cell wall mRNAs across ascomycota[3]. With Atlanta Cook's group, we solved the structure of Ssd1 and are investigating its mechanisms of RNA binding.

We begin to marshal evidence across the dikaryota to build a model for Ssd1-mediated spatiotemporal regulation of cell wall synthesis. Using both model fungi and non-model fungal pathogens, we begin to probe functional divergence relevant to stress resistance and pathogenesis. In *S. cerevisiae*, Ssd1-regulated mRNAs have distinct spatiotemporal expression during the cell cycle, ruling out a simple mechanism by which Ssd1 controls mRNA localisation. By contrast, we find increased production of Ssd1-regulated cell wall proteins in *ssd1Δ* mutants. Ssd1's role as a translation repressor is directly demonstrated by our finding that Ssd1-binding motifs inserted into reporter mRNAs repress translation. We hypothesise that Ssd1-mediated translational repression, in co-operation with other RNA-binding proteins, allows "just-in-time" local translation of cell wall components near sites of cell wall synthesis across fungal cell cycles and morphological transitions. Disruption of this regulatory circuit has profound, pleiotropic impacts on cell wall synthesis, cell division, and stress resistance.

Overall, using diverse approaches, we provide insight into a fungal-specific mechanism of post-transcriptional regulation that has implications for the local translation of fungal cell wall proteins central to fungal cell biology.

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594B **Functional analysis of genes induced during the aerial hyphae collapse leading to perithecium formation in *Fusarium graminearum*** Sung-Hwan Yun, Yun-Seon Choi, Da-Woon Kim Medical Biotechnology & Medical Sciences, Soonchunhyang Univ

Fusarium graminearum, the causal agent of Fusarium Head Blight in cereals, predominantly produces sexual fruiting bodies (perithecia) on previous crop residues in the fields. In contrast, perithecia formation has been artificially induced by removing aerial hyphae grown on carrot agar culture of *F. graminearum*. Even under artificial culture conditions, the *F. graminearum* Z3643 strain frequently formed perithecia in the central region of the agar culture where aerial hyphae had naturally collapsed. To elucidate the molecular mechanism underlying hyphae collapse-mediated sexual development in *F. graminearum*, we identified a total of 699 differentially expressed genes (DEGs) during the aerial hyphae collapse on agar medium. Among these, 87% were likely not transcriptionally controlled by the *MAT* loci, the master regulators of sexual development in *F. graminearum*. Through the generation of transgenic mutants of Z3643, each carrying a single deletion of the selected 35 DEGs, we confirmed that five genes played crucial roles during hyphae collapse and/or perithecium formation. Notably, the deletion strain of a gene encoding a putative phospholipid-translocating ATPase, which was highly induced during hyphal collapse but was not regulated by *MAT*, displayed no evident hyphal collapse and failed to produce perithecia on carrot agar. In conclusion, these findings suggest that aerial hyphae collapse, when possibly occurring on crop residues, may serve as a physical signal triggering sexual development in *F. graminearum* under natural conditions.

595B **Investigating the role of chromatin dynamics in *Histoplasma* morphogenesis** Nebat Ali, Mark Voorhies, Anita Sil UCSF
The ability to sense and adapt to the environment is a hallmark of clinically relevant microbial pathogens. This phenomenon is exemplified in thermally dimorphic fungi such as *Histoplasma,* where temperature is a critical signal that triggers a dramatic shift between cell states. At ambient temperatures, *Histoplasma* grows as filamentous hyphae that can be aerosolized and inhaled into the lungs of mammals, where elevated temperature (37°C) is sufficient to induce the switch to growth as a pathogenic budding yeast. Prior studies aimed at understanding the regulation of this switch identified a key network of transcription factors (TFs) Required for yeast phase growth (Ryp 1,2,3,4) that globally reprogram the transcriptome to establish yeast cells at 37°C. Given that chromatin state can influence TF activity and DNA-binding, we sought to investigate if chromatin dynamics underlie the global gene expression changes required to establish cell state. Studies in related fungi that undergo morphology changes have identified histone modifying enzymes that are critical for proper initiation and maintenance of morphogenesis. In *C.albicans,* histone deacetylases (HDACs) work in conjunction with TFs to direct the regulatory landscape of the cell throughout transitions. To broadly query a putative role for HDACs in *Histoplasma,* we sought to test the morphological effect of chemical compounds that act as HDAC inhibitors (HDACi). We selected a panel of HDACi to comprehensively target all classes of HDAC orthologs conserved in *Histoplasma.* Interestingly, treatment of *Histoplasma* yeast cells with a Class-I HDACi disrupts yeast-phase growth and induces improper hyphal growth at 37°C. Transcriptome analyses of Class-I HDACi-treated cells reveals large global

changes in gene expression and disruption of the canonical Ryp regulon at 37°C. Furthermore, HDACi treatment triggers inappropriate accumulation of hypha-specific transcripts at 37°C, suggesting a potential role for Class I HDACs in temperature-dependent transcription. Phylogenetic analysis reveals Histoplasma encodes two highly conserved Class I HDACs: Rpd3 and Hos2. Genetic studies leveraging CRISPR/Cas9 gene editing are currently underway to investigate the function of Rpd3 and Hos2. Preliminarily, strains undergoing disruption of Rpd3 display aberrant morphology. These studies will uncover how chromatin remodeling factors contribute to regulating this critical developmental switch in thermally dimorphic fungi.

596B **Exploring RNA thermosensors that drive development and virulence in thermally dimorphic fungal pathogens** Murat Can Kalem[1], Mark Voorhies[2], Anita Sil[2] [1]Microbiology and Immunology, University of California San Francisco, [2]University of California San Francisco

Human body temperature is a key signal sensed by fungi to elicit developmental programs that facilitate pathogenesis. The molecular mechanisms of temperature sensing and thermosensors remain enigmatic. We hypothesized that fungal transcriptomes harbor temperature-responsive RNA structures. We are exploring RNAs as potential thermosensors in the thermally dimorphic fungal pathogens *Histoplasma* and *Coccidioides.* They are the ideal systems to investigate RNA thermosensors since host temperature is the main signal that triggers the reprogramming of morphology and gene expression. *Histoplasma* and *Coccidioides* grow as multicellular hyphae in the soil but transition to a yeast or spherule form in the host in response to elevated temperature.

RNA is multifunctional due to its ability to fold into complex three-dimensional structures that can interact with other molecules, such as RNA-binding proteins (RBPs). We adopted a global and unbiased RNA structure probing approach, DMS-MaP-seq, to discover temperature-responsive RNA elements and structures. DMS-MaP-seq, along with efficient rRNA depletion, gives us the opportunity to explore the role of RBPs and helicases on thermally regulated structures. RBPs and helicases may be differentially expressed or functional at various temperatures and act on structural elements. Some *Histoplasma* RBPs and helicases have differential translation efficiencies at 22˚C and 37˚C, including the helicase Ded1. We are knocking down Ded1 in *Histoplasma* and investigating its role in regulation of morphology.

In a complementary candidate approach, we are exploring the role of RNA guanine quadruplex (rG4) structures in thermosensing and development. *Histoplasma RYP2,* a transcription factor that is required for yeast-phase growth, has a longer 5' UTR only at room temperature with two putative rG4 structures. The longer 5' UTR correlates with a robust decrease in translation. rG4 structures modulate translation, RNA decay, and phase separation. We utilized carboxy pyridostatin (cPDS), which stabilizes rG4s, to begin dissecting the role of rG4s in morphology. cPDS promoted hyphal growth in *Histoplasma* and altered *Coccidioides* development. This work highlights that RNA structure is crucial for temperature-responsive fungal development. Interrogation of structure-function relationships and effectors contributing to thermosensing will continue to unravel fundamental mechanisms of RNA regulation.

597B **Predicting culture conditions for secondary metabolite production based on binding targets of biosynthetic gene cluster-specific transcription factors** Fan Lu, Shuhui Guo, Ruiwen Chen, Lakhansing Pardeshi, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau

Fungi possess remarkable capabilities for synthesizing diverse secondary metabolites (SMs) with significant application potential in medicine, agriculture, and industry. SM biosynthetic genes are often organized in clusters on fungal genomes, where each cluster is responsible for synthesizing a specific SM. Genome sequencing has identified many novel SM biosynthetic gene clusters (BGCs) in fungi, representing a rich resource for drug discovery. However, most SM BGCs remain transcriptionally silent under standard laboratory conditions, presenting challenges for their identification. In many BGCs, a transcription factor gene is embedded within the cluster, playing a crucial role in activating other biosynthetic genes within the same cluster. However, whether these cluster-specific transcription factors can bind and regulate genes outside their cluster is not clear. We hypothesize that BGC transcription factors may regulate non-BGC genes with functions essential or favorable for the biosynthesis of the intended SM, such as metabolic reprogramming to produce necessary metabolic precursors, response to specific environmental cues, or physiologies specific for developmental stages. If this were true, it would be possible to deduce the conditions favorable for SM production based on the genome-wide targets of the BGC transcription factor for a given SM. To demonstrate this, we conducted Chromatin Immuno-Precipitation followed by Sequencing (ChIP-Seq) to map the genome-wide binding sites of AflR – the well-studied transcription factor of sterigmatocystin (ST) biosynthesis in *Aspergillus nidulans*. Our results revealed extensive AflR binding to genomic regions beyond the ST BGC, exerting control over numerous physiological processes. More importantly, we successfully devised specific growth conditions that promote ST production based on the AflR binding target information. Taken together, this

work provides valuable insights into the regulation of ST and introduces a novel approach to activate cryptic SM BGCs, enabling the discovery of novel secondary metabolites.

598B **The effects of phase separation on chromatin modifications, transcriptional regulation and virulence in the human fungal pathogen *Candida albicans*** Qing Lan, Zhengqiang Miao, Ruiwen Chen, Pin Wu, Songlin Wu, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau

Candida albicans is an opportunistic pathogen that can live in the human body as a commensal and cause deadly infection when the immune system is compromised. *C. albicans* can colonize different niches and survive the wide range of stresses elicited from the host during infection. These abilities are mediated by rapid and dynamic transcriptional responses, which are tightly controlled at transcription levels (e.g. transcriptional activation, elongation, and termination) and chromatin modification. The phenomenon of liquid-liquid phase separation can rapidly trigger the formation and dissociation of cellular compartments for biomacromolecules like proteins and nucleic acids without physical barriers. The process is reversible and tunable by factors relevant to the infection process, such as pH, temperature, and salinity. Phase separation has been shown to play crucial roles in controlling various biological processes and pathways in many organisms. We hypothesize that phase separation is important for the transcriptional responses of pathogens during adaption to diverse environmental conditions and infection. This study investigated the role of phase separation on the transcription and pathogenicity processes in *C. albicans.*

599B **Transcription factor ManS regulates mannanase gene expression in *Aspergillus nidulans*** Haruno Watanabe[1], Nuo Li[2], Kyoko Kanamaru[2], Makoto Kimura[2], Tetsuya Kimura[1], Tetsuo Kobayashi[2], Emi Kunitake[1] [1]Graduate school of Bioresources, Mie University, [2]Nagoya University

Aspergillus strains produce mannanolytic enzymes which are widely used as industrial enzymes. Induction of the mannanase genes is mediated by the transcriptional activator ManR in *A. oryzae*, which is essential not only for mannanase but also for cellulase gene expression. By contrast, the cellulase regulator ClrB in the model fungus *A. nidulans*, which is the ortholog of ManR, only partially involved in regulation of the mannanase genes, and our previous studies revealed that ManS, a paralog of ManR/ClrB, functions as the major transcriptional activator of the mannanase genes. In this study, we performed transcriptional analysis of the mannanase genes in the *manS* and/or *clrB* deletants and also examined the DNA binding properties of ManS and ClrB *in vitro* to further elucidate the regulatory mechanisms of the mannanase genes by these two transcription factors.

Transcription of the mannanase genes were induced in the presence of β-1,4-mannobiose. Deletion of *manS* or *clrB* affected transcription of the genes differently depending on the gene; the expression of *manC* and *manE* was regulated primarily by ManS, while the expression of *manB* was regulated cooperatively by ClrB and ManS.

To determine the recognition sequence of ManS, electrophoretic mobility shift assay (EMSA) was performed using the His-tagged and FLAG-tagged DNA binding domains of ManS and ClrB, repectively (His-$ManS_{164}$ and FLAG-$ClrB_{118}$). Various DNA fragments derived from the 1,000 bp upstream of *manE* was subjected to EMSA, and the short fragments that showed strong binding to His-$ManS_{164}$ were further analyzed by introducing mutations. Finally, $CGGN_{16}CCG$ (-502 to -481) was identified as the ManS binding site. $CGGN_{16}CCG$ was also present in the *manB* and *manF* promoters, and His-$ManS_{164}$ did bind to $CGGN_{16}CCG$ in the *manB* promoter. As a single exception, the *manC* promoter did not possess the sequence. DNA binding studies revealed that the binding sites is located in the -210 to -171 and -185 to -146 regions, and that the CGG triplets in the regions are required for the binding.

Since *manB* expression was dependent on both ManS and ClrB, we investigated the possibility that His-$ManS_{164}$ and FLAG-$ClrB_{118}$ bind to the *manB* promoter simultaneously. EMSA revealed formation of a heterodimer of His-$ManS_{164}$ and FLAG-$ClrB_{118}$ on the $CGGN_9CGGN_6CGG$ sequence (-117 to -94), implying that this is the cause of the cooperative regulation by ManS and ClrB.

600C **Study on environmental responses and peptidase genes transcriptional regulation in *Aspergillus oryzae* PrtR** Rika Numamzawa[1], Yukako Tanaka[2], Sawako Nishioka[2], Ryotaro Tsuji[1], Hiroshi Maeda[2], Yoshiyuki Itoh[3], Michio Takeuchi[1], Mizuki Tanaka[1], Youhei Yamagata[1] [1]United Graduate School of Agricultural Science, Tokyo Univercity of Agriculture and Technology, [2]Applied Biological Science, Tokyo Univercity of Agriculture and Technology, [3]Smart-Core-Facility Promotion Organization, Tokyo Univercity of Agriculture and Technology

Aspergillus oryzae is used as an enzyme source for producing traditional Japanese fermented foods. *A. oryzae* has a strong ability to secrete many hydrolytic enzymes, mainly amylases and peptidases. Although the regulation of amylase production has been

well studied, little is known about the regulation of peptidase production. It is thought that *A. oryzae* would recognize the external environment and produce peptidases as needed. However, the mechanism from recognition of external environmental signals to peptidase production is not clear. If this signaling network is elucidated, it may be possible to produce peptidases selectively from many peptidase genes in *A. oryzae*. In this study, we focused on PrtR, an ortholog of the transcription factor PrtT, which positively regulates the transcription of extracellular peptidase genes in some *Aspergillus.* We aimed to elucidate the role of PrtR in the network from the recognition of environmental signals to peptidase production.

First, we identified peptidase genes regulated by PrtR using *prtR* gene-deficient strain. It was shown that PrtR was involved in the transcription of almost all extracellular peptidase genes. Furthermore, PrtR optimizes transcription of peptidase genes in response to culture conditions.

Next, localization analysis was performed using GFP-PrtR-expressing strain. The results showed that PrtR localized to the nucleus when protein was used as a nitrogen source. Meanwhile, PrtR was generally localized to the cytoplasm when NH_4Cl was used. This result was consistent with the *prtR* mRNA levels when cultured with each nitrogen sources. This suggests that PrtR is controlled in mRNA levels depending on the nitrogen source. It was also shown that the excess amount of *prtR* mRNA was degraded depending on the nitrogen source.

Furthermore, LC-MS/MS analysis identified the phosphorylated amino acids of PrtR. Transcription of the peptidase gene was also enhanced in PrtR with these amino acid replaced with alanine. This suggested that PrtR would be activated form in the dephosphorylated state.

In summary, *A. oryzae* recognizes environmental nitrogen sources and would dephosphorylate PrtR to the activated state. The activated PrtR localizes to the nucleus and optimizes peptidase gene transcription in response to the nitrogen source. PrtR, itself would be also regulated at the mRNA level in response to the nitrogen source.

601C **Srr1, a conserved transcription factor regulates postmeiotic spore morphogenesis and ballistospory in mushroom-forming fungi** Zhihao Hou[1,2], Zsolt Merenyi[1], Yashu Yang[3,4], Yan Zhang[1,5], Arpad Csernetics[1], Balazs Balint[1], Botond Hegedus[1], Csenge Foldi[1,2], Hongli Wu[1], Mate Viragh[1], Xiao-Bin Liu[1], Nikolett Zsibrita[1], Wei Gao[3,4], Laszlo G Nagy[1] [1]Synthetic and Systems Biology Unit, Institute of Biochemistry, HUN-REN Biological Research Centre Szeged, [2]Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, [3]Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, [4]State Key Laboratory of Efficient Utilization of Arid and Semi-Arid Arable Land in Northern China, [5]College of Plant Protection, Shandong Agricultural University

Spore formation is the most widespread means of reproduction and dispersal in fungi. In the Basidiomycota, spores also have industrial importance and their active discharge represents the highest known acceleration in nature. In this study, we characterized a highly conserved C_2H_2-type zinc finger transcription factor *srr1* (sporulation-related regulator), which we identified based on its high expression in the gill of multiple mushroom-forming fungi. The Δ*srr1* mutant of *Coprinopsis cinerea* showed a spore-less white cap phenotype, with no significant influence on mycelium growth or the fruiting body developmental process. The knock-down of *srr1* ortholog in *Pleurotus cornucopiae* had a spore-poor phenotype, indicating the conserved role of *srr1* among Agaricomycetes. In the Δ*srr1* mutants, spore development was arrested when spore initials emerge at the sterigma tips after meiosis. RNA-Seq revealed 154 up- and 559 down-regulated genes in Δ*srr1* compared to the wild type, suggesting that *srr1* is mostly an activator. The motif GTGGCDNAWS was inferred as the binding site of srr1 among 260 direct targets. Taken together, *srr1* has a conserved role in sporulation and ballistospory in mushroom-forming fungi, with its target genes representing potential candidates for further studies of sporulation and the mushroom industry.

602C **A novel reporter system to identify arginoketides in soil that mediate cross-kingdom microbial interactions** Maira Rosin[1], Mario K. C. Krespach[1], Maria C. Stroe[1,2], Nils Jaeger[3], Kirstin Scherlach[4], Volker Schroeckh[1], Thorsten Heinzel[3], Christian Hertweck[4], Axel A Brakhage[1] [1]Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), [2]Dept of Microbiology, Karlsruhe Institute of Technology (KIT), [3]Dept of Biochemistry, Friedrich Schiller University Jena, [4]Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI)

In all habitats on earth microorganisms form consortia with many different species closely living together in the soil. Interspecies communication in these communities are decisive for function of microbial communities and further lead to the induction of otherwise silent natural product biosynthesis gene clusters. One prominent example is the interaction of the fungus *Aspergillus nidulans* and the bacterium *Streptomyces rapamycinicus*. Upon co-cultivation, the streptomycete is able to reprogram the

epigenetic machinery of the fungus by activation of the histone acetyltransferase GcnE which leads to the induction of the otherwise silent *ors* biosynthesis gene cluster in *A. nidulans* [1,2]. By inhibitor studies with the pan-sirtuin inhibitor nicotinamide and analyses of several histone deacetylase mutants, we identified the silent information regulator SirE as the histone deacetylase terminating the induction of the *ors* BGC by *S. rapamycinicus* [3]. Furthermore, we discovered that the compound family of arginoketides including azalomycin F produced by *S. iranensis* and *S. rapamycinicus* serve as the long sought-after bacterial signals for this induction [4]. To estimate the induction of silent gene clusters, we developed a fungal reporter system encoding the gene for the green fluorescence protein (GFP) coupled to the nanoluciferase gene and the gene of interest. Thus, enabling the qualitative and quantitative measurement of the transcriptional activation of genes. Here, this construct was translationally fused to the *orsA* gene of the orsellinic acid biosynthesis gene cluster of *A. nidulans.* Transformants showed fluorescence and luciferase activity upon addition of *S. iranensis*, azalomycin F or the pan-sirtuin inhibitor nicotinamide to the culture. Interestingly, extracted soil also led to an increased nanoluciferase activity and green fluorescence indicating that arginoketides are indeed present in the soil. Further, with this reporter we were able to identify several bacterial strains, isolated from a random soil sample, that induce green fluorescence in the fungus [4]. This indicates that arginoketides can be found around the world and playing an important role in mediating microbial interactions in the soil.

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603C **Understanding the mechanisms that regulate H3K27me3 in the model fungi *Neurospora crassa*** Felicia Ebot Ojong, Aileen R Ferraro The University of Georgia

Neurospora crassa is a filamentous fungus with a rich history in epigenetics research. Several conserved epigenetic pathways operate in *N. crassa* to silence gene expression, including RNAi, DNA methylation, H3K9 methylation, and H3K27me3, which is absent in yeast models. Like higher eukaryotes, H3K27me3 is catalyzed by a conserved Polycomb Repressive Complex 2 (PRC2). Neurospora also contains the HCHC complex, which contains Histone Deacetylase 1 (HDA-1) and is targeted to H3K9me3 by Heterochromatin protein 1 (HP1). The regulation of constitutive heterochromatin is also accomplished by the HCHC deacetylase silencing complex, composed of HP1, CDP-2, HDA-1 and CHAP. The HCHC complex removes acetyl groups from histones in constitutive heterochromatin. We carried out a genetic screen to identify genes required for Polycomb repression in N. crassa. We found that HDA-1 is required for repression of PRC2-targeted genes. In the absence of HDA-1, H3K27me2/3 is lost from typical PRC2-targeted domains and accumulates aberrantly at constitutive heterochromatin domains marked by H3K9me3. We also showed that CDP-2, another HCHC mutant, is important for proper localization of the H3K27me2/3 methylation mark. Together, our results show that H3K9me3 recruits' components of the HCHC deacetylation complex to prevent aberrant recruitment of PRC2 to constitutive heterochromatin domains.

604C **Clade-wide exploration of fungal sRNAs reveals hints of conservation** Nathan Johnson[1,2], Fabian Gonzales[1], Barbara Bernal[1], Luis Larrondo[2,3], Jose Miguel Alvarez[2,4], Elena Vidal[1,2] [1]Center of Genomics and Bioinformatics, Universidad Mayor, [2]Millennium Institute for Integrative Biology (iBio), [3]Pontificia Universidad Catolica, [4]Universidad Andres Bello

Small regulatory RNAs (sRNAs) are the functional units of RNAi and are a widespread and ancient form of genomic regulation. In fungi, sRNAs have several important roles, including protecting genomic integrity and pathogenesis, where there is evidence of *trans*-species silencing. However, many unknowns persist in terms of the genomics and evolution of fungal sRNAs. While sRNA processing and mechanisms have long been established, there is also extreme variation between fungi - some having functionally lost this pathway. Fungi also vary in the sizes of sRNAs produced by a locus and we know little of their sRNA transcriptional units (loci/genes). Conservation of sRNA loci and sRNA-target relationships are common in plants and animals, where little evidence has been found so far in fungi. This project is focused on exploring the genomics of sRNAs in fungi, focusing on breadth (all available species) and on consistency (common pipeline specific to fungi). This methodology allows for much more powerful comparisons between species, allowing us to explore their characteristics and evolution. This is key for precisely identifying any conserved sRNA loci that exist between within fungi. We have explored annotations that exist in publications for the most-described sRNA class in fungi: microRNA-like RNAs (milRNAs). These are sRNAs derived from hairpins that are similar to microRNAs from plants and animals, but generally lack the same clear definition. By compiling all available annotations, we found over 1,700 loci annotated from more than 40 fungal species. Careful reanalysis of this set showed a much smaller number of loci that passed basic tests of being hairpin-derived. With this filtered list, we were able to assess basic dimensions about milRNAs. Genomic comparisons of filtered loci showed several sRNA loci which are conserved with relatively distant species, including expression of the yet-undescribed hairpin. Ultimately, this analysis resulted in a localized and complete annotation of all these loci, including a classification of their supporting evidence and confidence. We are now expanding the scope to focus on all species with available sequencing. Our pipeline shows advantages in terms of consistency and sensitivity for a wide range of loci - key factors considering

the variability of data across fungi. Using these tools, we have shown basic details of sRNA loci across nearly 100 fungal species, allowing for more detailed analyses to come.

605C **A conserved oxylipin alarm blocks the fungicidal effects of echinocandins in pathogenic aspergilli** Dante G Calise[1], Sung Chul Park[1], Jin Woo Bok[1], Gustavo H Goldman[2], Nancy P Keller[1,3] [1]Dept of Medical Microbiology & Immunology, University of Wisconsin - Madison, [2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, [3]Dept of Plant Pathology, University of Wisconsin - Madison

Humans readily inhale spores of the ubiquitous mold *Aspergillus fumigatus*. Small enough to reach the alveoli of the lung, these spores are rapidly cleared by a healthy immune system without development of disease. However, in immunocompromised individuals, germination and tissue invasive hyphal growth can lead to a life-threatening infection termed invasive aspergillosis (IA). The recommended first line treatment of IA is with triazole antifungals, but in cases of poor clinical response to these membrane targeting drugs, salvage therapy with the cell wall active echinocandins is crucial for effective treatment. The echinocandin antifungals—caspofungin, micafungin, and anidulafungin—are limited in that they are only fungistatic against aspergilli due to their inability to kill established hyphae. However, *in vitro,* treatment of *A. fumigatus* with inhibitory concentrations of caspofungin results in the death of approximately fifty percent of germinating conidia by fungicidal lysis of their growing tips. Surviving germlings are further inhibited fungistatically and display severely stunted hyphal growth developing into highly branched chitin rich microcolonies. As they grow, caspofungin treated hyphae continue to undergo tip lysis, but the fungicidal effect is limited to the apical most hyphal compartment by the blocking of septal pores. Our lab recently found that the fungal oxylipin 5,8-diHODE, produced by *A. fumigatus* and related aspergilli, induces hyphal growth reminiscent of echinocandin treatment with increases in lateral branching, septation, and cell wall chitin. Here, we uncover an endogenous mechanism of antifungal tolerance in aspergilli whereby 5,8-diHODE activates echinocandin tolerant growth. We found that treatment of wild type *A. fumigatus* with echinocandins induced robust production of 5,8-diHODE by the enzyme PpoA. Further, we found that cotreatment with 5,8-diHODE blocked the fungicidal lysis of germinating conidia by caspofungin and micafungin. This protection against echinocandin tip lysis was also conserved in the related species *A. flavus* and *A. nidulans*. Lastly, we found that the transcription factor ZfpA was required for both induction of PpoA by caspofungin and full protection by 5,8-diHODE. Together, our findings reveal 5,8-diHODE to be an inducible and protective signal to activate echinocandin tolerant growth programs among pathogenic aspergilli.

606C **Hdp2 is the central transcriptional regulator during the early stage of plant infection of *Ustilago maydis*** Matteo Jurca[1], Jonas Ulrich[1], Lukas Baumann[1], Kerstin Schmitt[2], Oliver Valerius[2], Gerhard Braus[2], Joerg Kaemper[1] [1]Genetics, Karlsruhe Institute of Technology, [2]Georg-August-University Göttingen

In the phytopathogenic basidiomycete *Ustilago maydis*, the switch from the saprophytic to the pathogenic stage of its life cycle is controlled by a closely interconnected network of transcription (txn) factors. We have employed a combination of genome-wide expression analyses, Chip-Seq and Co-IP-MS experiments to dissect the combinatorial function of key txn factors during the transition. Pathogenic development is initiated by the activation of the bE/bW txn factor that activates *rbf1* as a direct target gene. Rbf1 is the master txn factor during the transition phase, however, ectopic expression of Rbf1 prevents subsequent *in planta*development. Expression of initially Rbf1-induced genes, as well as of more than 100 genes induced early after plant penetration, is mediated by Hdp2 via direct binding. A significant fraction of Hdp2 regulated genes encodes effector proteins required for pathogenic development, emphasizing the central role of Hdp2 during pathogenic development. Interestingly, prior to plant penetration, *hdp2* is expressed via a Rbf1-dependent promoter, *in planta* expression is maintained by an distinct promoter regulated by Biz1. The two mRNAs lead to two Hdp2 variants that differ at their N-termini, however, the two variants regulate identical sets of genes. The advantage of two distinct promoter regions is the independent integration of two specific sets of signals each without interference, a concept that is generally contributed to the gene regulation of higher eukaryotes. A closer comparison of the *U. maydis* transcriptome during the saprophytic and pathogenic stage revealed several other genes with alternative transcriptional start sites, indicating that *U. maydis* employs the concept of independent promoter platforms in a broader way.

607C **Methylation of H3K36 and H3K27 mediated by ASH1 and PRC2 co-defines heterochromatin in Magnaporthe oryzae** David Rowe[1], Aidan McVey[2], David Cook[2] [1]Kansas State University, [2]Plant Pathology, Kansas State University

Epigenetic histone marks serve a critical role in transcriptional availability and other DNA templated processes. The function of epigenetic histone marks is not always singular, and one chemical histone modification may influence another, termed cross-talk. We previously detailed that trimethylation of H3-Lys36 (H3K36me3) is ubiquitous across the *Magnaporthe oryzae* genome, present at both transcriptionally active and repressed regions, and that loss of H3K27me3 mediated by polycomb repressive complex 2 (PRC2) impacted H3K36me3 levels at a subset of loci. We aimed to further understand how H3K36me3 mediated by two histone

methyltransferases, SET2 and ASH1, engage in cross-talk with H3K27me3. Following a reverse genetics approach, we employed Cas9 to generate single and double knock out strains for SET2 and ASH1 in *M. oryzae* and used chromatin immunoprecipitation sequencing (ChIP-seq) to determine their impact on H3K36me3 and H3K27me3. We report that SET2 and ASH1 both contribute a substantial amount of total genomic H3K36me3, differing from previous reports in *M. oryzae* based on western blot analysis. We find that SET2-mediated H3K36me3 is enriched at actively transcribed genes, while ASH1 deposited H3K36me3 is enriched at transcriptionally repressed genes, some transposable elements, and 80% of predicted effectors that are marked by H3K36me3. The deposition of H3K36me3 at different genomic regions by SET2 and ASH1 is consistent with genome-wide ChIP-seq reported in *Neurospora crassa*. Our ChIP-seq results show that ASH1-mediated H3K36me3 co-occurs with 87.7% of H3K27me3 domains deposited by PRC2. Interestingly, loss of ASH1 but not SET2 caused a substantial reduction in H3K27me3 levels. Reciprocally, loss of H3K27me3 caused a substantial reduction in ASH1-mediated, but not SET2, H3K36me3 levels. This bidirectional reinforcement between ASH1-mediated H3K36me3 and PRC2-mediated H3K27me3 highlights the prominence of histone cross-talk and demonstrates that a detailed understanding is still needed for these well studied marks to discern their combined impact on transcriptional regulation and genome stability in filamentous fungi.

608C **Translational regulation by inositol through a deeply conserved fungal upstream open reading frame** Cheng S Wu[1], Ivaylo P Ivanov[2], Ananya Dasgupta[1], Thomas E Dever[2], Matthew S Sachs[1] [1]Dept of Biology, Texas A&M Univ, [2]National Institute of Child Health and Human Development, National Institutes of Health

An important emerging class of regulatory upstream open reading frame (uORF)-encoded peptides cause ribosomes to stall in response to metabolites. We discovered such a uORF in the 5'-leaders of fungal mRNAs specifying the first enzyme necessary to synthesize the central metabolic molecule inositol, inositol-3-phosphate synthase. This uORF's initiation codon is often a near-cognate start codon such as ACG in a good translation initiation context, but can be an AUG in poor context in some species. Based on experimental data, we have named the conserved uORF-encoded peptide the inositol regulatory peptide (IRP). Analyses of IRP function in *Neurospora crassa in vivo* showed that the endogenous gene (*inl*, NCU06666) is controlled at the translation level by exogenous inositol. *In vivo* and *in vitro* (cell-free translation system) analyses showed that inositol regulation is sensitive to mutations in conserved IRP residues. In fungal and mammalian cell-free translation systems, the IRP functioned either as a uORF or when fused in-frame with a reporter gene. Ribosome profiling using the *N. crassa* cell-free translation system showed inositol caused ribosome stalling at the wild-type uORF but not mutated uORF stop codons. The wild-type but not mutated IRP also regulated the expression of reporter genes in transfected mammalian cells in response to exogenous inositol. These data are consistent with models for IRP regulation in which inositol or a closely related molecule interacts directly with ribosomes, the nascent IRP, or both, to interfere with peptidyltransferase center activity. Stalling would act directly to reduce leaky scanning. Inositol-regulated ribosome stalling would thus regulate inositol-3-phosphate synthase biosynthesis and inositol metabolism across many fungal phyla.

609C **Functional characterization of polyketide synthase (PKS) gene PKS5 in *Fusarium oxysporum* f. sp. *vasinfectum* race 4** Yi Zhou, Huan Zhang, Won Bo Shim Plant Pathology and Microbiology, Texas A&M

Fusarium oxysporum f. sp. *vasinfectum* (*Fov*) is a fungal pathogen causing Fusarium wilt, with significant yield losses across the US Cotton Belt. While races 1 and 2 were previously responsible for Fusarium wilt in US cotton, the emergence of race 4 (*Fov4*), initially identified in California, has since spread to New Mexico and Texas. Significantly,*Fov4* exhibits heightened aggression and virulence toward Pima cotton, capable of causing disease in neutral to alkaline soil without nematodes. However, the study on the mechanisms of Fov4 virulence is very limited. Our study aims to uncover the role of polyketide synthase (PKS) genes in *Fov4* pathogenesis. By comparing the genomes of *Fov1* and *Fov4*, we found two PKS genes specific to *Fov4.* PKS are key enzymes involved in the biosynthesis of one of the most important groups of fungal secondary metabolites, polyketides, often known to play critical roles in fungal development and pathogenicity. This study focuses on the study of *PKS5* in *Fov4* virulence via the generation of a gene disruption mutant using a CRISPR/Cas9 system. The *PKS5* disruption mutant *ΔPKS5* showed reduced vegetative growth when grown on carbon source media amended with cotton roots when compared to the wild-type strain. Additionally, *ΔPKS5* exhibited reduced virulence in causing cotton root rot, accompanied by a significant decrease in the expression levels of fusaric acid biosynthesis genes *FUB11* and *FUB12.* The results suggest polyketide produced by PKS5, along with fusaric acid, plays an important role in the development and virulence of *Fov4* in cotton.

610C **Roles for phosphatases in Neurospora growth and circadian rhythmicity** Adrienne K Mehalow[1], Jennifer J Loros[2], Jay C Dunlap[1] [1]Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, [2]Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth

Post-translation modifications (PTMs) play a key role in regulation of physiological processes, including timing of the circadian clock. The mostly intensely studied PTM is phosphorylation, which is provided by the enzymatic activity of kinases. Due to their exquisite substrate specificity, kinases have been widely investigated across many fungal species and in higher organisms.

Phosphatases supply an opposing enzymatic activity, but until recent years their biology was broadly understudied. The realization that phosphatases are not promiscuous enzymes, but instead adhere to a separate and specific set of substrate recognition rules has spurred new interest in their biology.

An initial characterization of 28 *Neurospora crassa* phosphatases was performed a decade ago. Since that time, increased annotation across all fungal species and more advanced bioinformatic tools have allowed us to identify and characterize fully half-again more previously unannotated phosphatases in *N. crassa*. We present an expanded list of phosphatases characterized by homology groups. Using the *N. crassa* knockout collection we have screened deletion mutants for circadian period, circadian output, photobiology, sexual reproduction, and growth phenotypes. Knockouts of non-essential phosphatases which were absent from the collection were constructed and screened. In addition, we generated copper-regulated constructs of the essential phosphatases.

Nearly all knockouts were robustly rhythmic, both at the level of core circadian clock and developmental output. As phosphatase action has been implicated in clock function, this suggests multiple phosphatases cooperate to provide functional redundancy and maintain the correct phosphorylation status of key circadian clock proteins. This more complete catalog of phosphatases will be of interest to circadian biologists, as well as those studying the many diverse biological processes which are regulated by phosphorylation.

611C **Chromatin structural changes alter *cyp51A* expression in TR34-containing mutant strains of *Aspergillus fumigatus*** Sanjoy Paul[1], Mark A. Stamnes[2], Abigail Deaven[3], Chandler Goldman[3], Zachary A. Lewis[3], Scott Moye-Rowley[2] [1]Molecular Physiology and Biophyics, University of Iowa, College of Medicine, [2]Molecular Physiology and Biophysics, UNIVERSITY OF IOWA, COLLEGE OF MEDICINE, [3]Microbiology, University of Georgia

Aspergillus fumigatus is the primary human filamentous fungal pathogen. Disease associated with this organism is complicated by the increasing incidence of resistance to the primary antifungal drugs used to treat aspergillosis, the azole compounds. Aspergillosis associated with azole resistance organisms has a mortality of ~30%, even with the best standard of care. The principal route to azole resistance involves the duplication of a short element in the *cyp51A* promoter region, either 34 or 46 bp. Together, these duplications are present in as many as 80% of resistant clinical isolates. These alleles are referred to as TR34 (tandem repeat of 34 bp) or TR46. The presence of TR34 or TR46 has been shown by several labs to drive elevated transcription of *cyp51A* and is required for the observed increase in azole resistance. We have found that the transcription factor AtrR binds to a short element contained in the 34 bp repeat called the AtrR response element (ATRE) and is required for function of this 34 bp region, both as a single copy and in the TR34 context. To identify factors that work with AtrR to control expression of *cyp51A* and other ATRE-containing target genes, we used a biochemical approach to identify proteins that co-purify with a tandem affinity purification (TAP)-tagged form of AtrR (AtrR-TAP). Mass spectrometric analysis of these co-purifying factors identified several as chromatin remodeling proteins including the Arp4 protein, Ash1 histone methyltransferase, Ngg1 (component of the SAGA histone acetylase complex) and a RSC complex subunit (RscE). The abundance of these chromatin remodeling factors led us to examine the chromatin structure of both TR34 and TR46 versions of the *cyp51A* promoter using Assay for transposase-accessible chromatin (ATAC)-seq. We found that the presence of either the TR34 or TR46 duplication in the *cyp51A* promoter was sufficient to lead to an increase in chromatin accessibility for this gene, even in the absence of the known azole drug induction seen for *cyp51A*. Our data argue that the duplication of a small region in the *cyp51A* promoter, either 34 or 46 bp, is sufficient to trigger increased accessibility to this critical region, with subsequent transcriptional induction of the *cyp51A* gene and accompanying azole resistance.

612C **The transcription factor Ndt80 is a negative regulator of virulence in *Aspergillus fumigatus*** Vijendra Arya[1], Hong Liu[2], Mark A. Stamnes[3], Scott G. Filler[2], William Scott Moye-Rowley[3] [1]Molecular Physiology and Biophysics, University of Iowa, [2]Harbor-UCLA Medical Center, [3]University of Iowa

Aspergillus fumigatus is a ubiquitous filamentous fungus that is the leading cause of life-threatening invasive aspergillosis (IA) in patients. Using an overexpression approach, we identified a new *A. fumigatus* transcription factor that is a negative regulator of virulence. We designated this transcription factor Ndt80 on the basis of its structural similarity to its ortholog in *Candida albicans*. Using an immunosuppressed murine model of IA, we found that mice infected with a strain overproducing Ndt80 from the *gpdA* promoter (*gpdA-ndt80*) were avirulnet compared to mice infected with wild-type parent strain. By contrast, a *ndt80*Δ deletion mutant had wild-type virulence. To investigate the cause of the virulence defect of the *gpdA-ndt80* strain, we investigated its interactions with host cells vitro. These experiments revealed that the *gpdA-ndt80* strain had a 70% reduced capacity to invade pulmonary epithelial cells and caused 40% less damage to these cells, relative to the wild-type strain. By contrast, the *gpdA-ndt80* strain was as resistant as the wild-type strain to killing by bone marrow-derived macrophages. The reduced

capacity of *gpdA-ndt80* strain to invade and damage pulmonary epithelial cells likely explains its negative role in mammalian virulence. To determine the suite of genes that are governed by Ndt80, we constructed a *gpdA-ndt80*-3X FLAG strain and performed a chromatin immunoprecipitation-highthroughput sequencing experiment. We found that Ndt80 binds to the promoter regions of more than 700 genes, with the largest number of these genes sharing a function as transcriptional regulators. RNA-seq analysis indicated that overproduction of Ndt80 led to the repression of genes involved in cell wall biogenesis. This effect on expression could help explain the observed defect in pulmonary cell invasion. Together these data provide the first evidence that Ndt80 has a direct role in negatively regulating virulence attributes in *A. fumigatus*.

613C **Exploring Microbial Interactions in the Context of Antimicrobial Compounds** Hanna Roucka[1], Alyssia Gonzalez[2], Jeffrey Hollomon[2], Kurt Dahlstrom[3] [1]Microbiology, University of Georgia, [2]University of Georgia, [3]University of Georgiak
Fungal and bacterial interactions in soil participate in crucial roles in maintaining ecosystem health and function, such as nutrient cycling, disease suppression, and soil structure. Therefore, understanding how specific species of fungi are included or excluded from these communities is becoming increasingly important due to the impacts of climate change on crop development posing threats to global food security as warmer and drier soils see a decline in microbial diversity. Importantly, natural antibiotics are used by many microbes to control which species may live in a variety of microbial communities. Phenazines, produced by some bacteria, are redox-active molecules that impact the associations between fungi and bacteria in soil ecosystems through antimicrobial action. These molecules, such as phenazine-1-carboxylic acid (PCA), have antimicrobial properties that eliminate many fungi from the community, yet some sensitive species remain. The fungus *Aspergillus calidoustus* was isolated from PCA-replete soil with a physically associated novel bacterium, *Paraburkholderia edwinii*. When exposed to PCA, *P. edwinii* appears to exhibit a protective response over *A. calidoustus,* presenting as a morphological change to form bacterial aggregates which sequester PCA from the environment. To address the lack of understanding of this protective response, I will identify the downstream targets of *hrcA*, a known regulator of the "toxin sponge" protection response. This will aid us in mapping the genetic machinery necessary for *P. edwinii* to form aggregates, and to sequester and process PCA. In addition to this, I will evaluate the transcriptional regulation of PCA conditions in both individual colonies and co-cultures of *P. edwinii* and *A. calidoustus*, which will provide direction for how microbes regulate their transcription to form functional partnerships. Identified candidate genes will subsequently be deleted and complemented from either organism to assess the respective conditional characteristics.

614C **Investigating the role of long non-coding RNA *afu-182* in azole response in opportunistic pathogen *Aspergillus fumigatus*** Nava R Poudyal, Sourabh Dhingra Dept of Biological Sciences, Clemson University

Aspergillus fumigatus (AF) is a leading cause of aspergillosis in immunocompromised patients, and drug resistance has exacerbated the problem, with mortality rates reaching >90% for infections caused by drug-resistant isolates. Only 5-7% of invasive aspergillosis cases are caused by drug-resistant isolates; however, mortality rates still reach 50% for infections caused by drug-sensitive AF isolates for infections caused by drug-sensitive AF isolates. Thus, there is a knowledge gap in understanding fungal azole response. Here, we characterized long non-coding RNA, *afu-182*, as a negative regulator of azole drug response in Aspergilli. Our data show that *afu-182* controls fungal pan-azole response without a change in the minimum inhibitory concentration. Interestingly, clinically relevant biofilm of the D*afu-182* strain is recalcitrant to azole drugs, whereas overexpression of *afu-182* makes fungus more susceptible to azole drugs. Importantly, *afu-182* is indispensable for azole-mediated fungal clearance in a murine model of invasive pulmonary aspergillosis, highlighting a role of *afu-182* in virulence and providing a novel genetic link between low rates of successful treatment outcomes for infections caused by azole-susceptible isolates.

615C **Functional Characterization of a lncRNA in Stress Response and Pathogenesis of *Aspergillus fumigatus*** Ritu Devkota[1,2], Alexandra Randazza[3], Lela Lackey[3], Sourabh Dhingra[1,2] [1]Biological Sciences, Clemson University, [2]Eukaryotic Pathogen Innovation Center, Clemson University, [3]Clemson Center for Human Genetics, Clemson University

Aspergillus fumigatus is a saprophytic fungus that can cause a collection of diseases in an immunocompromised population termed aspergillosis; the most severe amongst them is invasive pulmonary aspergillosis (IPA). Azoles are the major classes of antifungal drugs used to treat invasive pulmonary aspergillosis. However, in recent years there has been an increase in fungal resistance to azole drugs exacerbating the problem. In addition, the fungal response to azole drugs is not entirely understood, resulting in poor disease outcomes associated with azole-susceptible strains.

It is becoming increasingly clear that lncRNA-mediated regulation is vital in stress response; however, their roles in fungi are lacking. Here, we have identified a lncRNA *afu-853*, which acts as a regulator of multiple stress response including azole response in *A. fumigatus*. Structural analysis showed that *afu-853* has flexible structure and can take many potential conformations *in vitro*. Thus,

we aim to characterize the role(s) of ncRNAs in antifungal drug response and pathogenesis. This study aims to provide a novel genetic link between ncRNAs and stress regulation including azole response in *Aspergillus fumigatus*.

616C **Bacterium Acts as Toxin Sponge to Protect Partner Fungus from Phenazine Assault** Alyssia Gonzalez[1,1], Hanna Roucka[1], Jeffrey Hollomon[2], Kurt Dahlstrom[2] [1]Microbiology, University of Georgia, [2]University of Georgia

Microbial communities are ecologically essential, functioning in nutrient cycling and organic decomposition. A community's function is dependent on its species-level composition, which is influenced by antimicrobial compounds (AMCs) produced within the community that exclude susceptible species. Many fungi are susceptible to a class of redox-active AMCs called phenazines. Paradoxically, many of these same fungi are isolated from phenazine-replete environments. Susceptible fungi can infiltrate phenazine-replete environments by physically associating with protective, phenazine-tolerant bacteria. Our lab has identified a model protective bacterial-fungal pairing, comprised of a bacterium, *Paraburkholderia edwinii*, and a filamentous fungus, *Aspergillus calidoustus*. During phenazine-1-carboxylic acid (PCA) challenge, *P. edwinii* forms anoxic bacterial aggregates in the center of the fungal colony. These aggregates act as toxin sponges, sequestering and reducing PCA, allowing the fungus to grow. We have identified a *P. edwinii* transcriptional repressor, HrcA, which negatively regulates this protection response. The Δ*hrcA* bacterial strain produces larger aggregates and provides better protection from PCA than WT. To test the hypothesis that HrcA represses transcription of genes promoting bacterial protection of the fungal partner, we conducted a chromatin immunoprecipitation sequencing assay (ChIP-seq) to identify the HrcA regulon. Candidate gene expression will be quantified by qRT-PCR in conditions where *P. edwinii's* protection response is active and inactive. I expect candidate gene expression to increase in the active protection condition. To further validate candidate gene function in the protection response, I will make in-frame deletions of the genes implicated in the HrcA regulon. I expect the generated mutant strains to provide less protection to its fungal partner than WT, validating these genes function in the protection response. Understanding how the bacterium activates its protection program will inform us on how microbial partnerships are used by fungi to enter AMC-replete environments.

617C **Plant-fungal reciprocity of gene expression patterns in the maize-*Cochliobolus heterostrophus* interaction** Rina Zuchman[1], Ofri Levi[1], Adriana Rightmyer[2], Hee-Jin Park[3], Vivian Ng[4], Daniel Peterson[4], Roni Koren[5], Scott E. Baker[6], B. Gillian Turgeon[3], Benjamin A Horwitz[7] [1]Biology, Technion, [2]Plant Pathology & Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, [3]Section of Plant Pathology & Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, [4]Joint Genome Institute – US Dept of Energy, [5]Technion, [6]Microbial Molecular Phenotyping Group, Environmental Molecular Sciences Division, Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, Washington, USA and DOE Joint BioEnergy Institute, [7]Technion - IIT

To study gene expression at critical stages in the interaction of *Cochliobolus heterostrophus* (Southern Corn Leaf Blight, SCLB) with its host plant, maize, RNASeq reads from infected leaves, harvested at times spanning the cycle of disease development from spore adherence to re-sporulation, were compared to uninfected leaves sampled at the same timepoints and to axenic fungal cultures. The large multicellular conidia germinate on the leaf surface. Germ tubes form small appressoria, hyphae penetrate the plant, proliferate between mesophyll cells and along vascular tissue, cause necrosis, and eventually re-conidiate. By 12 hpi, hyphae branch and proliferate. Temporally regulated transcripts mapping to the *Cochliobolus heterostrophus* strain C4 v6 database [1] include those encoding effectors, CAZymes and secondary metabolite (SM) biosynthetic enzymes. 22 predicted apoplastic protein effectors increased, showing several coregulation patterns over 8-72 hpi; a 10-member cluster of predicted cytoplasmic effectors increased at 24-72 hpi. Curiously, two sets of predicted cytoplasmic effectors were co-induced in stationary axenic liquid cultures. The 254 annotated glycosyl hydrolase (GH) genes include up and down-coregulated clusters, e.g., 13 coregulated genes whose expression increased over 8-72 hpi compared to 4 hpi. Within this cluster, there are specific temporal patterns. Of the SM genes, 14 of 25 annotated PKSs were expressed; of these, three showed significant regulation during infection, including T-toxin related synthases PKS1 and PKS2, upregulated in the first 12 hpi. Of the 13 NRPSs, 9 were expressed; 8 showed significant regulation, peaking at early, middle or late infection. The host plant, in parallel, up-regulates defense-related genes, and in late stages the photosynthetic apparatus is down-regulated. Several cell-death related fungal genes [2] are transiently upregulated at 8-12 hpi perhaps coinciding with pathogen cell death on the host at 12 hpi [3]. Thus, some expected patterns (e.g., multiple GH up-regulation late in infection) and some novel ones (e.g., genes whose annotation suggests they are cell death-related) were uncovered. These data will be analyzed to test whether they can help model the dynamics of localized pathogen cell death, survival within the lesion and proliferation.

[1] Grigoriev et al. (2014) Nucl. Ac. Res.; [2] Simaan et al. (2020) Curr. Genet.; [3] Shlezinger et al. (2011) PLoS Pathog.

618C **Unraveling crosstalk between Hog1 and general translation control in *Cryptococcus neoformans.*** David Goich, Amanda LM Bloom, John Panepinto University at Buffalo

Cryptococcus neoformans is an environmental fungus that causes severe opportunistic infections in immunocompromised individuals, particularly people living with HIV/AIDS. Upon entry into the host, *C. neoformans* rapidly alters its proteome to accommodate a variety of stressors, including elevated temperature, reactive oxygen species (ROS) in phagocytes, and changes in the nutrient environment. These changes are dependent in part on the p38 MAPK, Hog1, which regulates both transcription and translation during stress responses. We present data demonstrating that phosphorylation of the translation factor, eIF2α, is increased in *hog1*Δ during thermal stress. This phosphorylation mark is a conserved mechanism of general translation repression, and is fully dependent on the kinase Gcn2 in *C. neoformans*. Using polysome profiling, western blotting, and northern blotting, we draw associations between dysregulation of P-eIF2α, altered translational responses to stress, and compensatory activation of stress responses in *hog1*Δ. Overall, we observe both molecular and phenotypic compensation by the Gcn2 pathway in the absence of Hog1 across multiple stressors that are relevant to infection. Additionally, our data show stress-specific nuances in how these signaling pathways are connected, as well as a conserved link between the Hog1 and Gcn2 pathways in other pathogenic fungi. Future investigations will determine the molecular nature of the interface between these pathways, as well as its implications for pathogenesis.

619C **Unraveling the 6mA-regulated transcriptional regulatory networks in the early diverging fungus *R. microsporus*** Carlos Lax[1], Leo A Baumgart[2], Yu Zhang[2], Ghizlane Tahiri[1], Stephen Mondo[2], Ronan C O'Malley[3], Igor Grigoriev[2], Eusebio Navarro[4], Francisco E Nicolás[4], Victoriano Garre[4] [1]Departamento de Genética y Microbiología, Facultad de Biología, Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia, [2]U.S Dept of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, [3]Dept of Human Genetics, University of Chicago, [4]Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia

The cistrome is defined as the complete set of transcription factor (TF) binding sites (cis-elements) in an organism. Understanding the intricate regulatory mechanisms governing gene expression is pivotal for unraveling the complexities of fungal biology. Unfortunately, the lack of cost-effective and scalable approaches for TF binding has provoked detailed cistrome information to be restricted to a very few model species. Usually neglected and overlooked compared to higher fungi, early diverging fungi (EDF) possess unique biological features that remain mainly unexplored. In this work, we surveyed the transcription factor with a focus on the basal fungus *R. microsporus*. This EDF representative possesses a DNA epigenetic landscape dominated by 6mA, a rare epigenetic mark in eukaryotes that is associated with transcriptional regulation in this organism. Since some of the 6mA marked genes code for transcription factors, we aimed to characterize the indirect role of this epigenetic modification in transcriptional regulatory networks. DNA Affinity Purification sequencing (DAP-seq) offers a high-throughput and precision in identifying TF-DNA interactions. This pioneering approach allowed us to comprehensively examine 57 TF binding profiles belonging to the main fungal TF families. Our results uncover the binding patterns and functional regulatory networks governed by each TF family. Moreover, integrating these results with expression data has provided valuable insights into the dynamic nature of transcriptional regulation in response to environmental and growth conditions, including light exposure and zinc availability. We deepened into the binding dynamics of the 6mA-regulated white collar-2 (*wc2*) and characterized wc2-dependent and independent light-regulated genes in *R. microsporus*. Additionally, considering the pathogenicity of this species, we focus on the study of transcription factors that participate in the virulent response of *R. microsporus* to the host and identify the regulatory networks that govern the pathogenic capacity of this fungus. The identification and functional characterization of the binding patterns of the TF here studied and its conservation in other species could be a useful tool to reveal unknown regulatory networks in other fungal species, which could broaden the resources available to explore transcriptional regulation across the fungal tree of life.

620C **Argonaute proteins are important for RIPping in *Fusarium graminearum*** Zeyi Wang, Zhuyun Bian, Jin-Rong Xu Purdue University

The RID DNA demethylase is essential for repeat-induced point (RIP) mutation during sexual reproduction in Sordariomycetes. In this study, we used affinity purification and mass spectrometry analysis to identify putative FgRid1-interacting proteins in the wheat scab fungus *Fusarium graminearum*. One of them is FgAgo2 that is specifically expressed during sexual reproduction. The interaction of FgRid1 with FgAgo2 was confirmed by co-immunoprecipitation assays. To determine the role of AGO proteins in RIPping, we generated the *Fgago1 Fgago2* deletion mutant in a reporter strain containing direct repeats of an 802-bp sequence. The resulting double mutant had no detectable defects in vegetative growth, perithecium formation, and ascosporogenesis but was defective in ascospore discharge. However, in comparison with the wild type, the ripping efficiency was reduced over 80% in the *Fgago1 Fgago2* mutant. To determine the effects of *AGO* deletion on RIPing of unlinked repeats, we also generated the *Fgago1*

*Fgago*2 double mutant with two dispersed hygromycin phosphotransferase (*hph*) cassettes (one full-length and one truncated non-functional). Preliminary data showed that none of the 1,500 ascospore progenies isolated from this mutant with dispersed *hph* repeats mutant were sensitive to hygromycin. Sequencing analysis with 30 randomly selected ascospore progenies did not identify C-to-T mutations in both *hph* copies, indicating the importance of AGO proteins in RIPpping in *F. graminearum*. Further characterization of RIPping defects in ascospore progeny of both double mutants with linked and dispersed repeats is in progress.

621C **Determining the components and activity of DNA repair pathways in *Magnaporthe oryzae*** Tomas A McAnany[1], Jun Huang[2], Aasiya Nabi[3], David E. Cook[4] [1]Genetics, Kansas State University, [2]Duke University, [3]Sher-e-Kashmir University of Agricultural Sciences and Technology- Kashmir (SKUAST-K), [4]Kansas State University Plant Pathology Dept - Cook Lab

Magnaporthe oryzae, the causal agent of rice blast disease, is an important model pathogen given its worldwide distribution, threat to agriculture across a range of monocot hosts, and varied aggressiveness among strains. While significant progress has been made in understanding the genetics of host-range and virulence, it is less clear what specific mechanisms give rise to DNA variation given *M. oryzae's* mainly asexual reproduction. Previous research from our lab and others indicates that at least four DNA double strand break (DSB) repair pathways are active in *M. oryzae*, including classical non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), single-strand annealing (SSA), and homologous recombination (HR). While DNA DSB-repair is well studied in eukaryotes, there is not a full description of the genetic requirements, and most importantly, the general DNA repair outcomes for each repair pathway in filamentous fungi, especially related to MMEJ and SSA. We utilized a reverse genetics approach to identify the genetic requirements of previously characterized DNA DSB repair homologs, through the creation of gene deletion strains and characterizing repair outcomes in these genetic mutants. By disabling DNA end-resection at DSB sites, we determined that NHEJ repair results in INDELs up to 9 base pairs (bp) long, can include microhomology of up to 3 bp, incudes tandem duplications in ~50% of repairs, and does not result in any large deletions, such as those greater than 100 bp. Conversely, by disabling NHEJ and long-range end resection mediated by EXO1, we specifically identified an MMEJ-like repair profile with much larger INDELs, ranging from 21- 145 bp, and longer microhomology ranging from 3-6 bp. To define the genetic requirements in the individual DSB repair pathways, such as the polymerases and ligases involved, growth tests in the presence nucleotide depleting agents, which cause DSBs, are being used. Initial findings confirm that S-phase cells require end resection to repair DSBs during DNA replication, while NHEJ is dispensable. These results are helping define the outcomes and requirements of DNA DSB repair in *M. oryzae*, which is required to understand how DNA DSB repair impacts the creation of genome variation and pathogen evolution.

622C **DYRK-family kinases regulate *Candida albicans* morphogenesis and virulence through the Ras1/PKA pathway** Jessie MacAlpine, Zhongle Liu, Saif Hossain, Luke Whitesell, Nicole Robbins, Leah Cowen University of Toronto

Candida albicans is a member of the mucosal microbiota capable of causing superficial infections in healthy people and life-threatening systemic disease in immunocompromised individuals. The fungus employs several virulence traits to cause disease in humans, including its ability to transition from yeast to hyphal morphologies. Previous work identified that genetic or pharmacological inhibition of the dual-specificity tyrosine-phosphorylation regulated kinase (DYRK) Yak1 blocks *C. albicans* hyphal morphogenesis and biofilm formation. Here, we expand on this work to provide mechanistic insights into how Yak1 governs this important virulence trait. First, we find that Yak1 acts downstream of Protein Kinase A (PKA) and upstream of core transcription factors, including Efg1 and Flo8, to regulate hyphal morphogenesis. While Yak1 plays a pivotal role in inducing hyphal morphogenesis in response to multiple cues, it is dispensable for the yeast-to-hyphal transition in response to physiological concentrations of CO_2, highlighting that regulation by this kinase is environmentally contingent. We determined that the bypass of Yak1 is due to hyperactivation of the Ras1/cAMP/PKA pathway. Interestingly, deletion of another predicted DYRK gene in *C. albicans*, *POM1*, in a background lacking *YAK1* blocks the yeast-to-hyphal transition under physiological concentrations of CO_2; suggesting these kinases can act in parallel to regulate morphogenesis and highlighting a previously undescribed role for Pom1 in regulating *C. albicans* morphogenesis. Finally, we demonstrate that Yak1 is required for hyphal morphogenesis in a dermatitis model of *C. albicans* infection and that pharmacological inhibition of Yak1 with a beta-carboline attenuates morphogenesis in the dermal tissue. Overall, this work characterizes the role of Yak1 in regulating *C. albicans* hyphal morphogenesis, identifies a role for Pom1 in the yeast-to-hyphae transition, and suggests inhibition of Yak1 may serve as a therapeutic strategy to combat *C. albicans* dermatitis.

623A **Genomic Insights into *Fusarium graminearum*: Dual RNA Sequencing for Pathogenicity Gene and Fungicide Target Discovery** Erika Kroll[1,2], Martin Urban[1], Neil Brown[2], Ryan Ames[3], Carlos Bayon[1], Jason Rudd[1], Victoria Armer[1,3], Kim Hammond-Kosack[1] [1]Rothamsted Research, [2]University of Bath, [3]University of Exeter

Fusarium graminearum which infects wheat and other cereals is the causative agent of the highly destructive fungal disease Fusarium Head Blight (FHB). FHB causes devastating crop losses by dramatically decreasing grain quality before harvest. Furthermore, the pathogen produces harmful toxins which deem grains unfit for human or animal consumption. Faced with a growing population, climate change, environmental pressures, and fungicide resistance, the ability to control fungal plant pathogens has become a global concern requiring urgent solutions. With *F. graminearum* being one of the most economically important plant pathogenic fungi globally, developing new methods to control this pathogen has become paramount.

Recent advances in fungal genomics and NextGen sequencing technologies enabled a substantial number of RNA-sequencing (RNA-seq) studies to investigate the genetic interactions between *F. graminearum* and its cereal hosts during infection. This includes stage and tissue specific investigation of the biphasic *F. graminearum* infection process (1). Using this dataset, we performed a weighted gene co-expression network analysis (WGCNA) to generate the first fungal pathogen/crop dual co-expression networks in wheat. Virulence specific modules were identified and by studying these modules, we discovered a hub gene encoding a cell wall regulatory protein (*FgCWP1*). Through comprehensive analysis, we confirmed the pivotal role of *FgCWP1* in various biological processes, including morphogenesis, growth, cell wall stress tolerance, and pathogenicity. Further studies confirmed the observed phenotypes are partially due to the gene's involvement in regulating the fungal cell wall integrity pathway by modulating the phosphorylation of the MAP-kinase *MGV1*.

CWP1 orthologues are not limited to other *Fusarium* species but are distributed widely across the fungal kingdom, suggesting CWP1 could be a suitable fungicide target. Encouragingly, the restricted growth and loss of pathogenicity phenotypes observed in the *F. graminearum* mutant were replicated upon deletion of the orthologous gene in the distantly related wheat fungal pathogen *Zymoseptoria tritici*. Overall, this project and its results demonstrate the utility of an integrated network-level analytical approach to provide new targets for future fungicide development.

1. Dilks et al., (2019) PLoS Pathogens, 15(4), p.e1007666.

624A **Polymorphisms, host immune response, and clinical outcomes: Investigating clinical isolates of *Cryptococcus neoformans*** Perry Kezh, Kirsten Nielsen Microbiology and Immunology, University of Minnesota

Cryptococcus neoformans is the leading cause of fungal meningitis world-wide. Exposure to *C. neoformans* from environmental inhalation is common. Immunocompromised individuals are at risk of disease due to hematogenous dissemination and eventual crossing of the blood brain barrier to cause cryptococcal meningitis. Disease is especially prevalent in Sub-Saharan Africa and is associated with HIV/AIDs. Our previous studies identified 9 genes in Ugandan clinical isolates associated with a hypervirulent phenotype and an increase in the cytokine IFNγ. A deletion mutant in one of these genes, ITR4, recapitulated the hypervirulence phenotype and high IFNg production in the lab reference strain KN99alpha.To further explore the role of *ITR4*, as well as additional genes associated with hypervirulent and latent phenotypes, knockouts in the clinical isolates UgCl422 (hypervirulent) and UgCl223 (latent) were created utilizing the Crispr/Cas9 system. Subsequently, the deletion mutants were tested for immune response, production of virulence factors and survival in the mouse model. These data provide additional evidence of the close relationship between pathogen genetic polymorphisms and the host immune response and clinical outcome.

625A **The nematode-trapping fungus *Arthrobotrys flagrans* small-secreted protein NipA interferes with cuticle integrity in *Caenorhabditis elegans*** Jennifer Emser, Reinhard Fischer Karlsruhe Institute of Technology

The primary line of protection for animals often lies in their surface structures, which are frequently reinforced by polymeric proteins. Nematodes, such as *Caenorhabditis elegans*, rely on a robust cuticle as their first defense against external threats, necessitating pathogenic microorganisms to surmount this barrier during assaults via unconventional entry points. Nematode-trapping fungi have evolved specialized hyphal structures designed to capture and immobilize live nematodes. *Arthrobotrys flagrans* employs adhesive trapping networks to lure and capture the nematode prey. It subsequently breaches the nematode cuticle, establishing an infection bulb beneath the epidermis, from which it proceeds to colonize and digest the entire nematode organism. While lytic enzymes play a significant role, small-secreted proteins (SSPs) have emerged as crucial effectors in this intricate process. Here, we characterized NipA (nematode induced protein A), a key SSP in this context. *nipA* was transcriptionally upregulated in fungal traps, with the protein accumulating at the penetration site. The absence of NipA resulted in delayed penetration compared to wild type. Moreover, expression of *nipA* within the epidermis of *C. elegans* led to aberrant regulation of specific pathways and the formation of characteristic blisters. NipA cysteine residue 23, not involved in intramolecular disulfide bond formation, was required for blister formation. These findings shed light on the multifaceted role of NipA in the complex interaction between nematode-trapping fungi and the nematode preys.

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626A **Yeast – oomycete interaction in Arabidopsis phyllosphere via a membrane permease** Yiheng Hu[1], Daniel Gómez[1,2], Sinja Niemann[1], Johanna Bode[1], Maryam Mahmoudi[1], Alfredo Mari[1], Eric Kemen[1] [1]Microbial Interactions in Plant Ecosystems, Center for Plant Molecular Biology, University of Tübingen, [2]Cellular and Organismic Networks, Faculty of Biology, Ludwig-Maximilians-Universität München

Plants, like animals, are no longer defined as individuals without considering their associated microbial communities, or microbiomes. However, the extent to which plant microbiomes influence pathogen threats is still largely understudied. Obligate plant pathogens depend on living hosts to complete their lifecycle and are responsible for the majority of crop yield losses. Previous research has shown that plants infected by obligate pathogens have altered microbiomes, which suggests that interactions between the pathogen and other microbes in the plant microbiome play a role in the infection. Here, we identified a cross-feeding mechanism between the obligate oomycete *Albugo laibachii* and a key member of the plant microbiome, *Dioszegia hungarica*. We found that some *Dioszegia* species can provide thiamine through a membrane thiamine permease, and are beneficial to *Albugo* infection. The loss of function mutant of *D. hungarica* lost the capacity of promoting *Albugo* infection. We also screened the selection pressure of this gene within *Dioszegia* genome and across the fungal kingdom, and found that the purifying selection of this gene is uniquely intensified in genomes of *Dioszegia* species associated with *Arabidopsis* and expressing permease. These results indicate the natural selection of a microbial cooperative mechanism in a specific ecological niche, shedding light on novel biocontrol strategies for obligate pathogens.

627A **A DASH complex ortholog mediates pH adaptation and virulence in *Cryptococcus neoformans*.** Rebekah A Satalino[1], Ryker Heller[1], Allyson Hardin[2], Andrew Alspaugh[3], Michael S Price[1,3] [1]Dept of Molecular and Cellular Sciences, Liberty University College of Osteopathic Medicine, [2]Dept of Biology and Chemistry, Liberty University, [3]Dept of Medicine, Duke University

Cryptococcus neoformans is an opportunistic fungal pathogen responsible for approximately 15% of AIDS-related deaths per year. Despite advances in medicine and care for those with HIV/AIDS and other immunocompromised states, this pathogen remains a significant issue globally. One of the factors that contributes to its virulence is its ability to rapidly adapt to ambient pH differences it encounters during its infection cycle. Previous work at Duke University identified numerous genes displaying altered growth at alkaline pH in *C. neoformans*, including the gene CNAG_02291, a potential homolog of *DAM1* in *S. cerevisiae* involved in the attachment of microtubules to the kinetochore. It was our goal to determine whether this gene is involved in pH adaptation and virulence of *C. neoformans.* First, CNAG_02291 (hereafter *DCM1* for DASH complex with microtubules) was deleted in wild-type (WT) *C. neoformans* strain CM2049 using electroporation and integration with CRISPR-Cas9. To reconstitute the mutant to WT phenotype, the *DCM1* gene was cloned into plasmid pSDMA25 and transformed into the *dcm1Δ* mutant. The *dcm1Δ* and reconstituted strains were then evaluated for various growth/stress phenotypes. *dcm1Δ* exhibited poor growth on YPD pH 8 compared to WT, supporting the hypothesis that *DCM1* affects the ability of *C. neoformans* to adapt to alkaline pH. Virulence studies using *Galleria* and murine inhalation models showed statistically significant differences in virulence between the WT and *dcm1Δ* strains. This supports the hypothesis that this gene affects the virulence of *C. neoformans* in the host. Therefore, *DCM1* appears to be involved in pH adaptation and virulence of *C. neoformans*.

628A **Endophytic Fungi as Biofertilizing and Biocontrol Agents of Cranberry Plants** Bhagya Chattanahalli Thimmappa[1], Lila Naouell Salhi[1], Lise Forget[1], Matt Sarrasin[1], Peniel Bustamante Villalobos[1], Marcel Turcotte[2], Franz B. Lang[1], Gertraud Burger[1] [1]Dept of Biochemistry and Robert-Cedergren Centre for Bioinformatics and Genomics, Université de Montréal, Montreal, Quebec, Canada, [2]School of Electrical Engineering and Computer Science, University of Ottawa, Ottawa, Ontario, Canada

Traditionally, associations of endophytes with their host plant have been studied from a crop-disease perspective. In contrast, our research aims at the investigation of microbes that stimulate plant growth (**biofertilization**) and/or protect their host from

pathogens (**biocontrol**). While more than 90% of all land plant species are colonized by Arbuscular Mycorrhizal Fungi (AMF; Glomerales), **Ericaceae** are among the few plant groups harboring other fungi. This plant group readily grows in **nutrient-poor**, **acidic soil**, which is likely facilitated by their endophytes, but only little is known about these microbes and even less about ericaceous endosymbionts with biocontrol and biofertilization ability.

Here we report our work on a fungal endophyte of ***Vaccinium macrocarpon*** (American cranberry), called Endophytic Champignon 4 (**EC4**), which lives inside the cells of cranberry plant roots. Phylogenetic analysis based on 28S rRNA identifies EC4 as a member of ***Codinaeella***, a poorly explored fungal genus belonging to the Chaetosphaeriaceae (Sordariomycetes). In-plantae tests show that EC4 **stimulates growth** on a water-insoluble phosphate source (tricalcium phosphate) otherwise inaccessible to plants. We also demonstrate, by tests on plate and in-plantae, that EC4 **inhibits** the growth of a broad range of **pathogens** infecting cranberry and other plants. Analysis of the nuclear **genome** and **transcriptome** revealed that EC4 expresses numerous genes involved in **mineral uptake**, assimilation, and transport, but also in the production of **secondary metabolites** such as NonRibosomal Peptide Synthetases, PolyKetide Synthases and fungal cell wall degrading enzymes by EC4. The first groups of genes most likely give rise to host **growth promotion**, while the latter group is thought to be involved in **pathogens suppression**.

Fungal endophytes of Ericaceae have been reported before, but these belong to Helotiales (Leotiomycetes), which are phylogenetically distant to Chaetosphaeriaceae. In fact, EC4 is not only the **first** characterized *Vaccinium* endophyte but also the **first** member of the **Chaetosphaeriaceae** family for which genome and transcriptome sequences are now available. Given its biofertilization and biocontrol potential, EC4 has the potential to be employed as a biocontrol and biofertilizing agent in **sustainable agriculture**.

629A **Deletion of core septin gene *aspB* in *Aspergillus fumigatus* results in fungicidal activity of caspofungin** Rebecca J Busch[1], Carson Doty[1], Allie Mills[2], Flutur Latifi[1], Vjollca Konjufca[1], Laura Herring[2], José Vargas-Muñiz[1] [1]Southern Illinois University, [2]University of North Carolina at Chapel Hill

Septins are a family of GTP-binding proteins. Although highly conserved throughout many eukaryotes, their functions vary across species. In *Aspergillus fumigatus*, the etiological agent of invasive aspergillosis, septins participate in a variety of roles such as cell wall organization of conidia, septation, and response to anti-cell wall stress. Previous studies determined that the *ΔaspB* strain had a greater sensitivity to anti-cell wall drugs, especially the echinocandin caspofungin, yet mechanisms behind this augmented sensitivity are unknown. We performed cell viability staining post-caspofungin exposure and found that the *ΔaspA, ΔaspB*, and *ΔaspC* strains showed significant reduction in cell viability. Concomitant with the reduced viability, deletion strains are more susceptible to caspofungin on solid media. These results indicate that the septin cytoskeleton is important for *A. fumigatus* survival in the presence of caspofungin. Due to the potential of improved therapeutic outcome, we followed up using a neutropenic murine model of invasive aspergillosis. Deletion of the *aspB* gene resulted in improved survival, reduced pulmonary inflammation, and reduced fungal burden when treated with caspofungin when compared to the *akuB*^{KU80} wild-type or untreated *ΔaspB* strains. Quantitative proteomics analyses were used to find proteins involved in the septin-dependent adaptation to caspofungin. We identified four candidates with roles in cell wall integrity. Deletion of these candidate genes resulted in increase in susceptibility to caspofungin and moderate reduction in viability post-drug exposure. Taken together, these data suggest that septin AspB is essential in mediating the fungistatic response to caspofungin.

630A **Spores of arbuscular mycorrhizal fungi host surprisingly diverse communities of endobacteria** Olga Lastovetsky[1], Tancredi Caruso[2], Susanna Pylni[2], Fiona Brennan[3], David Wall[3], Evelyn Doyle[2] [1]School of Biology and Environmental Science, University College Dublin, [2]University College Dublin, [3]Teagasc - Crops Environment and Land-Use Programme

Arbuscular mycorrhizal fungi (AMF) are ubiquitous plant root symbionts which can house two endobacteria: Ca. Moeniiplasma glomeromycotorum (CaMg) and Ca. Glomeribacter gigasporarum (CaGg). However, little is known about their distribution and population structure in natural AMF populations and whether AMF can harbour other endobacteria. We isolated AMF from two environments and surveyed the surface-sterilized spores for endobacteria. Consistent with previous reports, we found that CaMg were extremely abundant (80%) and CaGg were extremely rare (2%) in both environments. Unexpectedly, we discovered an additional and previously unknown level of bacterial diversity within AMF spores which extended beyond the known endosymbionts, with as many as 277 other bacterial taxa detected in individual spores. Detailed analysis of endobacterial communities inside AMF spores revealed that: (i) CaGg were not limited in distribution to the Gigasporacea family of AMF, as previously thought, (ii) CaMg population structure was driven by AMF host genotype, (iii) a significant inverse correlation existed between the diversity of CaMg and diversity of all other endobacteria. The latter suggests the existence of competition dynamics between different bacterial populations inside AMF spores and provides a basis for generation of testable hypotheses regarding the function of CaMg in AMF biology

631A **Viro-Fungal Tag-Team: Aspergillus dsRNA virus drives fungal fitness and pathogenicity in the mammalian host** Vanda Lerer[1], Marina Rocha[1], John Adeoye[1], Neta Shlezinger[2] [1]The Koret School of Veterinary Medicine, The Hebrew University, [2]The Hebrew University

Fungal pathogens pose a significant threat to global health. As eukaryotes, they share considerable homology with their hosts, requiring the development of innovative, non-cross-reactive therapies. *Aspergillus fumigatus* accounts for approximately 65% of all invasive fungal infections in humans, with mortality rates from aspergillosis reaching nearly 50%. Fungal virulence in plant pathogenic fungi can be modified by mycoviruses, which are viruses that infect fungi. However, their impact on fungal pathogenesis in mammals has remained largely unexplored. Here, utilizing an *A. fumigatus* strain naturally infected with *Aspergillus fumigatus polymycovirus-1 (AfuPmV-1)*, we found that the mycovirus confers a significant survival advantage to the fungus under conditions of oxidative stress, heat stress, and within the murine lung. Thus, *AfuPmV-1* modulates fungal fitness, resulting in increased virulence and the progression of exacerbated fungal disease. Moreover, antiviral treatment reverses the exacerbated *AfuPmV-1M*-Mediated Virulence. Therefore, antiviral drugs that target viral replication represent promising "antipathogenicity" treatments against virus-bearing pathogenic fungi. Taken together, these data suggest that mycoviruses play a significant role as "backseat drivers" in human fungal diseases, presenting critical clinical implications.

632A **Airway epithelial cells as a novel intracellular host reservoir for *Cryptococcus* spores** Sebastien C Ortiz[1], Rachael Fortune-Grant[1], Robin C May[2], Rebecca A Drummond[3], Margherita Bertuzzi[1] [1]Manchester Fungal Infection Group, Faculty of Biology, Medicine and Health, University of Manchester, [2]Institute of Microbiology & Infection and School of Biosciences, University of Birmingham, [3]Institute of Immunology & Immunotherapy, Institute of Microbiology & Infection, University of Birmingham

The inhaled human fungal pathogen *Cryptococcus neoformans* causes over 200,000 deaths a year. Cryptococcal disease occurs by dissemination of the pathogen from the lung into the brain and importantly, can ensue years after exposure; however, the mechanisms of *Cryptococcus* dissemination and intracellular latency are still unclear. The inhalation of *Cryptococcus* spores initiate infections and recent evidence *in vivo* has shown that spores display unique host-pathogen interactions and are able to escape the lung and disseminate to the brain more readily than yeast. Due to the difficulties associated working with these basidiospores, little is known about how they interact with host cells. Airway Epithelial Cells (AECs), which cover the entire alveolar surface and comprise 24% of all cells in the human lung parenchyma, likely have instant and extensive contact with inhaled spores. A growing body of evidence has emerged demonstrating a role of AECs in host defence against inhaled pathogens, but in certain cases including diseased states, may be exploited by pathogens as a potential intracellular safe haven. Using state-of-the-art single-cell technologies, we demonstrated that *Cryptococcus* spores (15.4%) are more readily taken up by AECs, than yeast (0%) within 6 hours of infection. Furthermore, we demonstrated that while a fraction of internalized spores fail to germinate at all (16.3%), internalised spores can germinate and replicate. On occasion, *Cryptococcus* can even escape AECs in a non-lytic manner, leading us to hypothesise that **AECs may be a novel intracellular host reservoir that contribute to both dissemination and latency**. To this end our work has centred around characterizing the ability of spores, unlike yeast, to invade, escape, and persist in AECs, both in tissue culture and in murine models of disease. Understanding this novel intracellular host reservoir could elucidate both the mechanisms of how fungi disseminate out of, and remain latent in, host lungs.

633A **Morphotype-specific fungal factors drive uptake and clearance of *Aspergillus fumigatus* by airway epithelial cells** Sebastien C Ortiz, Patrick J Dancer, Thomas Easter, Kayleigh Earle, Rachael Fortune-Grant, Mike Bromley, Sara Gago, Margherita Bertuzzi Manchester Fungal Infection Group, Faculty of Biology, Medicine and Health, University of Manchester

Aspergillus fumigatus (*Af*) affects over 3,000,000 individuals annually, with invasive aspergillosis having mortality rates of over 50%. Airway epithelial cells (AECs), which cover the entire alveolar surface and comprise 24% of all cells in the human lung parenchyma, have instant, extensive, and likely prolonged contact with *Af* conidia upon inhalation. Recent evidence from our lab demonstrates that AECs provide a potent means of antifungal defense against *Af in vivo*, and that dysfunctional epithelial antifungal activity in at-risk patients may provide an opportunity for *Af* to exploit AECs as a safe haven to reside intracellularly. Relatively little is known about the fungal and host factors controlling *Af* uptake and clearance by AECs and the dependency of these processes on the morphotype-specific changes associated with fungal germination. To characterize how morphotype-specific fungal factors shape AEC-*Af* interactions, we locked *Af* into specific morphotypes using fluorescent auxotrophic *pyrG*$^{-}$ strains, evaluated internalization using imaging flow cytometry, and determined that swollen conidia, locked at 3 and 6 hours, are 2-fold more readily internalized than resting conidia locked at 0 hours. Using a combination of fluorescent lectins and cell wall mutants, we are now systematically evaluating morphotype-specific factors on *Af* surface for their role in mediating fungal uptake and clearance by AECs and determined that surface mannose likely dictates these interactions. Supporting this, mannose and the mannose-binding lectin Concanavalin A were able to reduce (by 88%) and abolish (100%) *Af* internalization, respectively. Through

the evaluation of candidate receptors, we have identified a receptor (Rc1) with known mannose binding affinity, as a key receptor in these interactions. When Rc1 is knocked out, there is a 68% decrease in *Af* internalization. Our work is now focused on systematically evaluating the role of Rc1 and related polymorphisms in Af-AEC interactions, and their importance in disease. Understanding how AECs contribute to antifungal clearance could provide novel avenues for the prevention and treatment of fungal diseases.

634A **Identification and functional characterization of *Fusarium graminearum* effectors** Nicholas Rhoades[1,2], Gabdiel Yulfo-Soto[2,3], Susan McCormick[4], Hye-Seon Kim[4], Guixia Hao[4] [1]NCAUR-ARS, USDA, [2]ORISE, [3]USDA, [4]REE-ARS-MWA, USDA
Fungal pathogens secrete small proteins called effectors that interfere with the plant immune system and promote infection and disease. *Fusarium graminearum* is the causal agent of Fusarium Head Blight (FHB), a disease on wheat, barley, and other grains. During infection, *F. graminearum* produces trichothecene toxins, predominately deoxynivalenol (DON), contaminating grain and reducing crop yields. DON also functions as a virulence factor to promote *F. graminearum* spread throughout the wheat head. In addition to producing DON, *F. graminearum* secretes hundreds of putative effector proteins, which can interfere with plant immunity and promote disease development. Effectors that are conserved in multiple species of fungi are known as core effectors. In this study, we aim to investigate and characterize the core effectors that are important for *F. graminearum* pathogenesis and use them as targets to control FHB and mycotoxin contamination. Using a combination of genome sequence analyses and artificial intelligence programs to predict putative effector coding genes, we analyzed 199 genome sequences representative of the 23 *Fusarium* species complexes, including 25 genomes within the *F. graminearum* species complex. These programs identified approximately 150 putative core effectors in *F. graminearum* (PH-1). Gene expression analyses of 50 selected candidates identified 12 effector genes that were highly induced over a seven-day infection period. To elucidate their role in FHB, we have generated deletion mutants for the ten most promising effector genes and performed FHB virulence assays on wheat heads. Of the ten tested, deletion of five effector genes significantly reduced initial infection in wheat spikes and one mutant also reduced infection in barley spikes compared to wild type. Complementation of the mutants and FHB spread assays in wheat are in progress.

635A **Comparative transcriptomic and histochemical analyses of *Microbotryum pavonius* infection on two *Dianthus* species. Is it a "generalist" or "specialist" fungus?** Derica G Tavares[1], Roxanne K Hayes[1], Tatiana Giraud[2], Rebecca Dangol[3], Joseph P Ham[3], Emmy Walters[3], Michael H Perlin[1] [1]Dept of Biology, University of Louisville, [2]Université Paris-Saclay, [3]University of Louisville
Members of the *Microbotryum* species complex of fungal pathogens infect wildflower species in the Carnation family, causing anther-smut disease, and the complex serves as a model for emerging infectious diseases through host shifts. Some species of *Microbotryum* are "specialists" while others are more "generalist." *Microbotryum* infections in *Dianthus* hosts show overlapping host specificities for four *Microbotryum* lineages, and the lineages frequently co-occur in single-host populations. Here, we examined whether there are mechanisms associated with host specificity and disease progression at early stages of infection by *Microbotryum pavonius* in *D. pavonius* (specific host) and *D. segueri* plants using gene expression analysis, confocal fluorescence microscopy, and scanning electron microscopy (SEM). *D. pavonius* and *D. segueri* seeds were inoculated with *M. pavonius* and seedling tissues were collected at 6-leaf and 12-leaf stages for RNA extraction for RNASeq analysis as well as for both microscopies. In addition, *D. pavonius* and *D. segueri* plants were allowed to grow for over two months to observe the complete life cycle of *M. pavonius* through the development of teliospores in the flower. DNA and RNA (for RNASeq data) were also isolated from buds of infected and uninfected plants. *M. pavonius* infection on seedlings affects the initial root growth, with larger seedlings in the uninfected control, but over time, the infected plants develop comparable growth characteristics. Confocal microscopy and SEM showed no obvious differences in early infection stages in *D. pavonius* and *D. segueri* in terms of haploid sporidia mating and production of filamentous dikaryons. Appressorium formation of mated cells was observed on plant tissue of *D. pavonius* and *D. segueri* seedling roots. Infected *D. segueri* plants flowered, but not *D. pavonius*, which requires vernalization. Infection status, evidenced by qPCR for fungal gDNA, did not always correlate with teliospore production in flowers: most infected *D. segueri* flowers produced teliospores, but some plants failed to do so, and flowers appeared healthy, even though PCR with *M. pavonius*-specific primers confirmed its presence in both types of buds. Thus, *M. pavonius* infection of *D. segueri* suggests this fungus may have a broader host range than originally suggested from field studies.

636A **Characterization of the Effector Protein MVLG_01732 from *Microbotryum lychnidis-dioicae* and Its Interactions with Host Proteins** Joseph P Ham[1], Michelle T Barati[2], Michael L Merchant[2], Derica G Tavares[1], Emma A Lamb[1], Michael H Perlin[1] [1]Dept of Biology and Program on Disease Evolution, University of Louisville, [2]Dept of Medicine, University of Louisville

Microbotryum lychnidis-dioicae is a pathogenic fungus that infects the flowering plant *Silene latifolia*, causing anther smut disease. The pathogenicity of this fungus relies on a suite of effector proteins that manipulate host cell processes to facilitate infection and colonization. Effector proteins are key elements in the fungal arsenal that suppress plant defense responses and alter host cell physiology to benefit the pathogen. This study focuses on the effector protein MVLG_01732, a novel protein identified in *M. lychnidis-dioicae* which plays a critical role in the interaction between the pathogen and its host. Through yeast two-hybrid

screening and subsequent pull-down immunoprecipitation assays, we have discovered that MVLG_01732 interacts with two pivotal host proteins: a calcium lipid-binding protein and the cellulose synthase interactive protein-1. The calcium lipid-binding protein is essential for signal transduction and membrane trafficking within plant cells, while cellulose synthase interactive protein-1 is a critical component of the cellulose synthase complex, a key enzyme in cell wall biosynthesis. The interaction between MVLG_01732 and these host proteins suggests a strategic disruption of cell wall integrity and signaling pathways, which are vital for plant defense. The pull-down immunoprecipitation assay also uncovered other proteins that MVLG_01732 may interact with which are used in plant development and defense and could be further studied for their interaction. Furthermore, the expression of MVLG_01732 was found to be upregulated during the early stages of infection, indicating its importance in the establishment of the disease. This study not only sheds light on the complex interactions between *M. lychnidis-dioicae* and its host but also opens up new avenues for the development of disease-resistant plant varieties. By understanding the role of effector proteins like MVLG_01732, we can better comprehend the molecular mechanisms of pathogenesis and host specificity in plant-fungal interactions, which is paramount for advancing plant protection strategies.

637A **Learning from the negative: Studying pathogen evolution from the "non-pathogen" perspective** David Rinker[1], Thomas J C Sauters[2], Karin Steffen[2], Camila Figueiredo Pinzan[3], Huzefa Raja[4], Manuel Rangel Grimaldo[4], Thaila Reis[3], Patrícia Alves de Castro[3], Nicholas Oberlies[4], Gustavo Goldman[3], Antonis Rokas[2] [1]Vanderbilt University, [2]Biological Sciences, Vanderbilt University, [3]University of São Paulo, [4]University of North Carolina Greensboro

Microbial trait evolution is an ever-evolving topic in biology. The canonical method of study involves direct investigation of the species that display the trait of interest. While great strides are made using this method, there is fantastic potential for defining trait evolution by studying closely related sister taxa that do not display the trait of interest. The repeated, independent evolution of pathogenesis in *Aspergillus* section *Fumigati* presents the opportunity to study the origins and building blocks of fungal pathogenesis. In this study, we define a phenotypic, genotypic, and transcriptional profile of the non-pathogenic fungus, *Aspergillus fischeri*, using 16 strains and contrast these findings with similar work in the pathogen *Aspergillus fumigatus* using 14 strains. We find low genomic variation between strains in *A. fischeri*, in contrast to the high variation across *A. fumigatus*. Constructing an *A. fischeri* pangenome, we find a large degree of core proteome conservation from *A. fumigatus* to *A. fischeri,* including 204/207 of the verified virulence genes. We also find a large degree of transcriptional heterogeneity at both 30°C and 37°C in *A. fischeri* prompting gene regulatory network comparisons within and between species. The transcriptional heterogeneity is mirrored in virulence assays as there is a great deal of intraspecific variation. Our work raises questions about the drivers of phenotypic breadth and highlights the grey area found between the categories of pathogen and non-pathogen when studying intraspecific strain diversity.

638A **Lichtheimia corymbifera as model system for mucormycosis** Kerstin Voigt[1,2] [1]Jena Microbial Resource Collection, University of Jena, [2]Jena Microbial Resource Collection, Leibniz Institute for Natural Product Research and Infection Biology

The basal fungal lineage order Mucorales comprise more than 30 species which are human pathogenic causing life-threatening infections known as invasive mucormycosis (IM). Mortality rates range from 40-80% in immunocompromised background. Rapid progression and difficult diagnosis are major hallmarks of the disease. Comorbidities such as diabetes mellitus, hematological malignancies, organ transplantations, and most recently COVID-19 predispose individuals to Mucormycosis. The increased incidences of these underlying diseases over the last years are leading to a worldwide spread of incidences for mucormycosis. Besides *Rhizopus*, *Mucor* and *Rhizomucor* spp., *Lichtheimia corymbifera* ranges among the most prominent causative agents of IM. In 1885, Arnold Paltauf published the first case of disseminated mucormycosis, which he named "Mycosis mucorina" and was most probably caused by *L. corymbifera*.The current abstract provides an overview about (i) the genome architecture, (ii) the interaction with immune cells of the innate immune system, (iii) expression signatures based on dual-transcriptomics and (iv) potenmtial virulence markers derived from spore surface proteomics, secretomics and iron uptake profiling. All these features are discussed in the light of potential targets for diagnostics and therapy.

639A **An acidophilic fungus is integral to prey digestion in a carnivorous plant** Pei-Feng Sun[1], Min R. Lu[1], Yu-Ching Liu[1], Yu-fei Lin[1], Daphne Z. Hoh[1], Huei-Mien Ke[2], I-Fan Wang[3], Mei-Yeh Jade Lu[1], Roland Kirschner[4], Ying-Chung Jimmy Lin[4], Ying-Lan Chen[3], Isheng Jason Tsai[1] [1]Academic Sinica, [2]Soochow University, [3]National Cheng Kung University, [4]National Taiwan University
Carnivorous plant leaves, such as those of the spoon-leaved sundew *Drosera spatulata*, secrete mucilage which hosts microorganisms potentially aiding in prey digestion. We characterised the mucilage microbial communities and identified the acidophilic fungus *Acrodontium crateriforme* as the ecologically dominant species. The fungus grows and sporulates on sundew glands as its preferred acidic environment. We show that the *A. crateriforme* has a reduced genome similar to that of other symbiotic fungi. Based on the transcriptomes when encountering prey insects, we revealed a high degree of genes co-option in

each species during fungus-plant coexistence and digestion. Expression patterns of the holobiont during digestion further revealed synergistic effects in several gene families including fungal aspartic and sedolisin peptidases, facilitating the digestion of sundew's prey, as well as transporters and dose-dependent responses in plant genes involved in jasmonate signalling pathway. This study establishes that botanical carnivory is defined by multidimensional adaptations correlated with interspecies interactions.

640A **A G-alpha protein mediates the interaction between *Aspergillus fumigatus* and *Pseudomonas aeruginosa* during biofilm formation** Gustavo H Goldman Ciencias Farmaceuticas, Universidade de Sao Paulo

Human chronic diseases, such as cystic fibrosis, create a pulmonary environment that allows the biofilm interaction between the filamentous fungus *Aspergillus fumigatus* and the bacterium *Pseudomonas aeruginosa* (AfxPa). *A. fumigatus* recognition of *P. aeruginosa* probably involves surface receptors that will activate signal transduction pathways important for coping with the bacterium. Screening of 21 null mutants of G-protein coupled receptors (GPCR), Mitogen-Activated Protein kinases (MAPK) receptors, a histidine kinase receptor, and G-alpha proteins revealed 7 mutants (two GPCRs, three MAPK receptors, a G-alpha protein, and one histidine kinase receptor) with reduced biofilm formation specifically in the presence of *P. aeruginosa*. Transcriptional profiling and mass spectrometry analysis of secondary metabolites (SMs) produced by of one of these mutants, Δ*gpaB* (*gpaB* encodes a G-alpha protein) showed GpaB controls the production of several SMs shown as important for the Af Δ*gpaB* xPa dual biofilm interaction, among them pyripyropene A. Pyripyropene A, is a SM previously shown as a potent inhibitor of a mammalian acyl-CoA cholesterol acyltransferase. Deletion of *pyr2*, encoding a non-reducing polyketide synthase, showed reduced Af Δ*pyr2* xPa growth, suggesting pyripyropene is important for this AfxPa interaction. Our results suggest pyripyropene plays an important role in the AfxPa dual biofilm interaction.

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641A **Polishing a pathogen: Annotation of a hybrid genome assembly reveals putative virulence and pathogenicity factors of *Calonectria* sp. 134-2022, a novel pathogen of *Baptisia australis*.** Fiona C Harrigian[1], Elizabeth Bush[2], Lina Rodriguez-Salamanca[2], Catalina Salgado-Salazar[1], Nicholas LeBlanc[1] [1]USDA-ARS, [2]Virginia Tech

Fungi in the genus *Calonectria* are pathogens of a variety of ornamental and horticultural crops, however most research on *Calonectria* focuses on them as pathogens of *Eucalyptus*. Novel *Calonectria* spp. continue to emerge, but very few have fully sequenced genomes. Without genomic data, it is not possible to determine the genetic mechanisms underlying the organism's pathogenicity. One *Calonectria* isolate (*Calonectria* sp. 134-2022) belonging to the *C. colhounii* species complex was recently observed to cause stem rot of *Baptisia australis* propagation seedlings. Considering these observations, it was important to strive for a high-quality genome, to identify putative virulence factors in the genome, and to determine concretely whether this *Calonectria* strain was the pathogen causing disease on *Baptisia australis*. Genome sequences were obtained using nanopore sequencing technology. Guppy basecaller, Porechop and Flye were used for basecalling, adapter trimming and genome assembly, respectively. A pathogenicity test was carried out in a growth chamber using seedlings of *Baptisia australis* inoculated with $1x10^5$ spores/mL. Nanopore sequencing produced 3.94 million reads with an N50 of 11.89kb. The assembly was 61,738,591 bp with 14 contigs and an N50 of 5,551,483 bp with an initial BUSCO completeness of 97.8%. After polishing the genome assembly with Illumina reads using Pilon, completeness increased to 99.8%. Among the proteins predicted using Augustus and annotated with PHI-base, there were 216 apoplastic effectors and 54 cytoplasmic effectors found in the assembly. In addition, 65 secondary metabolism biosynthetic clusters were identified using AntiSMASH, among them complete clusters of the known virulence factors AM-toxin and ACT-toxin. The fungal pathogen was reisolated from the infected plant tissue and used to infect more *Baptisia australis* seedlings producing consistent disease symptoms on root and foliar tissue. Spores reisolated from infected plant tissue were confirmed to be the same strain based on spore measurements. Sequencing the full genome of *Calonectria* sp. 134-2022 and creating a high-quality assembly enabled a better understanding of what host-pathogen interactions may occur during the infection process of the novel confirmed pathogen.

642A ***An N-glycosylated effector rescues pectin methylesterase enhancing Ustilago maydis virulence*** Chibbhi Kumarasamy Bhaskar, Minh-Quang Chau, Lay Sun Ma Institute of Plant and Microbial Biology, Academia Sinica

Pectin, a key plant cell wall component, is the primary defense against pathogens, significantly enhancing plant resilience. The degree of methyl esterification (DM) of pectin, which affects cell wall rigidity, is jointly regulated by two proteins: pectin methylesterases (PME) and its inhibitor PMEi. Unmethylated pectin, due to PME activity, becomes susceptible to degradation by pathogen polygalacturonases, facilitating pathogen penetration into plant tissues. Conversely, PMEi is upregulated to inhibit PME activity and block fungal infection. However, the mechanisms through which fungal pathogens overcome the defensive role of

PMEi remain to be explored. Here, we report that *Ustilago maydis* deploy the effector ALE1 to target maize PMEi in an N-glycosylation-dependent manner. ALE1 is N-glycosylated at position 71 and shows a conserved N-glycosylation-dependent virulence function among smut fungi. N-glycosylated ALE1 preferably targets maize PMEi45, the inhibitor of PME19. Overexpression of ALE1 increases the level of unmethylated pectin, implying rescue of PME activity from PMEi. The interaction between PME19 and PMEi45 has been revealed by Y2H assay and LC-MS/MS analysis. We thus propose that ALE1 targets PMEi45, freeing PME19 to demethylate pectin, leading to changes in plant cell wall composition that could favor fungal infection and disease development. Based on structural homology analysis, we are currently investigating the effect of ALE1 on multiple PMEi-PME interactions at the apoplast. Our study shall reveal the strategy of *U. maydis* deploying a single effector ALE1 to broadly negate PMEi regulation on multiple PME enzymes, thereby modulating plant cell wall favorable for fungal infection.

643A **Avc1 regulates adaptation to high CO_2 levels in the human fungal pathogen *Cryptococcus neoformans*** Benjamin Chadwick, Xiaorong Lin University of Georgia

The opportunistic pathogen *Cryptococcus neoformans* causes fatal systemic disease in immunocompromised individuals worldwide. As an environmental fungus, adaptation to host conditions is key for its survival and pathogenesis. A major difference between the host and ambient environment is the concentration of CO_2. CO_2 makes up ~5% of the air in the human body, which is nearly 125 times higher than in ambient air (~0.04%). The growth of many environmental isolates of *C. neoformans* is inhibited by host levels of CO_2, and the ability to tolerate this high level of CO_2 is correlated with virulence. How CO_2-sensitive environmental isolates have been able to adapt to the higher CO_2 level of the host has remained a mystery. Through experimental evolution and comparative genomics, we found that multiple environmental isolates of *C. neoformans* can adapt to high levels of CO_2 *in vitro* through stable mutation. Loss of function mutations in the gene *AVC1* was responsible for a gain of CO_2 tolerance in multiple environmental isolates. We deleted the *AVC1* gene in multiple CO_2-sensitive isolates and discovered a conserved effect of gain in CO_2 tolerance and gain of fitness *in vivo*. Overexpression of *AVC1* resulted in hypersensitivity to CO_2 and CO_2 dependent cell size enlargement. Taken together, these results implicate a critical role for Avc1 in regulating CO_2 tolerance and a potential mechanism for high CO_2 adaptation of *C. neoformans* environmental isolates.

644A **Investigating the immunogenicity of capsule and mannoprotein in a novel heat killed *Cryptococcus neoformans* vaccination model** Samantha Avina[1], Siddhi Pawar[1], Yina Wang[1], Keyi Wang[1], Amariliz Rivera[2], Chaoyang Xue[1] [1]Rutgers University, [2]Pediatrics, Rutgers University

Currently, no fungal vaccine exists for clinical use while fungal infections are responsible for over 1.5 million deaths every year and account for half the AIDS related deaths worldwide. Although anti-fungal drugs are available, they are limited in their applications. As populations susceptible to fungal infections continue to increase combined with the rise of anti-fungal resistance, an effective anti-fungal vaccine is highly desired. Our previous studies identified a *Cryptococcus neoformans* mutant strain *fbp1Δ* as a potential vaccine candidate. This mutant strain contains a deletion of the F-box protein Fbp1, a key subunit of the SCF E3 ligase complex necessary for ubiquitin-mediated proteolysis. Excitingly, we found that vaccination with heat-killed *fbp1Δ* (HK-*fbp1Δ)* can elicit protection against *C. neoformans* parental strain and its sibling species *C. gattii* in an interferon gamma (IFN-γ) dependent Type 1 immune response. The protection is preserved in $CD4^+$ T cell depleted animals, indicating that this vaccination approach may be able to impart protection in both immunocompetent and immunocompromised hosts, e.g., HIV/AIDS. While these findings show great potential for HK-*fbp1Δ* as a fungal vaccine candidate, we have yet to decipher the immunogenic factor(s) expressed by the *fbp1*Δ mutant that are responsible for the induction of the protective immune response observed. In current studies, we have identified that capsule plays an important role in HK-*fbp1Δ* vaccine mediated protection, as acapsular HK-*fbp1Δ* cells showed diminished protection against wild type challenge. Additionally, our studies focus on characterizing the role of mannoproteins in conferring immune protection in the HK- *fbp1Δ* vaccine candidate. Our studies have shown that Cytokine Inducing Glycoprotein 1 (Cig1), a GPI anchored mannoprotein with a PEST domain sequence, is upregulated in *fbp1Δ*. Furthermore, we have found that deletion of Cig1 in the *fbp1Δ* background resulted in decreased recruitment of anti-fungal effector T cells and diminished production of protective inflammatory cytokines by the host. With knowledge gained from these studies, we hope to improve our vaccine strategy and advance the long-term goal of generating a fungal vaccine as a tool in the arsenal against fungal infections.

645A **Do Fungi have an Immune System? The Neurospora crassa and Pseudomonas syringae pathosystem reveals an initial cellular reaction to bacterial proximity** Frances G Stark, Mari Torii-Karch, Ksenia Krasileva Plant and Microbial Biology, University of California, Berkeley

Recent comparative genomics and evolutionary analyses provided multiple lines of evidence for the fungal immune system and proposed hypotheses for mechanistic interactions. Fungi possess complex non-self, or “xeno-recognition”, surveillance systems

similar to protective, non-self surveillance systems in plants, animals, and bacteria. Understanding similarities of how different organisms across kingdoms respond to non-self requires leveraging existing model systems and their genetic toolkits. We leveraged two model systems, Neurospora crassa and Pseudomonas syringae DC3000 (pstDC3000) to dissect fungal response to bacteria. PstDC3000 preferentially surrounds N. crassa germlings on a solid surface, causing Propidium Iodide (PI) vital dye uptake, indicative of a cell death response, as early as ten minutes post bacterial proximity. Inoculating N. crassa with heat-killed pstDC3000 abolished the PI uptake. Deletion mutants of common or proposed cell death regulating genes in N. crassa and pstDC3000 did not abolish PI uptake including; multiple HET genes, VIB1, PhcA, T3SS, and eleven of the seventeen proposed NLR-like genes in N. crassa. To try and dissect initial cellular signaling events, we performed transcriptomics on N. crassa after pstDC3000 inoculation at ten minutes and one hour. Our study provides insight into an early transcriptional response in filamentous fungi exposed to bacteria alongside surveying fungal NLR-like deletion mutants.

646A **A look into the *Pyrenophora teres* f. *teres* colonization strategies on barley using a transformation-free staining and confocal microscope analysis** Ashley C Nelson[1], Gayan Kariyawasam[1], Nathan Wyatt[2], Janine Haueisen[3,4], Eva H. Stukenbrock[3,4], Pawel Borowicz[5], Zhaohui Liu[1], Timothy L. Friesen[2] [1]Plant Pathology, North Dakota State University, [2]Edward T Schafer Agricultural Center, USDA-ARS, [3]Evolutionary Biology, Max Planck Institute, [4]Environmental Genomics, Kiel University, [5]Animal Sciences, North Dakota State University

Laser scanning confocal microscopy is an invaluable tool in assessing plant microbe interactions at a cellular level. Here we use a transformation-free staining technique with propidium iodide (PI), which stains RNA and DNA, and wheat germ agglutinin labeled with fluorescein isothiocyanate (WGA-FITC), which stains chitin, to visualize fungal colonization of plants. Showcasing this, in tandem with the fungal pathogen *Pyrenophora teres* f. *teres* (*Ptt*) infecting barley, we show how high resolution images shed light on fungal colonization strategies and infection structures of fungal pathogens. In the *Ptt*-barley interaction, intracellular vesicles develop in epidermal cells directly below penetration points and serve as branching points for hyphal growth into the plant's mesophyll layer. Infected plant mesophyll layers are full of deliberate intercellular hyphal growth that maximizes its surface areas to grow around the individual mesophyll cells, exhibiting patterns we characterize as encasement, mesophyll cell trapping, thick layering, and branching. Encasement is the growth of hyphae in the mesophyll where it surrounds the cells on two opposing sides. Mesophyll cell trapping begins as encasement, but the hyphae continue to grow around the whole mesophyll cells surrounding it on all sides. Thick layering is the layered parallel growth of multiple hyphae and branching is the perpendicular growth of hyphae through numerous layers of mesophyll cells. We analyzed morphological differences between avirulent and virulent isogenic strains of *Ptt* and used the growth patterns mentioned above to assess their success in-planta. Hyphae of virulent strains were most intent on growing parallel to the length of the leaf, through the mesophyll layer as rapidly as possible, followed by lateral branching, explaining the net like lesions characteristic of this disease. Cell death was only observed behind the growing point of the fungus, where mesophyll cells were surrounded by the fungal hyphae. Comparatively the avirulent isogenic isolate was able to grow in-planta but had a fitness deficiency that inhibited its quick takeover of the leaf tissue. We believe the pathogen is maximizing fungal biomass to absorb nutrients at a high efficiency while delaying plant defenses before cell death is an advantage to the pathogen. *Ptt* has shown the potential of this technique to relook at the strategies of fungal pathogens and work in tandem with quantitative and molecular analysis.

647A **The key role of the biotic component in kiwifruit vine decline syndrome (KVDS) in Italy, an emerging multifactorial syndrome** Micol Guaschino[1,2], Marco Garello[1,2], Luca Nari[3], Davide Spadaro[1,2] [1]Dept of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, [2]AGROINNOVA, University of Torino, [3]Fondazione per la Ricerca, l'Innovazione e lo Sviluppo Tecnologico dell'Agricoltura Piemontese, Agrion

Kiwifruit vine decline syndrome (KVDS) is considered a multifactorial syndrome where abiotic and biotic stressors are involved. In this study, the microbial communities of kiwifruit soil, rhizosphere and root were characterized together with their associations with abiotic stressors. Several soilborne oomycetes belonging to the genus *Phytopythium* were previously associated to the syndrome onset. Association networks unveiled the correlation of *Phytopythium* spp. with the diseased status of orchard soils, whereas in the rhizosphere the oomycete ASVs were negatively associated with growth promoting fungal genera and AM fungi found in kiwifruit. Network analyses conducted for the rhizosphere communities mainly showed that associations were established between different *P. vexans* ASVs, thus revealing strain specific characteristics which require further investigation. The dynamic emerging from the analysis of the ecological processes driving rhizosphere community assembly, highlights the possibility of a dysbiosis phenomenon in the rhizosphere, driven by deterministic processes in the oomycete community. Differently, fungal and bacterial microbiotas showed mainly stochastic processes. The study highlights the importance of considering multifactorial aspects and their interactions in emerging pathosystems, where climate change plays a role in syndrome onset. The combination of different omics techniques is needed for a wider comprehension of oomycete pathogenesis in complex systems.

648A **A predatory fungus detects prey pheromones via G-protein-coupled receptors** Chih-Yen Kuo[1,2], Rebecca J Tay[1], Hung-Che Lin[1], Sheng-Chian Juan[1], Guillermo Vidal-Diez de Ulzurrun[1], Jason Hoki[3,4], Frank C Schroeder[3,4], Yen-Ping Hsueh[1,2] [1]Institute of Molecular Biology, Academia Sinica, [2]Molecular and Cell Biology, Taiwan International Graduate Program, Academia Sinica and Graduate Institute of Life Science, National Defense Medical Center, [3]Boyce Thompson Institute, Cornell University, [4]Dept of Chemistry and Chemical Biology, Cornell University

The ability to sense prey-derived cues is essential for predatory lifestyles. Under low nutrient conditions, *Arthrobotrys oligospora* and other nematode-trapping fungi develop dedicated structures for nematode capture when exposed to nematode-derived cues, including a conserved family of pheromones, the ascarosides. *A. oligospora* senses ascarosides via conserved MAPK and cAMP-PKA pathways; however, the upstream receptors remain unknown. Through genomic, transcriptomic, and functional analyses, we identified two families of GPCRs involved in sensing distinct nematode-derived cues. GPCRs homologous to yeast glucose receptors are required for ascaroside sensing, whereas Pth11-like GPCRs contribute to ascaroside-independent nematode sensing. Both GPCR classes activate conserved cAMP-PKA signaling to trigger trap development. This work demonstrates that predatory fungi use multiple GPCRs to sense several distinct nematode-derived cues, enabling robust prey recognition. Furthermore, identification of the ascaroside receptors in *A. oligospora* sheds light on the molecular mechanisms of cross-kingdom communication via conserved pheromones also sensed by plants and animals.

649A **Induction of *Aspergillus fumigatus* zinc cluster transcription factor OdrA/Mdu2 provides combined cellular responses for oxidative stress protection and multiple antifungal drug resistance** Christoph Sasse[1], Emmanouil Bastakis[2], Fruzsina Bakti[2], Annalena M. Höfer[2], Isabella Zangl[3], Christoph Schüller[3], Anna M. Köhler[2], Jennifer Gerke[2], Sven Krappmann[4], Florian Finkernagel[5], Rebekka Harting[2], Joseph Strauss[3], Kai Heimel[2], Gerhard H. Braus[2] [1]Institute of Microbiology and Genetics, Dept of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), University of Göttingen, [2]University of Göttingen, [3]University of Natural Resources and Life Sciences, Vienna (BOKU), [4]University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nürnberg, [5]Philipps University, Marburg

The filamentous fungus *Aspergillus fumigatus* represents a prevalent opportunistic human pathogen, which can develop resistance mechanisms against antifungal drugs. A genomic wide overexpression screen of 228 zinc cluster transcription factor encoding *zcf* genes of *A. fumigatus* revealed 11 genes conferring increased resistance to to the broadly applied azole voriconazole, to the polyene amphotericin B or to both. These include four *odrA-D* genes encoding oxidative stress and drug resistance factors, which provide even broader cellular stress protection. Thereby, the corresponding fungal OdrA/Mdu2 and AtrR/OdrD-dependent genetic networks are interconnected. OdrA/Mdu2 activates *atrR/odrD* transcription by direct binding to the promoter, whereas AtrR/OdrD functions as repressor of *odrA/mdu2* expression. *odrA/mdu2* overexpression provides combined resistance to amphotericin B, voriconazole, itraconazole and to reactive oxygen species generated by menadione. OdrA/Mdu2 mediated itraconazole resistance is evoked by direct regulation of the transporter encoding gene *mdr1*. Oxidative stress-inducing substances like amphotericin B and menadione promote OdrA/Mdu2 accumulation in the nucleus to regulate stress response genes like *mdr1* and the putative glutathione-S-transferase encoding gene *gstD*. The expression levels and external stress conditions fostering nuclear accumulation of OdrA/Mdu2 determine the regulation of the target genes. Hence, OdrA/Mdu2 provides a combined adaptation strategy for survival in nature or within a potential host, where this fungus represents the most common agent for human mold pneumonia worldwide. The OdrA/Mdu2 controlled genetic network highlights the tight connection between oxidative stress response and antifungal drug adaption to secure *A. fumigatus* survival in various hostile environments.

650A **Characterization of gene expression in *Peronospora effusa* and spinach during resistant and susceptible race-cultivar interactions** Kelley Clark[1,2], Chunda Feng[2,3], Amy G. Anchieta[2], Allen Van Deynze[1], James C Correll[3], Steve Klosterman[2] [1]Plant Sciences, University of California-Davis, [2]USDA-ARS, [3]Plant Pathology, University of Arkansas

The obligate oomycete pathogen, *Peronospora effusa,* causes downy mildew of spinach. Spinach downy mildew control is predominantly based on deployment of resistant cultivars. However, new races and novel isolates of the pathogen continue to emerge and overcome cultivar resistance. Currently there are 19 named races of *P. effusa*. Here we characterized the transcriptomes of spinach, *Spinacia oleracea*, and *P. effusa* during disease progression using the spinach cultivar Viroflay, two near isogenic lines NIL1 and NIL3, and two *P. effusa* races, R13 and R19, at 24 hours post inoculation and 6 days post inoculation. Differentially expressed gene (DEG) analysis in resistant spinach interactions of R13-NIL1 and R19-NIL3 revealed DEGs from protein kinase-like and P-loop containing families, which have roles in plant defense. Additionally, analysis of the expression of eight DEGs with homology to previously reported downy mildew resistance genes highlighted that some are differentially expressed during resistant spinach-*P. effusa* reactions but not during the susceptible ones. Examination of *P. effusa* gene expression during infection of susceptible cultivars identified expressed genes specific to R13 or R19, and included predicted genes encoding RxLR and Crinkler effector genes that may be responsible for race-specific virulence on NIL1 or NIL3 spinach hosts, respectively. These findings

provide insight into gene expression in both spinach and *P. effusa* during susceptible and resistant interactions and provide a library of candidate genes for further exploration and functional analysis. These resources will benefit spinach breeding efforts for disease resistance and increase our understanding of the virulence mechanisms of this obligate pathogen.

651A **Large-scale deletions and secondary metabolite changes caused by recent Helitron activity in the clonal Tropical Race 4 lineage of *Fusarium* infecting banana** Jelmer Dijkstra[1], Anouk van Westerhoven[1,2], Harold J. G. Meijer[3], Joost Houdijk[1], Yinping Li[1,4], Desalegn Etalo[1], Einar Martinez de la Parte[1], Carolina Aguilera-Galvez[1,5], Giuliana Nakasato Tagami[1], Gert H.J. Kema[1] [1]Laboratory of Phytopathology, Wageningen University and Research, [2]Theoretical Biology & Bioinformatics Group, Utrecht University, [3]Business Unit Biointeractions and Plant Health, Wageningen University and Research, [4]Institute of Pomology, [5]Biological Science, EAFIT University

Tropical Race 4 (TR4), or *Fusarium odoratissimum*, is one of the main fungal pathogens threatening the Cavendish banana production, which dominates both global production (>50%) and export (>95%). Besides Cavendish, TR4 can infect most other banana cultivars and is therefore also a threat to many smallholders whose livelihoods depend on banana production. In particular, the recent incursion and unsuccessful containment in Mozambique raises concerns as the surrounding Great Lakes region of eastern and central Africa is the region with the highest per capita banana consumption in the world. A recent analysis of TR4 isolates collected from several locations in Mozambique showed that strain M1 has at least two large-scale deletions in its core genome. These deletions encompass more than 1% of the reference TR4 genome (isolate II5). At both deletion positions, Helitron transposable elements (FoHeli1) were discovered that are also present at other positions in the reference TR4 II5 genome. In the most closely related strain not belonging to the TR4 clonal lineage (isolate 36102), no FoHeli1 copies were identified. Further analysis of all FoHeli1 copies and their locations in both strains reveal many recent transpositions in the otherwise highly clonal TR4 lineage. The largest deletion in M1 is approximately 450 kb, which overlaps with the predicted biosynthetic gene cluster for the production of fusaric acid. HPLC analysis confirmed the absence of fusaric acid production in M1 and - in contrast to published studies – that this loss did not reduce the level of virulence in greenhouse infection assays on Cavendish bananas. We developed a knockout strain of the FUB1 key enzyme, essential for fusaric acid production, to confirm the observations with the natural M1 mutant.

Overall, more insight into the role of different secondary metabolites in the infection of bananas is crucial for a proper understanding of TR4 pathogenicity. Additionally, the recent activity of FoHeli1 provides an important insight into TR4 genome dynamics.

652A **Genome of endophytic *Fusarium oxysporum* from the strawberry root microbiome lacks common virulence factors** Samantha Gebben-Hernandez, Fiona C Harrigian, Nicholas LeBlanc USDA-ARS

Fungi in the *Fusarium oxysporum* species-complex have primarily been studied as plant pathogens. However, many of these fungi commonly associate with plants as nonpathogenic endophytes and can have beneficial effects on host growth. Understanding the underlying genetic bases of endophytic interactions requires a high-quality genome assembly. The goal of this study was to generate a genome assembly for a novel endophytic strain of *F. oxysporum* isolated from roots of healthy strawberry plants. Genomic DNA was sequenced from a single spore isolate for 24 hours using Nanopore technology. An assembly was generated and polished using Flye, genes were predicted using Funannotate, and completeness was measured using a set of conserved orthologs and BUSCO. Homologs of proteins required for fungal production of the plant hormones indole-acetic acid (IAA) and gibberellic acid (GA) as well as Secreted In Xylem (SIX) effector proteins were identified using blast. Additional putative effector proteins were identified using SignalP and EffectorP and annotated with PHI-base. Approximately 3 million Nanopore reads assembled into a genome of ca. 50 Mb, composed of 86 contigs, with an N_{50} of 4,529,961 bp, and 99.4% completeness. The genome lacked SIX effectors and proteins required for production of plant hormones IAA and GA. Of the total 15,716 predicted proteins, 487 were identified as putative effectors. Only one putative effector showed homology with a known virulence factor of pathogenic *F. oxysporum*, a secreted metalloprotease (FoMep1). Outcomes from this research demonstrate the potential for using Nanopore data to generate fungal genome assemblies and will provide a platform for identifying genes required for host infection by endophytic forms of *F. oxysporum*.

653A **Do fungal terpenoids volatiles structure the mycosphere?** Erika Kothe[1], Katrin Krause[2] [1]Friedrich Schiller University, [2]Friedrich Schiller Univresity Jena

Fungi play a major role in structuring their soil habit. In addition to involvement in wood degradation and element cycling, adding structure *via* hyphal growth and excreting carbon sources for the use of bacterial commensals, they release a bouquet of terpenoid

volatiles. The effect of such terpenes was investigated for two major habitats in forest soils: rotting wood and ectomycorrhizal symbiosis. Using the wood-rotting basidiomycete, *Schizophyllum commune*, as well as the ectomycorrhizal *Tricholoma vaccinum*, a major impact of fungal terpenes on the microbiome and ectomycorrhizosphere could be established. In an early succession woodland, the effect of basidiomycetes on the bacteriome could be verified.

Here, we show that volatiles of *S. commune* are active against wood-decay fungi and bacteria found in its mycosphere. *Actinobacteria* and *Proteobacteria*,including *Pseudomonadaceae*, *Sphingomonadaceae*, *Erwiniaceae*, *Yersiniaceae* a nd *Mariprofundaceae*, dominated the microbiome in *S. commune* white rot. With the major sesquiterpenes β-bisabolol, β-bisabolene and (E)-γ-bisabolene, growth of the competing wood-degraders *Ganoderma lucidum, Flammulina velutipes* and *Kuehneromyces mutabilis* was severely inhibited, while reduced swarming motility and hence reduced distribution was seen with *Serratia marcescens* and *Bacillus subtilis* isolated from the same rotting wood.

In the ectomycorrhizal interaction, *T. vaccinum* terpenes are involved in host specificity. In addition, the ectomycorrhiza fungal community in a woodland determines the bacterial community. Thus, not only can the tree smell an ectomycorrhizal friend, but also soil-dwelling bacteria respond to fungal volatiles. We therefore can conclude that, indeed, basidiomycetes structure their mycobiome in soil providing a major ecosystem service.

654A **Uncovering putative secreted DNases as virulence factors in *Fusarium oxysporum* f. sp. *vasinfectum*** Miranda Otero, Jeffrey J Coleman Entomology and Plant Pathology, Auburn University

Fusarium oxysporum f. sp. *vasinfectum* (Fov), is the causal agent of Fusarium wilt, a vascular disease of cotton. Symptoms of Fusarium wilt include wilting, stunting, leaf necrosis, vascular staining, and plant death. This soil-borne fungal pathogen possesses a large host range, making host resistance the most effective management strategy. Since the discovery of root border cells and the nucleic-acid extracellular traps (NETs) they produce, limited preliminary research has been conducted on their function in host-pathogen interactions. It is proposed that some plant pathogens use extracellular DNases to degrade host root NETs to facilitate infection. To assess the potential role of extracellular DNases in Fov virulence, a selection of nine DNases in Fov race 4 isolate 89-1A were evaluated for *in planta* expression. These DNases were conserved across a wide variety of fungal genera. Root samples from infected susceptible Pima cotton cultivar DP744 were collected at 0, 2, 4, 6, 12, 24, 32, and 120 hours in a hydroponic test tube assay. Total RNA was extracted and RT-qPCR was performed in three independent experiments. Two endonucleases (nuc4 and nuc5) containing signal peptides were significantly upregulated three and five days after infection. These two endonucleases and a TatD DNase (nuc1) were individually knocked out using CRISPR/Cas9 transformation. Resulting mutants of all three DNases were reduced in virulence compared to the wild type isolate in hydroponic and pot assays. DNase activity of mutant and wild type strains will be assessed. Complement mutants had virulence levels restored to that of the wild type. These results suggest that putative extracellular DNases are involved in Fov virulence on cotton.

655A **Using *Nicotiana benthamiana* to understand non-host resistance against *Zymoseptoria tritici*** Abdelrahman M A Mohammad[1,2], Graeme J Kettles[1] [1]Biosciences, University of Birmingham, [2]Agricultural botany, Faculty of Agricultural sciences, Al-Azhar University

The ascomycete fungus *Zymoseptoria tritici* causes Septoria tritici blotch (STB), a damaging disease in all wheat-producing parts of the world. Breeding efforts have improved STB resistance through deployment of large-effect qualitative disease resistance (*R*) genes. However, once deployed in the field, newly-emergent fungal strains often overcome this resistance. Due to the paucity of genetic diversity among elite wheat cultivars, it is critical to identify new and effective sources of resistance. This study builds on the previous observation that several *Z. tritici* secreted proteins (effectors) are recognised in the non-host plant *Nicotiana benthamiana* but not in the natural wheat host (Kettles et al., 2016). This recognition is dependent on the co-receptors BAK1 and SOBIR1, suggesting the existence of cell-surface receptors that allow effector recognition. Using forward genetics, we aim to identify the immune receptors that mediate *Z. tritici* effector recognition in this non-host plant. To develop a mutagenised *N. benthamiana* population, seeds were chemically mutagenised with 0.4% Ethyl methanesulfonate (EMS). A total of 1500 mutagenised plants were cultivated and selfed to establish an M2 mutant population. Agrobacterium-mediated transient expression of three *Z. tritici* effectors (Zt9, Zt11, Zt12) were conducted on four plants from each of 750 lines, amounting to a total of 3000 individual plants. Of the 750 lines screened, five exhibited a lack of cell deathresponse to one or more of the three *Z. tritici* effectors. Subsequently, the five lines underwent backcrossing with wild-type plants to produce F1 progeny. Future work involves self-pollination of the F1 progeny to generate the F2 mapping population, re-screening and bulked segregant analysis. Our goal is to identify the causative mutations underpinning the loss of cell death phenotype in these five lines. Our most recent findings will be presented.

656A **Fungal small molecules modulate *Fusarium verticillioides* transcription** Daren W. Brown, Hye-Seon Kim, Robert H. Proctor Mycotoxin Prevention and Applied Microbiology Research Unit, USDA-ARS

Fusarium verticillioides is both an endophyte and pathogen of maize. During growth on maize, the fungus often synthesizes the mycotoxins fumonisins, which have been linked to a variety of diseases, including cancer in some animals. How *F. verticillioides* responds to other fungi, such as *Fusarium proliferatum*, *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium oxalicum*, that coinfect maize, has potential to impact mycotoxin synthesis and disease. We hypothesize that low molecular weight acids produced by these fungi play a role in communication between the fungi in planta/nature. To address this hypothesis, we exposed 48-hour solid maize kernel cultures of *F. verticillioides* to oxalic acid, citric acid, fusaric acid, or kojic acid and then compared transcriptomes after 30 minutes and 6 hours. Transcription of some genes were affected by multiple chemicals and others were affected by only one chemical. The most significant positive response was observed after exposure to fusaric acid which resulted in >2-fold upregulation of 225 genes, including genes involved in fusaric acid synthesis. Exposure of cultures to the other three chemicals increased expression of only 3 - 15 genes. The predicted function of two gene clusters that responded to kojic acid support possible roles in protecting the fungus from kojic acid and a role in chemical synthesis. These unique transcriptional responses support our hypothesis that these chemicals can act as signaling molecules. Ongoing studies with gene deletion mutants will indicate if the initial transcriptional response to the chemicals benefit *F. verticillioides*.

657A ***Candida albicans* biofilm formation through the lens of natural variation** Katharina Goerlich[1], Aaron P Mitchell[1], Scott G Filler[2], Norma V Solis[2] [1]Microbiology, University of Georgia, [2]The Lundquist Institute at Harbor-UCLA Medical Center

Studies of *C. albicans* biofilm regulation have rested upon mutants with dramatic defects in multiple clinical isolates, and has revealed important roles of adhesins like Als1, Als3, and Hwp1 in biofilm integrity. However, some biofilm regulatory mutants have phenotypes that depend strongly upon environmental or genetic context. We hypothesized that context-dependent mutants may affect novel biofilm determinants. We have used a synthetic genetic approach to augment context-dependent phenotypes and to define key target genes. Mutants wor3Δ/Δ, bcr1Δ/Δ, and ume6Δ/Δ all have context-dependent biofilm defects. We found that a wor3Δ/Δ bcr1Δ/Δ double mutant has a uniformly severe biofilm defect in both in vitro and in vivo assays. In contrast, wor3Δ/Δ ume6Δ/Δ double mutant biofilms remain context-dependent. RNA-Seq analysis revealed that the wor3Δ/Δ bcr1Δ/Δ double mutant has strongly upregulated expression of numerous cell wall and secreted protein genes including CFL5, PGA6, DAG7, and YWP1. We considered the hypothesis that increased expression of these genes may cause a biofilm defect. Thus far we have found that mutations in three of the target genes, ywp1Δ/Δ, dag7Δ/Δ, and pga6Δ/Δ, restore biofilm formation in the wor3Δ/Δbcr1Δ/Δ double mutant background. Ywp1 has been known as an adhesin or an anti-adhesin, depending on the assay system, while Dag7 and Pga6 have no known role in biofilm formation. Our findings show that two context-dependent mutants affect a novel anti-adhesion pathway. An interesting application of these findings is that secreted anti-adhesins like Ywp1 or, potentially, Dag7 and Pga6, may provide a strategy to disrupt pathogenic biofilms.

658A **Following Fungal Farts: Using random barcoded transposon-site sequencing (RB-TnSeq) bacterial libraries to explore the effects of volatiles from the filamentous fungus Trichoderma atroviride** Catharine Adams[1,2], Jose Manuel Villalobos Escobedo[1,2], Mitchell G Thompson[1,2], Adam M Deutschbauer[1,2], Louise Glass[1,2] [1]UC Berkeley, [2]Lawrence Berkeley National Laboratory

Plant-associated fungi provide their hosts with many important health related benefits, and can even protect the plant from invading microbial pathogens. Trichoderma atroviride IMI is a plant root-associated filamentous fungus with potent antimicrobial effects, and volatile organic compounds (VOCs) from T. atroviride have been shown to discourage growth of a range of pathogenic microbes. However, few studies have explored how these VOCs may impact plant beneficial root-associated microbes. We predict that both volatiles and secreted metabolites will significantly shape fungal-bacterial interactions in the rhizosphere.

To test this hypothesis, we used a co-culture method we call a “Thunderdome” to assess how VOCs from T. atroviride impact the physiology of six Plant Growth Promoting Bacteria (PGPB) selected from across the proteobacteria: Azospirillum brasilense Sp245 and Sinorhizobium meliloti 1021 (alpha-proteobacteria), Burkholderia phytofirmans PsJN and Herbaspirillum seropedicae SmR1 (beta), and Klebsiella michiganensis M5al and Pseudomonas simiae WCS417(gamma). We also tested whether the VOCs significantly impacted 15 additional bacterial species that were isolated from the switchgrass rhizosphere.

We found fungal volatiles inhibited the growth of most tested bacteria, even when HEPES buffer was added to the media to prevent lowering of pH by CO2 or other acidic volatiles. A crystal violet staining assay showed that, in Azospirillum brasilense Sp245, fungal volatiles specifically inhibited biofilm formation. By adding individual VOCs to bacterial media, we have found that sensitivity to each compound varies considerably across species. Using bacterial Random Barcode Transposon Sequencing (RB-TnSeq) libraries,

we are further investigating which bacterial genes in the different rhizosphere species are relevant for coping with specific VOCs produced by this fungus.

By elucidating the system wide effects of fungal derived VOCs in the rhizosphere, we can begin to design microbially driven strategies to enhance beneficial plant-rhizosphere relationships, and improve overall plant health.

659A **Mycorrhizal fungi drive plant uptake of the antioxidant ergothioneine from soil and contribute to crop nutritional content** Wade P Heller, Joseph E Carrara Sustainable Biofuels and CoProducts Research, USDA-ARS, NEA, ERRC

The amino acid ergothioneine (ERGO) has recently gained attention as an important antioxidant for human health and has been shown to prevent chronic diseases of the heart and brain. ERGO is produced only by fungi and certain types of bacteria in soils. As such, humans acquire ERGO exclusively through their diet, and express a transporter for tissue-specific localization, though no specific transporters are known outside of animal systems. Aside from mushrooms, plant-based foods are major sources of ERGO; however, plants do not synthesize ERGO and the mechanism by which they acquire it from microbial sources in the soil is unknown. Arbuscular Mycorrhizal Fungi (AMF) have been shown to play a role in the plant uptake of organic nitrogen compounds from the soil, including proteinaceous amino acids, which was the basis of our hypothesis that AMF may contribute to ERGO uptake as well. To test this, we used controlled inoculation trials of asparagus, black beans, oats, wheat, and potatoes, and found that AMF inoculation enhanced the ERGO concentration in all crops evaluated. Further, we found that the level of AMF colonization, which varied among inoculation treatments, positively correlated with plant ERGO concentration. Additional work to investigate the mechanism of AMF-mediated transport of ERGO is ongoing. Our results demonstrate a novel role for AMF in their contribution to the nutritional quality of crops by mediating the uptake of a specific bioactive molecule.

660A **Eukaryotic metagenome-assembled genomes recovered from seagrass leaves include a novel chytrid in the order Lobulomycetales** Cassandra L Ettinger[1,2], Jonathan A Eisen[3,4,5], Jason E Stajich[1,2] [1]Dept of Microbiology and Plant Pathology, University of California, Riverside, [2]Institute for Integrative Genome Biology, University of California, Riverside, [3]Genome Center, University of California, Davis, [4]Dept of Evolution and Ecology, University of California, Davis, [5]Dept of Medical Microbiology and Immunology, University of California, Davis

Fungi play pivotal roles in terrestrial ecosystems as decomposers, pathogens, and endophytes, yet their significance in marine environments is often understudied. Seagrasses, as globally distributed marine flowering plants, have critical ecological functions, but knowledge about their associated fungal communities remains relatively limited. Previous amplicon surveys of the fungal community associated with the seagrass, *Zostera marina* (ZM) have revealed an abundance of potentially novel chytrids. In this study, we employed deep metagenomic sequencing to extract metagenome-assembled genomes (MAGs) from these chytrids and other microbial eukaryotes associated with ZM leaves. Our efforts resulted in the recovery of five eukaryotic MAGs, including a single fungal MAG in the Chytridiomycota (75% BUSCO completeness), three MAGs representing diatoms in the Bacillariophyta (95%, 88% and 44% BUSCO completeness) and one MAG representing a haptophyte algae in the Prymnesiophyceae (61% BUSCO completeness). Whole-genome phylogenomic assessment of these MAGs suggests they all largely represent under sequenced, and possibly novel eukaryotic lineages. Of particular interest, the chytrid MAG was placed within the order Lobulomycetales, consistent with the identity of the dominant chytrid from previous ZM amplicon survey results. Annotation of this MAG yielded 5,650 gene models of which 77% shared homology to current databases. With-in these gene models, we predicted 121 carbohydrate-active enzymes and 393 secreted proteins (103 cytoplasmic effectors, 30 apoplastic effectors) paving the way for in-depth ecological exploration of the role of this chytrid within the ZM ecosystem. Exploration of orthologs between this MAG and existing Chytridiomycota genomes is currently ongoing and promises further insights into its evolutionary and ecological adaptations. Overall these five eukaryotic MAGs represent substantial genomic novelty and valuable community resources. Ongoing and future work will continue to unravel their evolution and ecology, contributing to a deeper understanding of the roles of fungi and other microbial eukaryotes in the larger seagrass ecosystem.

661A **Soybean frogeye leaf spot: Its control using a potent antifungal peptide** Ambika Pokhrel, Vishnu Sukumari Nath, Raviraj Kalunke, Dilip Shah Donald Danforth Plant Science Center

Cercospora sojina (*Cs*) is a necrotrophic fungus known to cause frogeye leaf spot (FLS) disease on soybean (*Glycine max*). Historically, FLS was more prevalent in the southern United States; however, in the recent years it has become frequent in midwestern and northern US making it more economically important. Application of foliar fungicides specifically quinone outside inhibitor (QoI) fungicides is the primary method used to manage FLS disease in the field. However, *Cs* has developed resistance against these QoI fungicides which is now confirmed in 15 different states within the US. Small cysteine-rich antimicrobial peptides (AMPs) with potent antifungal activity have the potential for development as spray-on biofungicides. Therefore, we screened

several AMPs for their ability to inhibit the growth of *Cs in-vitro* and *in-planta*. Among the candidate AMPs, a chickpea 32-amino acid nodule-specific cysteine-rich peptide NCR13_PFV1 with three disulfide bonds was shown to inhibit the growth of both fungicide sensitive and fungicide resistant *Cs* isolates *in-vitro* with a minimal inhibitory concentration of 0.18 µM. The curative *in-planta* antifungal activity of NCR13_PFV1 at 6 µM was assessed using foliar spray application on 3-week-old Blackhawk soybean plants which revealed that NCR13_PFV1 significantly reduced the FLS symptoms. Furthermore, quantification of the fungal DNA content using RT-PCR revealed that *Cs* DNA was significantly reduced in NCR13_PFV1 treated soybean leaves compared to the no peptide control. The mode of action of NCR13_PFV1 against *Cs* was assessed using confocal microscopy which revealed that this peptide can permeabilize the plasma membrane and is localized in certain hotspots inside the fungal cell. The last phase of this work involves identification of key subcellular targets of NCR13_PFV1 in cells of *Cs* using the confocal and super-resolution microscopy. In addition, RNA-seq and proteomics studies will be conducted to identify intracellular molecular targets of this peptide.

662A **Protein-protein networks in an integrated barley-powdery mildew interactome** Valeria Velásquez-Zapata[1], J. Mitch Elmore[2], Shivansh Patel[3], Schuyler Smith[4], Gregory Fuerst[5], Roger P Wise[6] [1]GreenLight Biosciences, Inc., [2]USDA-ARS / University of Minnesota, [3]Plant Pathology, Entomology and Microbiology, Iowa State University, [4]Pathology, Entomology and Microbiology, Iowa State University, [5]Pathology, Entomology and Microbiology, USDA-ARS / Iowa State University, [6]Plant Pathology, Entomology and Microbiology, USDA-ARS / Iowa State University

Disease phenotypes are the result of dynamic changes in gene and protein interactions at multiple levels in multiple compartments. To establish a regulatory network view of protein-protein interactions critical to pathogen infection and disease resistance in cereal grains, we are constructing protein networks of barley (*Hordeum vulgare* L.) in response to powdery mildew, caused by the ascomycete fungus, *Blumeria hordei* (*Bh*). The barley MLA nucleotide binding, leucine-rich repeat (NLR) receptor was used as a model regulator to interrogate cereal immune response, as it's alleles and orthologs confer recognition specificity to several diseases, including powdery mildew, stem rust, stripe rust and rice blast. Forty-seven representative *Bh* effector proteins, including AVR_{A1}, AVR_{A7}, AVR_{A9}, and AVR_{A13}, were selected from time-course RNA-sequencing on wild-type progenitor and immune mutant hosts, and used as baits in yeast-two hybrid next-generation interaction screens (Y2H-NGIS), followed by quantitation and ranking with NGPINT and Y2H-SCORES software, and subsequent binary confirmation. Results were integrated with the HvInt barley interactome, enabling assembly of a high-confidence host-pathogen network of 1085 proteins and 1497 interactions to infer immune activation and effector mechanisms for next-generation breeding to new and emerging pathogens.

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663A **Development of *in vitro* platform techniques for investigating bacteria-fungi interactions in the gut microbiome** Yujin Lee, Ji-Young Lee, Seong-Ryong Yu, Soojin Yu, Hyunjin Cha, Dong-Woo Lee, Yong-Sun Bahn Dept of Biotechnology, Yonsei University

The human gut microbiome plays a pivotal role in determining health and disease states. Despite its significance, limited research exists on the interaction between fungi and bacteria within the gut microbiota and their subsequent impact on the host. In this study, we aimed to establish in vitro methodologies to probe these interactions. We selected *Candida albicans* and *Saccharomyces cerevisiae* as representative intestinal fungi and *Escherichia coli* as the representative intestinal bacterium. For co-culturing, we opted for 0.5x blood-heart-infusion (BHI) media and cultivated them anaerobically at 37°C, simulating the intestinal milieu. We undertook RNA-sequencing-based transcriptional profiling to monitor fungal and bacterial gene expression changes during co-incubation. Mono-cultured cells of both fungus and bacterium served as controls, while co-cultured samples were harvested at 1, 6, and 24 hours post-incubation. Total RNA was isolated using the TRIzol method. Our findings highlighted that *E. coli* displayed the most dynamic expression shifts within the 1-hour of co-culture. Conversely, both *S. cerevisiae* and *C. albicans* demonstrated the most prominent transcriptional changes at the 24-hour mark. KEGG pathway analysis unveiled shared and unique alterations in up- and down-regulated pathways when *E. coli* was co-cultured with either *S. cerevisiae* or *C. albicans*. Future research will focus on refining multi-omics methodologies for deeper insights into bacteria-fungi interactions and elucidating various facets of these interactions.

664A **A fungal transcription factor *BOT6* triggers the transition of fungal infection strategy from mutualistic to pathogenic in plant-associated endophytic fungus *Colletotrichum tofieldiae*** Ren Ujimatsu[1], Junya Takino[2], Masami Nakamura[1], Atsushi Minami[2], Kei Hiruma[1] [1]Graduate School of Arts and Sciences, The University of Tokyo, [2]Faculty of Science, Hokkaido University

Plants host a wide variety of fungi refining their infection strategy from pathogenic to mutualistic depending on the host plant and environmental conditions. While extensive functional analyses have been conducted on numerous molecules such as secreted effectors and/or secondary metabolites in pathogens, the conditions leading to the transition of fungal infection strategy from mutualistic to pathogenic and vice versa, as well as the associated molecular mechanisms, remain largely elusive.

We previously discovered a specific strain of endophytic fungus *Colletotrichum tofieldiae* (designated Ct3) exhibits pathogenicity to the host *Arabidopsis thaliana*, while other Ct strains promote plant growth. A comparative transcriptomic and genetic analysis revealed that a gene cluster comprising putative ABA and botrydial (BOT) biosynthesis genes, denoted as putative ABA-BOT, was activated in Ct3 but not a beneficial Ct strain (Ct4) during the root colonization and is required for Ct3 virulence. We found that the gene cluster includes a Zn_2Cys_6 transcription factor named BOT6, exhibiting distinct evolutionary trajectories in contrast to ABA and BOT genes, despite its co-expression with the other genes in the same cluster.

We aim to characterize the BOT6. We generated Ct4 transformants that constitutively express *BOT6* and monitored the expression of ABA/BOT genes in Ct4 transformants grown in liquid media, where the wild-type Ct strains exhibit minimal expression of ABA-BOT. As a result, all the ABA/BOT genes were hyper-upregulated in the transformants. Moreover, metabolite analysis revealed that the transformants prolifically produced not only BOT precursors that we reveal have host ABA response-inducing activity but also an array of unidentified chemical compounds, implying that BOT6 not only regulates the putative ABA-BOT cluster but also regulate other metabolite genes outside of the cluster. Strikingly, despite the transformants showing reduced vegetative growth on a nutrient medium, the transformants turned to a pathogen severely inhibiting the growth of host plant, a sharp contrast to the WT without *BOT6* expression, which promotes plant growth. Based on these findings, we hypothesize that activation status of BOT6 can exert a global influence on other genes, thereby modulating its continuous fungal infection strategy ranging from beneficial to pathogenic. We will discuss the hypothesis, based on our currently ongoing approaches to identify the BOT6 targets.

665A **Effector proteins involved in defense of mushroom-forming fungi against their competitors** Marieke H van Maanen, Erik P W Beijen, Tijn Bakker, Spyros Kanellopoulos, Robin A Ohm Biology, Utrecht University

Mushrooms are prone to pests and diseases from a range of fungal and bacterial competitors, leading to substantial agricultural losses. Mushroom-forming fungi have evolved several methods to defend themselves, including the secretion of effector proteins capable of suppressing the competitor's growth. However, few secreted proteins involved in defense have been identified to date. Therefore, the aim of this research is to identify novel secreted proteins involved in defense of mushroom-forming fungi against their competitors.

To elucidate the secreted arsenal of mushroom-forming fungi against their competitors, we determined gene expression of the mushroom-forming fungi *Schizophyllum commune* and *Pleurotus ostreatus* during interaction with four fungal and bacterial competitors. Upregulation of transcripts encoding thaumatins and glycosyl hydrolases were observed as the main annotated groups for secreted proteins in *S. commune*, whereas redox-related proteins were predominantly present during interaction in *P. ostreatus*. However, most drastic changes in expression were observed in unannotated genes encoding secreted proteins, which lack known domains.

Ten putative effector proteins were expressed in *Pichia pastoris* and subjected to bioactivity assays to analyse their function. Four of these proteins (SP1 – SP4) exhibited notable effects on fungal growth, germination, and viability, but with distinct modes of action. For example, SP1 enzymatically breaks down components of the fungal cell wall, resulting in broad-spectrum inhibition of growth of competitor fungi. SP2 induces cell lysis in yeast cells. The variety of outcomes observed implies that these proteins play diverse roles in disrupting crucial cellular processes. Further investigation of function and regulatory network will contribute to the understandings of the defense system of mushroom-forming fungi.

666A **Role of Mac1-dependent copper acquisition and superoxide dismutase activity in *Fusarium oxysporum* pathogenicity** Rafael Palos Fernández[1], María Victoria Aguilar Pontes[1], Harald Berger[2], Lena Studt-Reinhold[2], Joseph Strauss[2], Antonio Di Pietro[1], Manuel Sánchez López-Berges[1] [1]Genetics, Facultad de Ciencias, Universidad de Córdoba, Campus de Excelencia Agroalimentario (ceiA3)., [2]Applied Genetics and Cell Biology, Institute of Microbial Genetics, University of Natural Resources and Life Sciences, Vienna (BOKU).

In fungi, copper acquisition is mainly carried out by specific high-affinity systems consisting of Fre metalloreductases and Ctr copper transporters, which are transcriptionally activated under copper limiting conditions by the copper-sensing transcription factor Mac1. Mac1 is required for full virulence in several animal pathogenic fungi, but its role in plant pathogenesis has not yet been

elucidated. In this work, we show that Mac1 controls the transcriptional response of the vascular wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* to copper limitation and that it is essential for growth under copper limiting conditions. Mac1 inactivation in *F. oxysporum* leads to a complete loss of virulence both on tomato plants and on the animal model *Galleria mellonella*. Importantly, we demonstrate that the role of Mac1 in *F. oxysporum* pathogenicity is exclusively related with the copper uptake mechanisms, since simultaneous overexpression of the high-affinity copper transporter *ctr3* and the metalloreductase *fre9* in a *mac1*Δ background restored growth under copper limitation and pathogenicity. In addition, in planta fungal burden quantification, together with fluorescence microscopy studies, revealed that these copper acquisition systems are required for the progression of the fungus inside the tomato plant roots and the colonization of the xylem vessels.

Besides being essential for growth, copper is also required for the activity of virulence-related enzymes such as superoxide dismutases (SODs). Inactivation of Mac1 in *F. oxysporum* leads to increased sensitivity to oxidative stress, indicating that Mac1 is required for efficient ROS detoxification in *F. oxysporum*. Interestingly, we found that the two SODs of *F. oxysporum* that use copper as a cofactor, Sod1 and Sod5, play a role in virulence. Furthermore, copper-cofactored SOD activity was reduced in *mac1*Δ under copper-limiting conditions. Collectively, our results show that Mac1-dependent copper acquisition is essential for growth and for virulence-related processes during copper limiting conditions ultimately affecting virulence of *F. oxysporum* both on plant and animal hosts.

667A **Feeling the heat: investigating the dual assault of *Zymoseptoria tritici* and heat stress on Wheat (*Triticum aestivum*)** Hannah R Blyth[1], Artin Zarsav[2], Daniel Smith[3], Kostya Kanyuka[4], Kirsty L Hassall[3], Jason J Rudd[5], Richard P Haslam[1] [1]Plant Sciences for the Bioeconomy, Rothamsted Research, [2]Forest Dynamics, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, [3]Intelligent Data Ecosystems, Rothamsted Research, [4]Agricultural Crop Research, National Institute of Agricultural Botany, [5]Protecting Crops and the Environment, Rothamsted Research

As a result of climate change, field conditions are increasingly challenging for crops. While research has shown how higher temperatures affect crop performance, its impact on host-pathogen relationships is unclear. Understanding the effects of combined abiotic and biotic stresses on crop plants and plant-microbial interactions is crucial in developing strategies to improve crop stress tolerance and manage diseases. Cells typically reconfigure metabolism in response to stress, and lipids have emerged as crucial stress response components. Lipids sense, signal, and mitigate temperature elevation effects, and lipid remodelling plays a central role in the plant and fungal response to heat stress. Our study uses a systems approach to examine the *Z. tritici*-wheat model system, combining transcriptomics, functional genomics, lipidomics, and phenotyping to decipher the impact of high-temperature stress on the plant-pathogen interaction.

Microscopy and RNA-Seq analyses confirm that *Z. tritici* responds to high-temperature treatments with morphological and transcriptomic changes. Temperature-related re-configuration of the transcriptome was associated with increased expression of genes on accessory chromosomes and 'accessory' pan-genome-derived genes. There was a down-regulation in metabolism-related gene expression, as indicated by GO enrichment and analysis of KOG classes. The decrease in 'lipid transport and metabolism-related' expression in response to increased temperature indicated large-scale lipid remodelling. Actual changes in lipid content and composition were then validated by LC-MS analysis. Heat-responsive fungal genes and pathways, including scramblase family genes, are being tested by reverse genetics to ascertain their importance for fungal adaption to elevated temperatures.

Based on long-term climate data from Rothamsted Research, an elevated temperature scheme was applied to wheat to study the impact of combined stress on the plant-pathogen interaction. We compared uninfected and infected wheat under typical and elevated temperatures. Our initial analysis of the transcriptomic dataset indicates a delay in the development of *Z. tritici*, followed by its adaptation to the warmer environment. Once the infection was established, the fungus exhibited resilience to the impact of higher external temperatures. Our results indicate that temperature elevations associated with climate change directly impact plant-pathogen interactions. Furthermore, the study demonstrates a need for further detailed understanding to sustain crop resilience.

668A **The SOVIG9 effector protein is involved in host-specific phytoalexin induction by *S. reilianum* in *Sorghum bicolor*** Lukas Dorian Dittiger, Shivam Chaudhary, Jan Schirawski Friedrich Schiller University

Sporisorium reilianum is the causal agent of head smut in sorghum and maize. It exists in two host-specific *formae speciales*, *Sporisorium reilianum* f. sp. *reilianum* (SRS) and *Sporisorium reilianum* f. sp. *zeae* (SRZ) that are pathogenic on sorghum and maize, respectively. While SRS-infected sorghum shows almost no visible symptoms in early infection stages, SRZ-infection induces defence responses including the generation of the reddish-brown phytoalexin (PA) luteolinidin. Using a classical genetics approach we identified an effector protein of SRS, SOVIG9, that suppresses the PA response of sorghum. Transcriptome

comparison of sorghum inoculated with SRS containing or lacking SOVIG9 showed upregulation of cell-surface signalling receptors in the mutant, among which were six wall-associated kinases. To test whether SOVIG9 inhibits a positive feedback loop of the receptor kinases by directly binding to these receptors, we are currently doing targeted yeast two-hybrid analyses. In accordance with the hypothesis of direct interaction with membrane-spanning kinases, sequence analysis of SOVIG9 revealed a putative N-myristoylation site, hinting for plasma membrane localization of the effector. Mutation of the SOVIG9 myristoylation site resulted in loss of effector function. We showed that only C-terminally tagged SOVIG9 fusion proteins localize to the membrane when transiently expressed in *N. benthamiana*. When complementing ΔSOVIG9 strains of SRS we found that C-terminally tagged effector versions lead to strong PA induction in sorghum, suggesting that the tags interfere with virulence function of the effector and that the non-functional effector fusion proteins might be recognized as avirulence proteins in sorghum. Interestingly, SOVIG9 exists in a second, shortened splice variant in SRZ. When this short SRZ splice variant is used to complement the SRS ΔSOVIG9 strains, the fungus induced PA in sorghum. In contrast, the other, longer splice variant, that results in a protein similar to the SRS effector, neither leads to induction of PA nor fully complements the gene deletion phenotype. In summary, we identified a myristoylated SRS effector protein functioning at the sorghum plasma membrane that suppresses PA induction. In contrast, the SRZ ortholog is alternatively spliced and recognized as avirulence protein in sorghum. We propose a model for SOVIG9-mediated host-specific PA induction by *S. reilianum* in *Sorghum bicolor.*

669A **Diversity in DNA-binding effectors across endofungal *Mycetohabitans* spp. supports variable functionality** Sara C. D. Carpenter[1], Adam J Bogdanove[1], Brian Lovett[2], Jason Stajich[3], Jessie Uehling[4], Matt Kasson[5], Morgan E Carter[6] [1]Cornell University, [2]USDA ARS, [3]UC Riverside, [4]Oregon State University, [5]West Virginia University, [6]UNC Charlotte

Mycetohabitans spp. (formerly *Burkholderia*) live intracellularly in the plant and human pathogen *Rhizopus microsporus* and manipulate their fungal host using a type III secretion system. Almost no bacterial type III effector proteins that target fungi are characterized, with the exception of *Burkholderia* TAL-like (Btl) proteins from different *Mycetohabitans* spp. Btl proteins resemble DNA-binding, transcriptional activator-like effectors from plant pathogens and have been found to impact either fungal stress tolerance or fungal development during bacterial colonization. However, the inadequate availability of *Mycetohabitans* spp. genomes has limited our knowledge of the diversity of Btl proteins in sequence and likely function. We sequenced and assembled nine *Mycetohabitans* spp. genomes using long-read PacBio technology, seven newly available and two resequenced. All strains had *btl* fragments, and most had at least one intact *btl* gene. In parallel, we queried ZygoLife project sequencing data of Mucormycota fungi for bacterial contigs with *btl* genes and found an additional 21 accessions with homology to *btl* genes. Our overall data set of almost 50 complete *btl* genes allowed us to distinguish preliminary clades of Btl proteins that have variations in key motifs and DNA-recognition sequence, likely representing functional groupings. Btl proteins in *M. rhizoxinica* were more highly, but not completely, conserved as compared to those from *M. endofungorum*. Btl proteins form a distinct clade from studied TAL effectors from *Xanthomonas* and *Ralstonia* plant pathogens, adding new insight into the evolution of TAL effectors within different symbiotic relationships. By targeting future functional studies to Btl proteins from each of our identified groups, we can assess the breadth of roles that these effectors play in bacterial-fungal symbioses and identify fungal targets.

670A **A dual-function G-protein coupled receptor activates mitochondria and reprograms fungal cells to form adhesive traps for nematode hunting** Xiaodi Hu[1], David Hoffmann[2], Mai Wang[1], Birgit Schreckenberger[1], Maria Stroe[1], Lars Schuhmacher[1], Elke Wohlmann[1], Markus Elstner[2], Reinhard Fischer[1] [1]Dept. of Microbiology, Karlsruhe Institute of Technology, [2]Dept. of Theoretical Chemical Biology, Karlsruhe Institute of Technology

The initiation of developmental processes requires differential gene expression but also metabolic activation. Here we show that genetic and metabolic reprogramming is achieved with a dual-function G-protein coupled receptor (GPCR). The nematode-trapping fungus *Arthrobotrys flagrans* produces adhesive trapping networks upon starvation and in the presence of *Caenorhabditis elegans* and recognizes its prey through nematode-specific pheromones (ascarosides)[1,2]. We show that ascarosides activate the fungal GPCR, GprC, at the plasma membrane and together with the G-protein alpha subunit GasA reprogram the cell. Besides canonical signaling, GprC resides in mitochondria and boosts mitochondrial respiration through the same G-alpha subunit[3]. This is the first example for mitochondrial localization of a GPCR in a lower eukaryote and resembles the cannabinoid receptor CB1 in human, although in that case the mitochondrial receptor inhibits respiration[4]. *C. elegans* SRBC66 and GPCRs of many fungi are predicted for dual localization suggesting broad evolutionary conservation of the mechanism. A SRBC64/66-GprC chimeric protein was functional in *A. flagrans,* and *C. elegans* SRBC64/66 and DAF38 share ascaroside-binding sites with the fungal GprC receptor despite low overall sequence conservation, suggesting 400-million-year convergent evolution of the receptors.

1 Hsueh, Y. P. *et al.* Nematode-trapping fungi eavesdrop on nematode pheromones. *Curr Biol* **23**, 83-86 (2013).

2 Yu, X. *et al.* Fatal attraction of *Caenorhabditis elegans* to predatory fungi through 6-methyl-salicylic acid. *Nat Commun* **12**, 5462 (2021).

3 Hu, X. *et al.* A dual-function G-protein coupled receptor activates mitochondria and reprograms fungal cells to form adhesive traps for nematode hunting. *Nat Microbiol*, *in revision.* (2024).

4 Hebert-Chatelain, E. *et al.* A cannabinoid link between mitochondria and memory. *Nature* **539**, 555-559 (2016).

671A **FLO1 proteins and CDAs mediate nematode egg attachment and infection by *Pochonia chlamydosporia*.** Carla Mariel Berosich[1], Miguel R Valverde-Urrea[1], Javier R Espinosa-Manzano[2], Luis Vicente R Lopez-Llorca[1], Federico R Lopez-Moya[1] [1]Marine Science and Applied Biology, University of Alicante, [2]fisiology. genetics and microbiology., University of Alicante

The nematophagous fungus *Pochonia chlamydosporia* (Pc) parasitizes nematode eggs. The adhesion and penetration are a key point during Pc nematode-eggs infection. The infection process is mediated by proteins and glicopolysaccharides secretions and transformation chitin into chitosan. The fungal cell wall plays an important role in the host adhesion and infection process, as well as key proteins and enzymes extracellular or present in the cell wall and membrane, such as adhesins. The fungal cell wall is mainly composed of beta 1-3 glucans (30-80%) and chitin or chitosan (1-15%). Phylogenetic analysis and modeling of FLO1 flocculation proteins and chitin deacetylase enzymes (CDAs) and their gene expression in the presence of *Meloidogyne javanica* eggs and chitosan were performed to determine their role during adhesion and penetration. To analyze the mechanism of action of Pc123 parasitism, we observed the behavior of Pc123 conidia and protoplasts with *Meloidogyne javanica* eggs. Then we also characterize the role of FLO1 during Pc appressoria differentiation. In turn, the CRISPR/Cas9 ribonucleoprotein (RNPs) technique and uracil auxotrophy as a selection marker were used to transform Pc123 protoplasts. Finally, uracil auxotrophic Pc123 transformants were used to generate knockout mutants in target (CDA and FLO1) genes important for the mechanism of adhesion and infection of nematode eggs by Pc123.

672A **From multi- to single-mycoviral infection in the plant pathogenic fungus *Botrytis cinerea*** Julián Méndez-García[1], Julio L Rodríguez-Romero[1,2], María A Ayllón[1,2] [1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA/CSIC), Pozuelo de Alarcón, Madrid, SPAIN, [2]Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), Madrid, Spain

The u se of mycoviruses has been explored as an innovative strategy for the biological control of fungal infections, as it was discovered that they can decrease the virulence of the fungi on their hosts. *Botrytis cinerea* is one of the most important plant-pathogenic fungi, as it is responsible of the gray mold disease in more than 200 crops worldwide. In a previous study, an RNA-Seq analysis allowed the detection of the mycovirome of *B. cinerea*, in pools of 248 field isolates, collected from vineyards in different regions of Europe (Italy and Spain).

The RNA-Seq analysis of the mycovirome of three independent field isolates allowed us to demonstrate that multi-mycoviral infection is a common phenomenon in natural populations of *B. cinerea*. This analysis showed the presence of more than 20 mycoviruses infecting one single field isolate.

To study the independent effect of mycoviruses on a fungus, it is necessary to obtain fungal strains infected with a single mycovirus, and, then, analyse the effect of this infection compared to the isogenic line free of this mycovirus infection. In this sense, we combined different mycoviral curing strategies, including single-spore or protoplast isolation, co-culture transmission and hyphal tipping. These strategies were performed consecutively from the three independent field isolates.

The mycovirome of the distinct resulting cured strains was also analyzed in separate steps of the curing pipeline, through RNA-Seq analysis and detection by PCR amplification. These curing steps reduced the mycoviral content to over ten mycoviruses in a single-spore strain, under ten in a strain after co-culture transmission, and one single-mycovirus infection after hyphal-tipping.

In conclusion, the newly obtained strains showed a reduction in the number of infecting mycoviruses. Additionally, this analysis also demonstrates that multimycoviral infection is frequently maintained in *B. cinerea*, pointing out the difficulties in obtaining a singlemycoviral-infected fungal strain. Despite the pointed difficulties of the process, we successfully navigated the way from a multi- to a single-mycoviral infected *B. cinerea* strain. Interestingly, phenotypic and virulence analysis showed differences between isogenic strains that only varies in mycoviral population.

673B **Interactions between Polyextremotolerant Fungi and Photoautotrophs are Enhanced by Excreted Melanin** Erin C Carr[1], Quin Barton[1], Wayne R Riekhof[1], Steven D Harris[2] [1]School of Biological Sciences, University of Nebraska-Lincoln, [2]Plant Pathology, Entomology, and Microbiology, Iowa State University

Polyextremotolerant fungi are a paraphyletic group of melanized fungi that can withstand multiple extreme conditions simultaneously. *Exophiala viscosa* is a newly characterized polyextremotolerant fungus isolated from a biological soil crust community in the Canadian Rockies. This fungus grows in xeric, nutrient deplete environments implying highly flexible metabolism and the potential to form lichen-like mutualisms with nearby algae and bacteria. However, the exact ecological niche and interactions between this fungus and its surrounding community is not well understood. A combination of whole genome sequencing, analysis of melanin regulation, and microbial interaction experiments have been performed to fully characterize *E. viscosa* and help decipher their fundamental niche within the biological soil crust consortium. Our results reveal that *E. viscosa* excretes melanin into its environment, which can provide increased abiotic resistance, and potentially a carbon source, to the biological soil crust community. We have been able to show that in a carbonless and nitrogenless environment, a tripartite culture of *E. viscosa*, algae, and cyanobacteria are capable of survival and form extensive clumps. However, an *E. viscosa pks1* deletion mutant that cannot produce melanin does not support tripartite interactions, primarily lacking in the essential cell clumping necessary for symbiosis. This study therefore provides new insights into the regulation of melanin production in polyextremotolerant fungi, and novel data about how polyextremotolerant fungi interact with algae and cyanobacteria.

674B **Insights into establishment and maintenance of lichen communities through structural bioinformatics and synthetic microbial consortia** Max Heinen[1], Vivien Rosenthal[2], Ciaran Kelly[3], Lukas Hüttebräucker[4], Björn Usadel[2], Bart Thomma[3], Markus Pauly[4], Florian Altegoer[1] [1]Institute of Microbiology, Heinrich-Heine University, [2]Institute of Biological Data Science, Heinrich-Heine University, [3]Institute for Plant Sciences, University of Cologne, [4]Institute for Plant Cell Biology and Biotechnology, Heinrich-Heine University

Lichens are among the most ancient and fascinating examples of complex microbial communities known as pioneers that can establish themselves in extreme environments. In lichens, an algal or cyanobacterial photobiont tightly associates with a fungal mycobiont to form complex morphological structures. These structures are formed by the mycobiont and provide shelter but the fungus also takes up essential nutrients and water. The photobiont in turn performs photosynthesis and supplies the mycobiont with macronutrients such as carbon. Recent findings have demonstrated that additional yeasts and bacterial species are also part of the lichen community. While photobionts can exist independently, the mycobiont depends on the photosynthetic partner and is rarely observed in a free-living form. It is therefore likely that the mycobiont drives the community composition. Despite intensive research on lichen biology, molecular mechanisms that govern the establishment and maintenance of these symbioses presently remain unknown.

In our project, we focus on *Peltigera* cyanolichens and established an Oxford Nanopore based “metagenomic” sequencing pipeline. We extracted DNA from *Peltigera spp*. samples collected from different sampling sites near Düsseldorf and assembled a first high-quality genome of *Peltigera rufescens*. Based on the genomic information obtained for the *P. rufescens* mycobiont, we have established a computational pipeline to predict potential candidate effector proteins. By implementing structure prediction tools, we classify and compare these proteins to gain insights into the molecular repertoire of the lichen fungus. In the future, we will expand our approach, include additional *Peltigera* species but also outgroups of non-lichenized fungi enabling comparative analyses. Ultimately, we aim to unravel the molecular mechanisms underlying how a lichen community is shaped and maintained.

Another key feature of lichens is the defined structural architecture and close association of the different species. We investigate fungal carbohydrate-binding proteins that allow establishment and maintenance of intercellular contacts between mycobiont and photobiont. The primary focus is on lichen lectins that are exploited through fungal surface display techniques. We currently establish a synthetic community using *Ustilago maydis*, *Saccharomyces cerevisiae* together with algal or cyanobacterial partners. An important aspect here is the establishment of a surface display system for *U. maydis*, that enables us to screen for potential lectins conferring a tight association in our synthetic communities.

The combined approach allows us to identify the key players involved in the symbiotic networking of the mycobionts with their community partners and gain insights into the molecular mechanisms underlying the initiation and maintenance of these mutually beneficial interactions.

675B **The Pec effector complex of *Ustilago maydis* interferes with carbohydrate metabolism in maize** Yoon Joo Lee[1], Sara Stolze[2], Hirofumi Nakagami[2], Gunther Doehlemann[1] [1]University of Cologne, [2]Max-Planck Institute for Plant Breeding Research

The corn smut fungus *Ustilago maydis* secretes a repertoire of effectors in a spatiotemporally regulated manner to induce the formation of plant tumors. *U. maydis* manipulates host's metabolic processes, redirecting starch and glucose accumulation towards growing tumors, thereby turning them into strong sinks. A comprehensive cell-type-specific transcriptome profiling of *U. maydis* during tumorigenesis revealed a set of highly upregulated effectors involved in hypertrophy. Here, we show that effectors Pec (Primary metabolite Effector Complex) 1-3 are hypertrophy-associated effectors, being essential for full virulence of *U. maydis* and interacting with each other inside the host, forming a potential complex. Moreover, we observed a reduction and mislocalization of starch accumulation in plants infected with *U. maydis* Pec single-, double-, and triple knock-out mutants, indicating that Pec effectors act cooperatively to disrupt the normal metabolic processes in maize. To gain insight into the potential cooperative mechanism of Pec effectors within the host, we performed a large-scale mass spectrometry analysis and identified Pec1 as the central Pec-effector. Notably, Pec1 interacts with maize SnRK1 (SNF1–related protein kinase 1), a key mediator of cellular energy and nutrient homeostasis activated by phosphorylation under energy deprivation. Quantitative phosphoproteomics analysis of Pec1 knock-out mutants compared to wild-type infected plants revealed that SnRK1, TPS (trehalose-6-phosphate synthase), and other glucose-stimulating enzymes are highly phosphorylated in response to wild-type infected plants. These results suggest that Pec1 disrupts the antagonistic relationship between SnRK1 and T6P (trehalose-6-phosphate) through TPS, leading to reduced uptake and increased extracellular sugars in *U. maydis* triggered tumor formation.

676B **LPMO (-like) proteins and their role for *Cryptococcus neoformans* growth and virulence** Corinna Probst[1], Magnus Hallas-Møller[2], Katja Johansen[3], James Andrew Alspaugh[1] [1]Medicine, Duke University, School of Medicine, [2]Dept of Plant and Environmental Science, University of Copenhagen, [3]University of Copenhagen

Fungi need to adapt quickly to environmental stresses to thrive. The fungal cell wall, which supplies support and integrity to the cell, is an essential compartment to react and interact with the surrounding environment. Rapid changes within the carbohydrate composition and architecture occur in response to environmental stresses. Lytic polysaccharide monooxygenases (LPMOs) are mononuclear copper-enzymes, secreted by microbes to assist in the first steps of remodeling and degradation of cell wall carbohydrates. While recent studies have established an important role for LPMOs in plant pathogenic microbes, studies about the role of LPMOs in human pathogenic microbes are scarce and limited to bacterial LPMOs.

The human fungal pathogen *Cryptococcus neoformans* (Cn) encodes for 4 potential LPMO or LPMO-like proteins. Initial phenotypic analysis of loss-of-function mutant strains, for 3 out of the 4 predicted LPMO-encoding genes, identified one gene, which encodes for the copper binding and release protein Cbi1, to function in copper import as well as maintaining cell wall integrity during low copper stress. Unlike *CBI1*, none of the other LPMO genes are regulated by copper. Interestingly, further analysis of their transcriptional profiles identified the *CEL1* gene to be up-regulated during a murine lung infection, as well as in conditions associated with stresses occurring within the mammalian host (high temperature and alkaline pH). In line with this finding, the *cel1Δ* mutant strain is avirulent in a murine model of infection. Downstream analysis of virulence associated phenotypes identified the *CEL1* gene to be required for thermotolerance as well as cell wall integrity, and efficient cell cycle progression in presences of host mimicking stresses. Cel1 is secreted but retained in its own cell wall. Based upon those findings, we propose that, in contrast to other known LPMOs which are mostly targeting exogenous carbohydrates, Cel1 likely promotes intrinsic fungal cell wall remodeling events essential for adaptation to the host environment.

Taken together, our recent work has demonstrated that 2 out of 4 encoded LPMO or LPMO-like proteins have important roles in adaptation during environmental stresses, showing the importance of LPMO and LPMO-like proteins for *Cn* growth and virulence.

677B ***Baf* against the wall: elucidating mechanisms of oxygen-driven adaptations in the human fungal pathogen *Aspergillus fumigatus*** Angus Johnson[1], Nicole E Kordana[1], Kaesi A Morelli[1], Caitlin H Kowalski[2], Robert A Cramer[1] [1]Dartmouth College, [2]University of Oregon

The ability to grow during oxygen limitation (hypoxia) is a critical virulence determinant in the human fungal pathogen *Aspergillus fumigatus*. Yet, the mechanisms facilitating fungal growth and viability in hypoxic environments, such as those that arise during infection, remain to be fully defined. An experimental evolution approach, performed in a low oxygen atmosphere to elucidate such mechanisms, surprisingly linked filamentous fungal colony morphology with low oxygen fitness. The evolved, hypoxia-fit strain produced colonies with a broad rim of vegetative growth and furrows radiating from the center of the colony. This hypoxia-adapted morphology was termed H-MORPH. Importantly, H-MORPH isolates are often recovered from aspergillosis patient

samples, but the implication of H-MORPH in the prognosis, diagnosis, and treatment of *Aspergillus* infections remains unclear. Both *in vitro*-generated and patient-derived H-MORPH strains are more fit in hypoxia, have altered biofilm architecture, decreased surface adherence, altered cell wall composition, and increased virulence in a murine invasive aspergillosis model. To define the implications of H-MORPH and why it emerges in clinical isolates, we are utilizing a family of recently evolved genes whose expression is sufficient to induce H-MORPH, the biofilm architecture factor (*baf*) genes. However, the mechanisms through which *baf* genes mediate the emergence of H-MORPH is unclear. By defining the genetic pathways that give rise to H-MORPH, we expect to delineate the mechanisms that contribute to the phenotypes that H-MORPH strains exhibit. We are currently conducting co-immunoprecipitation experiments with epitope tagged Baf proteins to identify potential interaction partners. Additionally, we are performing random mutagenesis experiments to identify mutations that suppress H-MORPH, and targeted genetic approaches based on gene candidates identified in clinical H-MORPH isolates to define possible downstream pathways. By defining the genetic pathways that give rise to H-MORPH via *baf* function, we expect to delineate the mechanisms that contribute to the phenotypes H-MORPH strains exhibit and explain the emergence of this morphotype in clinical *A. fumigatus* isolates.

678B **Herbicides as fungicides: Targeting heme biosynthesis in the maize pathogen *Ustilago maydis*** Djihane Yushrina Damo o[1], Matthias Kretschmer[2], James Kronstad[2] [1]Microbiology and immunology, University of British Columbia, [2]Microbiology and Immunology, University of British Columbia

Pathogens must efficiently acquire nutrients from host tissue to proliferate, and strategies to block pathogen access therefore hold promise for disease control. In this study, we investigated whether heme acquisition is an effective target for ablating the virulence of the phytopathogenic fungus *Ustilago maydis* on maize plants. We first constructed heme auxotrophs of the fungus by placing the gene encoding uroporphyrinogen decarboxylase (UROD) for heme biosynthesis under the control of nitrogen or carbon source-regulated promoters. These strains were unable to cause disease in maize seedlings thus demonstrating the inability of the fungus to acquire sufficient heme from host tissue to support proliferation. Subsequent experiments characterized the role of clathrin mediated endocytosis (CME) in heme uptake, the susceptibility of the fungus to heme toxicity, as well as the transcriptional response to exogenous heme. Later RNA-seq experiments identified a candidate ABC transporter, which was upregulated 100-fold in the regulatable strain in the presence of hemin. The ABC mutant showed differential susceptibility to heme in addition to other cell stressors. In addition, the cell surface-located ABC transporter was shown to play a role in melanization *in vitro* but however not *in vivo*. Finally, the importance of heme biosynthesis for *U. maydis* pathogenesis allowed us to demonstrate that the well-characterized herbicide BroadStarä that contains flumioxazin, an inhibitor of heme biosynthesis, was an effective antifungal agent for blocking disease on maize. Thus, repurposing herbicides for which resistant plants are available may be an effective strategy to control pathogens and achieve crop protection.

679B **Defining the Genetic Mechanisms of a Dissemination Prone Morphotype of *Cryptococcus neoformans*** Christian Moreau, Jessica Brown School of Biological Sciences, University of Utah

Systemic *Cryptococcus neoformans* infections are a serious public health concern, primarily affecting immunocompromised individuals and annually causing ~200,000 deaths worldwide. Infections occur following inhalation of yeast or spores and subsequent colonization of the host lungs. In serious cases, dissemination from the lungs to extrapulmonary organs such as the brain, drives mortality by causing meningoencephalitis. These infections are difficult to treat due to limited drugs and other factors, highlighting the growing need to understand pathogenic mechanisms.

During infection, *C. neoformans* exhibits vast size heterogeneity; ranging from 2-100µm in diameter. While highly encapsulated cells dominate infection early on in the lungs, smaller cells ranging between 4-10µm in diameter being to emerge as disease progresses. We have termed these small cells as “seed cells” due to their enhanced dissemination and ability to seed extrapulmonary organs. We predominantly find seed cells in extrapulmonary organs as they are able to escape from the host lungs and disseminate significantly faster than larger cells. This implicates seed cell emergence with extrapulmonary dissemination and consequently mortality. Despite this, the genetic mechanisms giving rise to these seed cells remains elusive.

To identify genes important for seed cell formation, we have developed *in vitro* techniques to induce seed cells. We have used these techniques in conjunction with a deletion mutant library to screen for genes necessary for seed cell formation. We have found that deleting the cryptococcal transcription factor Usv101 results in a significant reduction in the number of seed cells. *In vivo* murine infections with *Δusv101 C. neoformans* results in a significantly longer time-to-endpoint compared to mice infected with wild type *C. neoformans*. In these infections, *Δusv101 C. neoformans* cells are constitutively larger than wild type *C. neoformans* cells in all organs. This indicates that the delayed mortality of mice infected with *Δusv101 C. neoformans* may be due to delayed extrapulmonary dissemination as a result of their inability to produce seed cells *in vivo*. In the future, we plan to

investigate this by looking at dissemination profiles of *Δusv101 C. neoformans* at earlier timepoints in infection. In addition, we plan to understand the transcriptional network of Usv101 and how it influences seed cell formation.

Dissemination of *C. neoformans* from the host lungs to extrapulmonary organs is still poorly understood. The objective of this research is to determine the genetic mechanisms driving *C. neoformans* seed cell formation to further elucidate dissemination mechanisms driving mortality.

680B **Back to the roots: A powerful plant-fungus interaction system** Krizstina Kolláth-Leiß[1], Urska Repnik[1], Hannes Winter[1], Frank Kempken[1,2] [1]Christian-Albrechts-Universität, [2]Christan-Albrechts-Universität zu Kiel

Aside of being saprophytes, many fungi play a dynamic role in complex relationships with plants. These connections show a great deal of diversity, including mutualistic and pathogenic linkages. When pathogenic fungi take up biotrophic or hemibiotrophic lifestyles, they harm their plant counterparts while taking advantage of the nutrients in their host. On the other hand, mutualistic interactions such as mycorrhiza (MR) produce benefits that are reciprocal for plants and fungi. Mycorrhizal associations occur when filamentous fungi invade plant root systems in an effort to create a stable and mutually beneficial coexistence.

Our research takes advantage of two well-known model organisms, i.e. the grass *B. distachyon* and the ascomycete *Neurospora crassa* to investigate plant-fungal interactions in a novel and- reliable interaction, which makes it easy to conduct in-depth studies in cell biology and genetics. Although *N. crassa* is a saprophytic fungus that has been extensively investigated, little is known about its ecological characteristics. Conversely, *B. distachyon*, a sweet grass closely related to significant crops, demonstrates remarkable ecological flexibility and participates in a variety of fungal interactions, encompassing both mutualistic and harmful associations.

In-depth microscopic investigation was conducted utilizing electron, fluorescence, and confocal laser scanning microscopy to acquire a more thorough look at the interaction of *N. crassa* and *B. distachyon* roots. Our data indicate that most plant root tissue is unimpaired by fungal invasion, but single hyphae showed a clear growth within the apoplastic space. Even more impressive, we identified accumulation of fungal hyphae in some root cortex cells. These infected cells were often surrounded by non-infected, living neighbor cells, however in some cases whole cell layers of cortical cells showed fungal accumulation in a very similar manner. Our observations for the first time that *N. crassa* is interacting endophytically with plant roots. The consistent connection between the fungus and *B. distachyon* may suggest that the fungus naturally associates with Poaceae. These findings offer the scientific community new perspectives for examining the fungus' natural life cycle in more detail.

681B **The phosphorylation landscape of infection-related development by the rice blast fungus** Frank L.H. Menke[1], Neftaly Cruz-Mireles[1], Miriam Osés-Ruiz[2], Paul Derbyshire[1], Clara Jégousse[1], Lauren S. Ryder[1], Mark Jave Bautista[1], Jan Sklenar[1], Bozeng Tang[1], Xia Yan[1], Vincent Were[1], Dan MacLean[1], Nicholas J. Talbot[1] [1]The Sainsbury laboratory, [2]Instituto de Agrobiotecnología, Public University of Navarre

Fungal pathogens continue to pose substantial threats to global food security, causing some of the most devastating crop diseases worldwide. We present a pioneering investigation into the infection-related development of the rice blast fungus, *Magnaporthe oryzae*, employing a quantitative mass spectrometry-based phosphoproteomic approach. Our study maps 8,005 phosphosites on 2,062 fungal proteins, providing a detailed landscape of the phosphorylation events that orchestrate plant infection. This reveals a profound re-wiring of phosphorylation-based signalling cascades during fungal infection, shedding light on the dynamic molecular responses elicited by *M. oryzae*. To unravel the broader significance of these findings, we undertook a comparative analysis of phosphosite conservation across 41 fungal species, revealing phosphorylation signatures linked to plant-associated fungal lifestyles, biotrophic and hemibiotrophic fungal infections. As the Pmk1 MAP kinase is a key orchestrator of plant infection in many plant pathogenic fungi, we then used parallel reaction monitoring to identify Pmk1 MAPK substrates. Our investigation defines 32 putative substrates of Pmk1, revealing a complex network of regulatory interactions. This led to identification of a novel regulator, Vts1, which undergoes Pmk1-dependent phosphorylation and is indispensable for the manifestation of rice blast disease. Vts1 is a SAM domain protein that plays an essential function in appressorium morphogenesis, septin aggregation and re-polarisation. Using an analogue-sensitive Pmk1 mutant, parallel reaction monitoring, and subsequent site-specific mutagenesis, we show that a single Pmk1-dependent phosphorylation event is necessary for its function as a virulence determinant during rice blast disease.

682B **Knottins are a new family of secreted virulence factors that are required for the virulence of the intracellular fungal pathogen *Histoplasma capsulatum*** Rosa A Rodriguez[1], Dinara Azimova[1,2], Bevin English[1], Jane Symington[1], Sarah Gilmore[1,3], Mark Voorhies[1], Anita Sil[1] [1]UC San Francisco, [2]Assembly Biosciences, Inc, [3]AlloVir

Intracellular pathogens secrete virulence factors to aid in replication and survival. Although many paradigms exist for bacterial secreted effectors, much less is known about fungal secreted factors that modulate pathogenesis in mammalian hosts. *Histoplasma capsulatum* (*Hc*) is a thermally dimorphic human fungal pathogen that grows within macrophages and triggers host cell death through the induction of the Integrated Stress Response (ISR). The mechanisms by which *Hc* induces host cell death are still poorly understood. In this work, we take a bioinformatic and genetic approach to identify and study novel *Hc* effectors. Known fungal effectors in plant pathogenic fungi tend to be small, secreted, cysteine-rich and highly expressed in the pathogenic form of the organism. Using these criteria, we identified multiple putative *Hc* effectors, some of which contain homology to the cysteine knot gene family called knottins. Knottins contain a 6-cysteine motif that creates three disulfide bridges forming a stable knot structure. We wrote a naive algorithm to mine the *Hc* genome and identified a total of 26 putative knottins. Furthermore, we observed a unique and massive expansion of putative knottin genes in all *Histoplasma* species; this expansion was not observed across the fungal kingdom. We characterized four knottins named *KNOT1*, *KNOT2*, *KNOT3*, and *KNOT4* and discovered that all four proteins localize to the host cytosol during *Hc* infection of macrophages. Using CRISPR-Cas9, we generated deletion mutants and discovered that *KNOT1*, *KNOT3*, and *KNOT4* were all required for both optimal *Hc* intracellular growth and lysis of macrophages. In contrast, *KNOT2* was dispensable for intracellular growth but required for optimal macrophage lysis. Intriguingly, these knottin proteins were not required to stimulate the ISR, which was previously shown to be required for optimal macrophage lysis. Finally, we characterized *KNOT2* and *KNOT4* in the mouse model of infection. Mice failed to succumb to infection with mutant *Hc* strains lacking *KNOT2* or *KNOT4*. Interestingly, the *knot4Δ* mutant displays a modest decrease in fungal burden in the mouse, whereas the *knot2Δ* mutant can grow essentially to wild-type levels. These data indicate that *KNOT2* and *KNOT4* are dispensable for fungal burden and may instead manipulate other host processes such as the immune response. Taken together, this work identifies a new family of virulence factors and highlights how *Hc* is using a varied arsenal of effectors to cause disease.

683B **Genomic signature of host specialization in the anthracnose pathogen *Colletotrichum lupini*** Sabrina Sarrocco[1], Sioly Becerra[2], Andrea Menicucci[3], Daniele Da Lio[1], Flora Pensec[4], Elena Baraldi[3], Antonio Prodi[3], Michael R. Thon[2], Serenella A. Sukno[2], Giovanni Vannacci[1], Gaetan Le Floch[4], Riccardo Baroncelli[5] [1]University of Pisa, [2]University of Salamanca, [3]University of Bologna, [4]University of Brest, [5]Dept of Agri-Food Science and Technology (DISTAL), University of Bologna

Colletotrichum spp. are among the most important plant pathogens of numerous economically important crops worldwide. While some species infect a wide diversity of hosts, some show a strong host specialization. The biological diversity of *Colletotrichum* and the presence of very closely related species with different characteristics such as host range makes it an excellent model to investigate genomic signatures associated with host specialization.

An example is provided by Colletotrichum lupin*i*, a member of the acutatum species complex that contrasts with other members of the latter by its host specificity. We sequenced several strains covering the global distribution and the diversity of this pathogen as well as all closely related species. A comparative genomics approach reveals one species-specific genome region present in all isolates sequenced of *C. lupini* but absent in any other *Colletotrichum* spp. analyzed. The identified region is between 80 and 100 Kb and is characterized by a high concentration of transposable elements and the highest Tajima's D value across the genome suggesting that this region is under strong balancing selection. This region harbors 26 genes, 11 of which have been predicted to form a secondary metabolite biosynthetic gene cluster. Evolutionary analyses suggested that these genes have been acquired horizontally by a distantly related fungus.

Disruption of the metabolic pathway (by knocking out the hybrid NRPS-PKS gene), and confirmed by the lack of several metabolites, renders the strain less virulent than the wild type, highlighting its role in plant interactions. Our findings indicate that horizontal acquisition of a secondary metabolite biosynthetic gene cluster provides a means by which plant pathogenic fungi may reshape their host range.

684B **The characterization of antifungal resistance in pathogenic Candida to improve development of resistance biomarkers and species identification.** Karolina Czajka[1], Krishnan M Venkataraman[2], Danielle Brabant-Kirwan[3], Stacey A Santi[3], Chris Verschoor[3], Vasu D Appanna[2], Ravi Singh[4], Deborah P Saunders[5], Sujeenthar Tharmalingam[4] [1]Medical sciences, NOSM University, [2]Laurentian University, [3]HSNRI, [4]NOSM University, [5]Health Sciences North Sudbury

Candidiasis is a highly pervasive fungal infection posing major health risks globally, especially for immunocompromised populations. Pathogenic Candida yeast species have evolved intrinsic and acquired resistance to a variety of antifungal

medications. This study aimed to characterize the resistant samples among a sample set of 80 clinical isolates retrieved from oral candidiasis infections in patients aged 65 or over at the time of prescription and two weeks after treatment. The predominant species observed were C. albicans (n = 57), N. glabrata was the second most common (n = 15), and there were a few C. dubliniensis, P. kudriavzevii, C.parapsilosis and C. tropicalis. Fluconazole was prescribed as the first line treatment for most cases. The top 8 resistant samples identified in susceptibility testing were: one P. kudriavzevii (fluconazole resistance), one C. tropicalis (azole resistance), one C. albicans (azole resistance) and five C. glabrata (one echinocandin resistant; two azole resistant and two azole susceptible-dose dependent resistance). Another study aim is to determine differential gene expression in resistant samples with whole transcriptome RNA sequencing, followed by sequencing of relevant DNA regions to detect any genetic alterations. Resistance can be conferred via gain-of-function mutations in target pathway genes, drug efflux pumps, or transcriptional regulators of the first two. Additionally, the development of point-of-care testing (POCT) for species identification would be helpful in candidiasis management given the intrinsic fluconazole resistance of the pathogenic species C. auris, N. glabrata and P. kudriavzevii. The unique microbial genetic sequences of potential biomarkers will be used to customize a general microbial detection platform. This system has already been developed and augmented with advances in CRISPR diagnostics and isothermal amplification for a simple lateral-flow assay readout.

685B **A set of effector proteins modulate host-specific virulence of *Sporisorium reilianum* f. sp. *reilianum*** Shivam Chaudhary, Lukas Dorian Dittiger, Jan Schirawski Dept of Genetics, Friedrich Schiller University

Sporisorium reilianum is a biotrophic basidiomycetous fungus and the causal agent of head smut disease in maize and sorghum. This soil-borne pathogen exists in two formae *speciales*, *Sporisorium reilianum* f. sp. *reilianum* (SRS) and *Sporisorium reilianum* f. sp. *zeae* (SRZ), that can penetrate and proliferate within the leaves of both plants but can cause disease only in sorghum or maize, respectively. To understand the mechanism of host-specific plant infection, we used a classical genetics approach of hybridization combined with next-generation sequencing and GWAS analysis. This led to the identification of a nine-effector gene cluster weakly conserved between SRS and SRZ and whose parental origin from SRS is linked to capacity of spore formation in sorghum. Effector genes were upregulated during the initial phase of biotrophy as determined by qRT-PCR. Complete-cluster deletion mutants of SRS showed substantially reduced virulence on sorghum. We found that nearly every single effector protein is necessary for full virulence of SRS on sorghum as shown by virulence analysis of gene deletion strains, contains a fully functional secretion signal peptide as determined by yeast secretion trap assay and localizes to the plant cytoplasm when transiently expressed in *Nicotiana benthamiana*. We selected the two effectors with strongest effect on virulence for further analysis. We will present the results of comparative RNA sequencing of wildtype and gene deletion strain-inoculated sorghum leaves. In addition, we are searching for putative interaction partners using yeast two-hybrid screening of a cDNA library generated from *S. reilianum*-infected sorghum plants. Based on these data, we will present a model of the role of the effectors in host-specific virulence of *S. reilianum* f. sp. *reilianum* on sorghum.

686B **Phosphate limitation remodels the cell wall to influence caspofungin tolerance, capsule attachment and titan cell formation in *Cryptococcus neoformans*** Xianya Qu[1], Kabir Bhalla[2], Linda C Horianopoulos[3], Guanggan Hu[1], Armando Alcazar Magaña[4], James W Kronstad[1] [1]Microbiology and Immunlogy, Michael Smith Laboratories, University of British Columbia, [2]Michael Smith Laboratories, University of British Columbia, [3]Wisconsin Energy Institute, University of Wisconsin–Madison, [4]Life Sciences Institute, University of British Columbia

There is a pressing need for new antifungal drugs to treat invasive fungal diseases. Unfortunately, the echinocandin antifungal drugs that are fungicidal against other important fungal pathogens are ineffective against *Cryptococcus neoformans*, the causative agent of life-threatening meningoencephalitis in immunocompromised people. Contributing mechanisms for echinocandin tolerance are emerging for *C. neoformans* with connections to calcineurin signaling, the cell wall and membrane composition. In this context, we discovered that phosphate limitation impairs the tolerance of *C. neoformans* to the echinocandin caspofungin. Our previous analysis of phosphate uptake in *C. neoformans* revealed that mutants lacking three phosphate transporters are impaired for formation of the polysaccharide capsule and virulence in mice. In this study, we investigated the underlying mechanisms and found that phosphate limitation results in cell wall changes that result in capsule shedding. This explains the reduced siz of capsule on mutants lacking phosphate uptake. We also found an influence on the calcineurin pathway including calcium sensitivity and endoplasmic reticulum in response to phosphate limitation. Furthermore, we found membrane and lipid composition changes consistent with the role of phosphate in phospholipid biosynthesis and consistent with previous studies implicating membrane integrity in caspofungin tolerance. Finally, we identified a contribution of phosphate to titan cell formation, a cell type that displays modified cell wall and capsule composition. Overall, our analysis reinforces the myriad of functions of phosphate including a major contribution to cell wall structure to impact capsule attachment, a key virulence factor. The impact

on the cell wall also established an important link with caspofungin tolerance and this finding may open new avenues for overcoming tolerance through combinations of drugs.

687B **Metabolic Plasticity Contributes to Structure and Function of *Aspergillus fumigatus* Biofilms** Katie Quinn[1], Charles Puerner[2], Sandeep Vellanki[2], Nicole E Kordana[2], Caitlin H Kowalski[3], Robert A Cramer[2] [1]Microbiology and Immunology, Dartmouth College, [2]Dartmouth College, [3]University of Oregon

Aspergillus fumigatus can cause invasive aspergillosis (IA) in immunocompromised individuals by forming complex, multinucleated biofilms. These biofilms, a known virulence factor, contribute to the chronic nature of these infections in part through mediating substantial resistance to contemporary antifungal drugs. Adaptations to the infection environment, specifically to reduced oxygen availability, contribute to the phenotypic heterogeneity of these biofilms. Strikingly, *A. fumigatus* experimentally evolved in a low-oxygen environment forms a distinct biofilm morphology termed H-MORPH. Intriguingly, H-MORPH strains are recovered from patient samples. H-MORPH strains possess increased growth in low-oxygen environments and virulence in murine models of IA. How H-MORPH mediates these important phenotypes is ill-defined.

H-MORPH strains have increased growth on alternative carbon sources found at the site of infection including acetate, lactate, and ethanol, all products of eukaryotic cell low oxygen metabolism. These data suggest metabolic rewiring contributes to their altered biofilm structure and function. As such, we hypothesize that these H-MORPH strains are partially carbon catabolite de-repressed.

To test this hypothesis, we utilized the CRISPR Cas-9 system to generate alcohol (*alcA*) and aldehyde (*aldA*) dehydrogenase null mutants in our wild-type and isogenic H-MORPH strain background. As expected, all null mutants have a significant growth defect when ethanol is the sole carbon source. Interestingly, H-MORPH biofilm architecture is altered with the loss of *alcA* and *aldA*, but not in wild-type. Consistent with an important role for ethanol catabolism in H-MORPH biofilm form and function, null mutants also display adherence defects predicted to impact the host-fungal interaction *in vivo.* Ongoing experiments include testing how these alcohol and aldehyde dehydrogenase mutants alter disease initiation and progression in murine models of IA. Given the clinical relevance of HMORPH, understanding the role of carbon catabolism regulation in virulence is important for understanding disease initiation and progression.

688B **Atpenin A5 - Elucidating the function of a succinate dehydrogenase inhibitor produced by the poplar pathogen *Sphaerulina musiva*** Cole Sawyer[1,2], Kelsey Sondreli[3], Tomas Rush[1,2], Sameer Mudbhari[1,2], William Alexander[2], Carrie Eckert[1,2], Jared LeBoldus[3], Paul Abraham[1,2], Joanna Tannous[1,2] [1]University of Tennessee Knoxville, [2]Oak Ridge National Lab, [3]Oregon State University

Production of poplar trees for biofuel production is a vital mission of the Dept of Energy which has largely been hampered by fungal infection. *Sphaerulina musiva*, the causal agent of leaf spot and stem canker disease, is the most economically impactful poplar pathogen. The traditional geographic range of this fungus is limited to the eastern coast of the United States, but breeding efforts of poplar strains has led to the anthropogenic movement of this pathogen westward. Recently, the succinate dehydrogenase inhibitor and potent fungicide Atpenin A5 was found to be encoded in the genome of *S. musiva*. The exact role of this metabolite in the biology of *S. musiva* and its downstream effects on a naïve ecosystem is currently unknown.

Bioinformatic prediction of atpenin A5 production was performed using antiSMASH across a 122-member population. Eight hypovirulent strains from a British Columbia clade were found to be missing the backbone PKS-NRPS from the atpenin a5 biosynthetic gene cluster. This finding led us to hypothesize that the metabolite might exacerbate infection on poplar trees. To study this possibility, atpenin A5 production was disrupted by targeting a pathway-specific transcription factor predicted to control regulation of the corresponding gene cluster. Five mutants were generated using a CRISPR-Cas9 ribonucleoprotein knockout protocol and subjected to Oxford nanopore sequencing. No off target effects of Cas9 were detected in three selected mutants. Absence of atpenin A5 production and significant down-regulation of its corresponding gene cluster was confirmed via metabolomics and transcriptomics. Comparing mutant and wild-type strains revealed a minor, strain-specific effect on 1 of 7 poplar genotypes. As Atpenin A5 is a known antifungal compound, we then tested the metabolite using co-culture assays with two beneficial, poplar-associated fungi. The ectomycorrhizal fungus *Laccaria bicolor* showed reduced hyphal growth in the presence of atpenin A5 positive strains of *S. musiva*. The dark septate endophyte *Hyaloscypha finlandica* showed no reduction in growth, indicating resistance to the compound. Further analysis of atpenin-like clusters using cBlaster located an intact atpenin A5 cluster in the genome of *H. finlandica.* Herein, we successfully disrupted atpenin A5 production in a non-model plant pathogen. Atpenin A5 likely does not play a substantial role during infection but instead mediates fungal-fungal antagonism between beneficial and detrimental poplar-associated fungi.

689B **Nutrient competition drives dynamic interactions between pioneering pyrophilous fungi** Monika S. Fischer, Neem Patel, Hannah Savin, Matthew Traxler University of California, Berkeley

Fire is an increasingly common disturbance affecting North American ecosystems. The first year following fire is a critical time of activity in the soil that lays the foundation for the rest of post-fire recovery and succession. To identify fungi that are active immediately post-fire, we collaborated with the Blodgett Forest Research Station on two experimental forest fires in the mixed conifer forest of the Sierra Nevada mountains in eastern California, USA. We then collected soil samples immediately post-fire, and continuing at least once/month for 15 months to ultimately generate a high-resolution time-series illustrating fungal community dynamics over time (via ITS amplicon seq). These data demonstrated that fire selects for a distinct cohort of pyrophilous fungi that are highly dynamic, patchy, and variable between sites. We combined our dataset with other publicly available datasets into a meta-analysis, which illuminated that a cohort of pyrophilous fungi were consistently present and dynamic across studies and ecosystems within the first year following fire. We isolated seven of these common pyrophilous fungi, sequenced their genomes, and have been working with them under laboratory conditions to examine how their metabolisms and interactions could explain the patterns we observed in the field. An initial series of experiments illuminated a complex interaction network which we are now using as a hypothesis road-map to investigate the mechanisms that drive each interaction, beginning with isolates of the genera *Pyronema, Tricharina,* and *Geopyxis*. *Pyronema spp.* are fast growing metabolic generalists capable of utilizing a broad variety of carbon sources from glucose to charcoal. *Pyronema spp.* are weak competitors in direct competition, however they are particularly good at depleting their environment for nitrogen, which in turn reduces the growth of several other pyrophilous fungi. *Tricharina praecox* grows significantly less when following *Pyronema spp.*, but *Geopyxis carbonaria* can rescue the growth of *T. praecox* by secreting essential nitrogen source(s). In total, we demonstrate that fire generates a dramatically altered and patchy nutrient landscape which drives the metabolism and behavior of pioneering pyrophilous fungi.

690B **The role of mycotoxins in governing interactions between the maize colonists, *Aspergillus flavus* and *Fusarium verticillioides*** Tim Satterlee, Jaci A. Hawkins, Trevor R. Mitchell, Lincoln F. Adams, Anthony Pokoo-Aikins, Anthony E Glenn, Scott E. Gold Toxicology & Mycotoxin Research Unit, USDA-ARS

The mycotoxigenic fungi, *Aspergillus flavus* and *Fusarium verticillioides,* commonly co-colonize maize in the field, yet their direct interactions at the chemical communication level have not been well characterized. Here we examined if and how the two most infamous mycotoxins produced by these species, aflatoxin and fumonisin, respectively, govern interspecies growth and mycotoxin production. We showed that fumonisin producing strains of *F. verticillioides* suppressed the growth of *A. flavus* while non-producers did not. However, while aflatoxin did not inhibit *F. verticillioides* growth, it did suppress fumonisin production. No fumonisin was detectable when *F. verticillioides* was challenged with a high dose of aflatoxin. With these findings, expression of the respective biosynthetic gene clusters was investigated for these two fungi. While no strong effect was seen on genes in the aflatoxin gene cluster when exposed to fumonisin, in preliminary analysis the key fumonisin biosynthetic cluster gene, *FUM1,* was unexpectedly induced when *F. verticillioides* was challenged with aflatoxin but, consistent with suppressed fumonisin production, so was the recently identified repressor of fumonisin synthesis, *ZBD1,* laying directly adjacent to the cluster. We also assessed the expression of *veA* and *laeA,* global regulators of fungal secondary metabolism, and found that expression of both is altered in *A. flavus* and *F. verticillioides* when exposed to their competitor's mycotoxin. Based on this, we initiated exploration into the roles of other mycotoxins produced by *A. flavus* and *F. verticillioides* in their interactions. This work gives insights into the ecological roles of mycotoxins and why these fungi may produce them as weapons in the interspecies battle for resource acquisition.

691B **The Kynurenine Pathway Contributes to Iron uptake and Virulence in *Cryptococcus Neoformans*** Christopher WJ Lee[1], Anna Brisland[2], Xianya Qu[1], Leandro Da Silva[1], Guanggan Hu[1], James Kronstad[1] [1]Microbiology and Immunology, University of British Columiba, [2]University of British Columiba

The basidiomycete fungus *Cryptococcus neoformans* serves as a useful model for investigating mechanisms of fungal pathogenesis. This pathogen is the causative agent of cryptococcal meningitis in immunocompromised patients and is listed in the critical priority group of the World Health Organization fungal priority pathogens list. Here, we investigate the role of the kynurenine pathway and characterize its role in cryptococcal virulence. The kynurenine pathway plays a major role in the biosynthesis of nicotinamide adenine dinucleotide (NAD+) and therefore mitochondrial regulation. By generating a collection of kynurenine deletion mutants consisting of *bna2Δ* (tryptophan 2,3-dioxygenase), *bna3Δ* (kynurenine aminotransferase), *bna5Δ* (kynureninase), and *bna1Δ* (3-hydroxyanthranilate 3,4-dioxygenase), we show that mutants defective in the kynurenine pathway have impaired mitochondrial function and impaired response to reactive oxygen species. Moreover, we show that this pathway uniquely contributes to *C. neoformans* role in iron uptake mechanisms which is essential for host virulence. *bna1Δ*, *bna2Δ*, and *bna5Δ* all displayed growth defects in low iron conditions in addition to sources of host iron such as transferrin, ferritin, and lactoferrin relative to wildtype.

We further characterized the transcriptome of *bna5Δ* in low and high iron conditions and found significant upregulation of mitochondrial and iron uptake related pathways to further support a role for the kynurenine pathway in iron uptake. All mutants except *bna3Δ* also displayed significantly reduced capsules compared to the wildtype implying a role in capsule formation. Moreover, the *bna5Δ* was found to be avirulent in a mice model of cryptococcosis and was significant reduced in fungal burden compared to the wildtype. These findings highlight key interactions between iron and mitochondrial pathways and support a key role for the kynurenine pathway in cryptococcal virulence.

692B **Glutathione metabolism impacts fungal virulence by modulating the redox environment** Braydon Black[1,2], Guanggan Hu[2], Xianya Qu[1,2], Leandro BR da Silva[2], Armando A Magaña[2,3], Daniel FQ Smith[4], Linda C Horianopoulos[2,5], Mélissa Caza[2,6], Arturo Casadevall[4], James Kronstad[1,2] [1]Microbiology & Immunology, University of British Columbia, [2]Michael Smith Laboratories, University of British Columbia, [3]Life Sciences Institute, University of British Columbia, [4]W. Harry Feinstone Dept of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, [5]Wisconsin Energy Institute, University of Wisconsin–Madison, [6]Larissa Yarr Medical Microbiology Laboratory, Kelowna General Hospital
Pathogens must overcome the hostile conditions of their hosts to survive, proliferate and cause disease. The fungal pathogen *Cryptococcus neoformans* is particularly adept at mitigating host environments and has developed an arsenal of defense mechanisms to evade oxidative and nitrosative agents released by phagocytic cells during infection. Among these mechanisms, melanin production is crucially linked to both fungal virulence and defense against harmful free radicals that facilitate host innate immunity and clearance of invading pathogens. Thus, processes for maintaining cellular redox homeostasis by acquiring or maintaining intracellular reducing equivalents are crucial for pathogen survival and the spread of disease. Our work employs comparative global metabolomic and genetic approaches to demonstrate that metabolism of the antioxidant glutathione (GSH) is inextricably linked to the redox-active processes that facilitate melanin production, and that genetic perturbations in GSH biosynthesis affect fungal growth and virulence. Furthermore, we show that disruption of GSH biosynthesis leads to changes in the extracellular redox environment that prevent melanin formation by mutant cells. These findings highlight the importance of redox homeostasis and metabolic compensation in the adaptation of pathogens to the host environment and suggest new avenues for antifungal drug development.

693B **Surprising strain-specific molecular determinants of *Aspergillus fumigatus* pathogenicity revealed by new cancer small molecule therapies** Katherine E Doss[1], Matthew R James[1], Andrew Wishart[2], Tobias M Hohl[3], Michail S Lionakis[2], Robert A Cramer[1] [1]Dartmouth College, [2]National Institutes of Health, [3]Memorial Sloan Kettering Cancer Center

Clinical risk factors for diseases caused by *Aspergillus fumigatus* have expanded beyond neutropenia and high-dose corticosteroid therapy. As treatments move toward small molecule drugs that target specific host pathways, novel immune states that facilitate infection have emerged. An example of this precision therapy is the drug ibrutinib (IBT) that inhibits Bruton's tyrosine kinase (BTK) and is used for the treatment of B-cell malignancies like chronic lymphocytic leukemia. Patients receiving IBT are at a higher risk of *A. fumigatus* infection. Surprisingly, reference *A. fumigatus* strains AF293 and CEA10 are not pathogenic in the setting of IBT treatment or genetic BTK-/- deficiency in mice. Rather, only certain strains of *A. fumigatus* are pathogenic in IBT-treated and BTK-deficient mouse models. These data challenge the long-standing paradigm that any *A. fumigatus* strain can cause disease in an immune compromised host.

Mechanistic studies of IBT-mediated susceptibility to *A. fumigatus* revealed an unexpected role of p40phox, a component of the neutrophil NADPH oxidase, and RAC2, which regulates NADPH oxidase. IBT treatment thus results in defective production of reactive oxygen species (ROS) that are a crucial aspect of host defense against *A. fumigatus*. We have tested the hypothesis that *A. fumigatus* strain-specific pathogenicity is ROS-mediated. To test this hypothesis, we are defining the genetic network that mediates *A. fumigatus* responses to NADPH oxidase-dependent host defense mechanisms. By utilizing the recently available protein kinase, phosphatase, and transcription factor null mutant collections, we are identifying key regulators of the fungal ROS response. In addition, to define the genetic and phenotypic variants associated with the strain-specific pathogenicity in the setting of BTK inhibition, we are utilizing both whole genome sequencing and ROS-related phenotyping of a unique collection of *A. fumigatus* isolates from patients on IBT. Preliminary results in both a biofilm and germling model suggest that five of seven isolates show reduced susceptibility to hydrogen peroxide. Using these approaches will allow us to define the cause-and-effect relationship between allelic variants in the *A. fumigatus* population and murine model disease outcomes. Defining these mechanisms is expected to promote new insights into both fungal pathogenicity and host response in specific patient populations.

694B **Quiescence in Candida albicans: defining the morphological, physiological and gene expression properties of an environmental tolerance induction mechanism** Ozan B Imir, David J J Gresham Biology, New York University

Candida albicans is the leading cause of fungal infections in the United States and its infection rates are on the rise globally. Studying Candida albicans is vital, as it causes numerous superficial and systemic infections annually, particularly harming immunocompromised individuals. The rising drug resistance in Candida albicans, amidst limited treatment options, urgently demands investigation into the factors behind resistance development. This study zeroes in on quiescence, a critical mechanism that halts growth in response to environmental stimuli.We hypothesized that quiescence could be a contributing mechanism for improved resistance to therapy in Candida albicans. By defining quiescence in Candida albicans, this study aims to provide a deeper understanding of how environmental factors like nutrient availability and downstream signaling pathways could influence fungal tolerance to stress and drug treatments. Our results show that nitrogen and carbon limitations significantly reduce the growth rate of Candida albicans in a concentration dependent manner. Furthermore, we observe an increase in temperature tolerance when Candida albicans is subjected to starvation due to continual culturing. A critical finding of this study is the reduced susceptibility of Candida albicans to fungicidal drugs, specifically caspofungin and micafungin, during quiescent states. Starvation-induced quiescence leads to a dose-dependent increase in cell survival, challenging the efficacy of these commonly used antifungals. Understanding the dynamics of quiescence in Candida albicans opens new avenues for therapeutic strategies and better management of fungal infections, particularly in immunocompromised patients where such infections are most perilous.

695B **Impaired Carbon Catabolite Repression pathway is associated with a decrease of virulence during infection in *Sclerotinia sclerotiorum*.** Shantala Mounichetty, Amandine Arnal, Sylvain Raffaele, Laurence Godiard LIPME

Sclerotinia sclerotiorum is a highly damaging plant necrotrophic fungus with a wide host range, including the model plant *Arabidopsis thaliana*. This Brassica produces a defense compound called camalexin, to which *S. sclerotiorum* is tolerant *in vitro* and *in planta*. Its closed sister species, *Sclerotinia trifoliorum* is sensitive to camalexin and unable to colonize *A. thaliana* due to this camalexin production. In response to this compound, a transcriptional reprogramming in *S. sclerotiorum* was highlighted and not in *S. trifoliorum*. An enrichment of WWCCCCRC binding motifs in the promoters of up-regulated genes provided clues to putative regulators of this response during infection (Kusch et al., Isme J 2021). One candidate, homologous to the transcription factor CreA, is involved in Carbon Catabolite Repression pathway (CCR), an ubiquitous pathway in microorganisms. Functional studies showed impaired CCR functionality associated to decrease of virulence on *A. thaliana* in deleted mutant strains of *S. sclerotiorum*. Camalexin metabolization seems linked to this decrease of virulence. This study suggests the role of fungal metabolic regulations in thwarting host defence responses and in controlling host specific defence compound. Finally, this should reveal dedicated fungal actors of transcriptional response to host chemical cues that underpin host range expansion in the *Sclerotiniaceae* species.

696B **Identification of secreted proteins from *Fusarium solani* f. sp. *phalaenopsis*, the pathogen causes leaf yellows of moth orchids** Wei-Chin Tsao[1], Chih-Li Wang[1,2,3] [1]Dept of Plant Pathology, National Chung Hsing University, [2]Master Program for Plant Medicine and Good Agricultural Practice, [3]Smart Sustainable New Agriculture Research Center (SMARTer)
Fusarium solani f. sp. *phalaenopsis* is the causal agent of leaf yellows of *Phalaenopsis* spp., which poses a significant obstacle to the orchid industry. The pathogen induces chlorotic leaves and necrotic rot at the base of the leaf sheath, leading to leaf drop and eventual death. However, to the best of our knowledge, no study has been conducted on the fundamental molecular biology of this industrially crucial pathogen, hindering the exploration of knowledge related to the pathogenesis of the Fusarium-phalaenopsis pathosystem. In this study, we performed a series of procedures on protein purification and identification to reveal potential necrosis-inducing proteins or effectors from cultural filtrate of FuZ10s. Necrosis-inducing proteins were purified from cultural broth of FuZ10 by ion-exchange chromatography and size-exclusion chromatography. Further, LC- ESI-Q-TOF MS was adopted to identify proteins contained in each sub-fraction (Q13, Q30, Q35, N28 and N33) with the annotated FuZ10s protein database. A total of 118 secreted proteins were identified. Among them, 31 proteins including 26 predicted effector proteins and 5 non-effectors proteins were selected for investigating the capability of cell death induction through Agroinfiltration-transient expression approach on *Nicotiana benthamiana.* Notably, four effector candidates (g9058, g6858, g2448, g7192) induced cell death. Specifically, proteins g6858, g2448 and g7192 induced cell death when fused with the SP ^{NbPR1a}, while their counterparts lacking signal peptide did not, suggesting that the three effector candidates might induce cell death in the apoplast of *N. benthamiana.* In contrast, one effector g9058 induced cell death with or without the signal peptide, indicating that it might induce cell death in the cytoplasm of *N. benthamiana.* Nevertheless, four effector candidates did not induce cell death on leaves and petals of phalaenopsis through transient expression. It is assumed that protein may not trigger cell death in phalaenopsis or may be due to insufficient concentration of assessed proteins for cell death induction. Thus, considering the effect owing to various

factors, we may employ the heterogeneous protein expression approach to purify these four proteins and assess the response on phalaenopsis. Moreover, the gene knock-out strategy will be conducted to assess the gene functions.

697B **Toward identification of host cell-death inducing genes in an NLR-dependent manner in *Colletotrichum higginsianum*** Katsuma Yonehara[1], Naoyoshi Kumakura[2], Ken Shirasu[1] [1]RIKEN, The University of Tokyo, [2]RIKEN
Colletotrichum higginsianum, a causal agent of anthracnose disease, is a hemibiotrophic fungus infecting various cruciferous plants including *Arabidopsis thaliana*. This fungus completes its biotrophic-necrotrophic cycle by controlling the expression of pathogenicity-related genes, including virulence genes encoding candidate secreted effector proteins. Meanwhile, *A. thaliana* upregulates the expression of genes encoding intracellular nucleotide-binding domain, leucine-rich repeat immune receptors (NLRs), which, in general, recognize alien effectors and induce hypersensitive responses including host cell death. These clues imply the presence of virulence genes recognized by NLRs and causing host cell death in the transition of the infection phases. However, identifying virulence genes that contribute to the outbreak of host cell death in an NLR-dependent manner has been challenging mainly due to the redundancy of virulence genes and the absence of an effective screening system. To address these issues, we have recently established an efficient multiple-gene disruption method in *C. higginsianum*. Additionally, we are using an oxicam-type compound tenoxicam, which has been reported to enhance the efficiency of *Agrobacterium*-mediated transient gene expression in *A. thaliana* leaves, providing a novel screening system. We are also taking an advantage of a bacterial type-three secretion system derived from *Pseudomonas syringae* pv. *syringae* 61., which has been reported to deliver candidate effector proteins into *A. thaliana* cells efficiently. The aim of this study is to use these systems to identify previously undefined virulence genes causing host cell death in *A. thaliana* in an NLR-dependent manner during the infection of *C. higginsianum*.

698B **Inducing Novel Endosymbioses by Bacterial Implantation into Fungi** Gabriel H. Giger[1], Chantal Ernst[1], Ingrid Richter[2], Thomas Gassler[1], Patrick Kiefer[1], Christoph G. Gäbelein[1], Orane Guillaume–Gentil[1], Kirstin Scherlach[2], Miriam Bortfeld-Miller[1], Tomaso Zambelli[3], Markus Künzler[1], Christian Hertweck[2,4,5], Julia A. Vorholt[1] [1]Institute of Microbiology, ETH Zurich, [2]Leibniz Institute for Natural Product Research and Infection Biology, HKI Jena, [3]Institute for Biomedical Engineering, ETH Zurich, [4]Institute of Microbiology, Friedrich Schiller University Jena, [5]Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena
Endosymbioses, marked by the intimate partnering of cells within cells and their metabolisms, have profoundly influenced life and have driven evolutionary transitions and innovations, including the emergence of eukaryotes. Furthermore, endosymbioses play an important role in the interactions across kingdoms, including those between fungi and bacteria. Uncovering new endosymbiotic relationships is particularly challenging because it mostly relies on retrospective analysis of systems that have naturally evolved. By combining atomic force microscopy, optical microscopy and nanofluidics, we developed a FluidFM-based approach for bacteria implantation to follow the fate of artificially induced endosymbiosis in fungi. As a model system, we use the wide-spread filamentous fungus *Rhizopus microsporus*. The injection of *Escherichia coli* and pre-adapted endosymbiont bacteria of the Burkholderia family into *R. microsporus* resulted in striking differences in host response. While injected *E. coli* was not transmitted to fungal spores, *Mycetohabitans rhizoxinica*, known for forming endosymbiotic relationships in a different *R. microsporus* strain, reached the spores in this non-host fungus and was vertically transmitted. This bacterium, which naturally synthesizes rhizoxin congeners to aid the host in acquiring carbon and defending against predators, maintained its metabolic functions within the new host. Vertical transmission of novel endosymbionts impacted host fitness, however, positive selection mitigated these fitness constraints and stabilized the endosymbiotic relationship during adaptive laboratory evolution. The approach provides an experimental framework to investigate the initial stages of endosymbiosis and to empirically test cost-benefit trade-offs.

699B **Updating the *Zea mays* root infection cycle of *Colletotrichum graminicola*** Anina Y Rudolph[1], Carolin Schunke[1], Christoph Sasse[2], Luis Antelo[3], Jennifer Gerke[2], Gerhard Braus[2], Stefanie Pöggeler[1], Daniela Nordzieke[1] [1]Genetics of Eukaryotic Microorganisms, Georg-August University Göttingen, Institute of Microbiology and Genetics, [2]Molecular Microbiology and Genetics, Georg-August University Göttingen, Institute of Microbiology and Genetics, [3]Johannes Gutenberg University Mainz, Institute of Biotechnology and Drug Research (IBWF)

Colletotrichum graminicola is a hemibiotrophic fungus, which causes corn anthracnose. Lesions on leaves, stalk rot, death of seedlings, top dieback, and stunting, characterize the disease. *C. graminicola* produces two morphological and developmental different asexual spores, oval and falcate shaped conidia. Our previous work provided evidence that both types have different infection strategies and roles in the infection cycle with falcate conidia being more efficient in maize leaf infection. In 2008, Sukno et al. described a mainly symptom free maize root infection outgoing from roots dipped in a falcate conidia suspension. As a follow-up study we investigate whether oval conidia can infect roots. In our study, both spore types induced the formation of hyphopodia, runner hyphae, microsclerotia, and acervuli on maize roots dipped in spore solutions. However, only oval conidia and derived germlings showed typical characteristics of root pathogenic fungi, e.g. a chemotropic response to signaling molecules in maize root exudates (MRE) of the host, which is absent for falcate conidia and derived germlings. Within MRE, we found that tricyclic

diterpenoids, not peroxidases as described for *Fusarium* species, are responsible for the attraction of *C. graminicola*. Since the pheromone receptors Ste2 and Ste3 mediate plant-derived peroxidase recognition in other fungi, we tested if diterpenoid sensing is based on a similar mechanism in *C. graminicola*. We showed that the a-pheromone receptor CgSte3 is responsible for sensing MRE and tricyclic diterpenoids. Next, we established a natural root infection based on chemotropism, in which maize seeds are co-incubated with oval conidia. For wildtype and ΔCgste3 co-incubations, we found a strong and significant reduction of plant biomass and length. Only for wildtype there is also progression of the fungus inside stem tissue, absent for ΔCgste3, indicating either a reduced or delayed root infection process or an additional function of CgSte3 in plant colonialization. We also investigated germination and propagation in soil. The ability of oval conidia to germinate in soil demonstrates better adaptation to root infection. In contrast, falcate conidia do not germinate in soil even after prolonged incubation time or in the presence of its host or MRE. In summary, the current study underpins the importance of both conidial types for the success of corn anthracnose, since both spore types are adapted to infect distinct plant tissues.

700B **Misregulation of a secreted glucanase negatively affects virulence of the wheat pathogen *Zymoseptoria tritici*** Cristian Carrasco[1], Diego Rebaque[1,2], Parvathy Krishnan[3], Gemma Lopez[1], Hugo Mélida[4], Asier Largo[4], Antonio Molina[5], Andrea Sánchez-Vallet[1] [1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, [2]KTH (Royal Institute of Technology), [3]Technical University of Denmark, [4]Universidad de León, [5]Universidad Politécnica de Madrid

The plant cell wall has a double role in plant-pathogen interactions. It acts as a major defence barrier against pathogens, and it is a major source of nutrients for microorganisms colonizing the host. During infection cell wall-derived oligosaccharides are released by the activity of a diverse set of secreted cell wall degrading enzymes (CWDEs) from the pathogen. Here, we demonstrated that mixed-linked glucan (MLG) oligosaccharides are highly accumulated in wheat at late stages of *Zymoseptoria tritici* infection when the pathogen initiates the necrotic phase. We identified a gene encoding for a β-glucanase, *ZtGH45*, which is only expressed at the necrotrophic phase. We showed that ZtGH45 hydrolyses wheat cell wall glucans, and releases MLG oligosaccharides, which act as elicitors of resistance to *Z. tritici*. We finally demonstrated that misexpression of *ZtGH45* impairs fungal virulence by triggering an early host immune response. These results demonstrate that the balance between cell wall degradation and release of inducers of resistance, such as wall damage-associated molecular patterns (DAMPs), by fungal CWDEs governs the outcome of host invasion. We suggest that this balance drives the evolution of fungal CWDE expression regulation to favour plant colonisation.

701B **Characterization of *Podospora anserina* glycolipid transfer protein HET-C and of its involvement in the response to antagonistic *Serratia* bacterial species** Asen Daskalov[1], Kamalraj Subban[1], Marina Lamacchia[2], Matti Kjellberg[3], Alexandra Granger-Farbos[4], Annick Breton[4], Bénédicte Salin[4], Peter Mattjus[5], Sven J. Saupe[2], Mathieu Paoletti[6] [1]ImmunoConcEpT, CNRS UMR5164, [2]IBGC, CNRS UMR5095, [3]Dept of Chemistry, University of Helsinki, [4]IBGC UMR5095, [5]Dept of Biochemistry, Åbo Akademi University, [6]CNRS UMR7266

Podospora anserina het-c gene (*het*erokaryon incompatibility determinant) encodes a 208-aa glycolipid transfer protein (GLTP), which is highly conserved in fungi but also plants and animals, including humans. The *het-c* gene is multi-allelic in populations of *P. anserina* with more than 10 identified alleles. Different alleles of *het-c* trigger a regulated cell death (RCD) reaction during anastomosis with strains expressing different alleles of *het-e* and *het-d*. The latter two genes are paralogs of the NOD-like receptors (NLR or NLR-like) family in fungi. NLRs are multi-domain intracellular sensors playing crucial role in innate immune systems in various eukaryotic and prokaryotic taxa. It has been hypothesized that the RCD reaction in *Podospora* is triggered by direct binding of HET-C by the NLRs HET-E and HET-D. Remarkably, a HET-C homolog in plants, ACD11 (Accelerated Cell Death 11) appears to be guarded by an NLR protein named LAZ5 (Lazarus 5) and involved in plant immunity. These trans-kingdom evolutionary parallels have prompted the hypothesis that *het-c* and *het-c*-dependent cell death are integral for fungal organismal defense.

While *het-c* role in Podospora has been previously related to ascospore formation, not much is known about the molecular mechanisms and biological roles of the gene, nor about its involvement in organismal defense. We set-up to investigate the role of *het-c* in both homeostasis and biotic interactions. Our preliminary data indicates that HET-C is involved in the fungal response towards antagonistic bacteria of the *Serratia* genus. The HET-C2 allelic variant of the protein is degraded during the fungal-bacterial interactions in dependence of a bacterial secreted protease, previously identified as a virulence factor. The HET-C degradation appears allele specific. We find that the GLTP activity of HET-C is important for the response to bacteria but not necessary for its role in heterokaryon incompatibility. Moreover, fragments of HET-c alone appear to exhibit cytotoxicity in *P. anserina*. Based on these preliminary results, we investigate a model, in which HET-C is directly targeted by the protease to subsequently trigger the fungal defenses.

702B **Critical Roles of Extended *O*-Mannosylation in Interaction with Host Cells and Functional Characterization of Mannoproteins in *Cryptococcus neoformans*** Eun Jung Thak[1], hyun ah Kang[1], J Andrew Alspaugh[2] [1]Chung Ang University, [2]Duke University School of Medicine

Cryptococcus neoformans is an opportunistic fungal pathogen causing life-threatening meningoencephalitis in immunocompromised individuals. *C. neoformans* assembles two types of *O*-linked glycans on its surface proteins, the more abundant major *O*-glycans that do not contain xylose residues and minor *O*-glycans containing xylose. The double deletion of *KTR3* and *CAP6* (*ktr3*Δ *cap6*Δ) completely blocked the mannose addition at the second position of *O*-glycans, resulting in the accumulation of proteins with *O*-glycans carrying only a single mannose. Tunicamycin (TM)-induced phosphorylation of the Mpk1 mitogen-activated protein kinase (MAPK) was greatly decreased in *ktr3*Δ *cap6*Δ strain. Transcriptome profiling of the *ktr3*Δ *cap6*Δ strain upon TM treatment revealed decreased expression of genes involved in the Mpk1-dependent cell wall integrity (CWI) pathway. Consistent with its defective growth under several stress conditions, the *ktr3*Δ *cap6*Δ strain was avirulent in a mouse model of cryptococcosis. Associated with this virulence defect, the *ktr3*Δ *cap6*Δ strain showed decreased adhesion to lung epithelial cells, decreased proliferation within macrophages, and reduced transcytosis of the blood-brain barrier (BBB). *O*-glycan extension in the Golgi apparatus plays critical roles in various pathobiological processes, such as CWI signaling and stress resistance and interaction with host cells in *C. neoformans*. Several Cryptococcal mannoproteins (MPs) are reported as key antigens stimulating host CD4 (+) T-cell response. Chitin deacetylase 1 (CDA1) that converts chitin into chitosan, is essential for the cell wall integrity of *C. neoformans*, and MP88 is a major protein associated with extracellular vesicles (EV). In this study, we investigated the structure of *O*-glycans of Cryptococcal MPs and effect on their function on the interaction with host cells. The purified CDA1 and MP88 with the altered *N*-/*O*-glycan structures will be valuable tools to examine the relationship between glycan structure and function in adhering to host cells and inducing the cytokine secretion of host cells. The information generated by this study would deepen our understanding of glycan-based host-pathogen interaction and expected to be useful for the development of antifungal drugs for fungal-specific targets.

703B **Zymoseptoria tritici effector interactions with plant PR-proteins** Elisha Thynne[1], Mohammad Abukhalaf[2], Andreas Tholey[2], Eva H Stukenbrock[1] [1]Christian-Albrechts University, [2]Institute for Experimental Medicine, Christian-Albrechts University

Zymoseptoria tritici is the major fungal pathogen of wheat in Europe, and is responsible for the disease known as Septoria tritici blotch (STB). Despite the importance of the pathogen, the molecular interactions between wheat and the fungus are poorly understood. We have identified a number of effectors that are able to suppress host immune responses; however, despite the activity of these effectors, the wheat host still expresses high levels of pathogenesis-related (PR) proteins. PR proteins are stress expressed proteins that can signal for defence responses and/or actively inhibit the growth of a pathogen. As these PR proteins are in high abundance during *Z. tritici* infection, the pathogen must have evolved mechanisms to tolerate their presence. We have identified various effectors that putatively target PR proteins. Of note among these are a class of structurally conserved fungal effectors that appear to interact with lipid transfer proteins (LTPs; PR-14). LTPs have been reported with antifungal activity, and so we are screening whether these LTP-binding effectors can protect *Z. tritici* from wheat LTP proteins antifungal activity.

704B **Fine root endophytes form a unique type of symbiosis with plants** Alan Wanke[1], Victor Rodriguez Morelos[2], Alex Williams[3], Alexandra Dallaire[4,5], Scarlet Au[1], Silvia Pressel[2], Katie Field[3], Sebastian Schornack[1] [1]Sainsbury Laboratory, University of Cambridge, [2]Natural History Museum, [3]University of Sheffield, [4]Royal Botanic Gardens Kew, [5]Dept of Biochemistry, University of Cambridge

Arbuscular mycorrhizal (AM) fungi from the Glomeromycotina form endophytic mutualistic relationships with the majority of land plants, enhancing nutrient uptake and promoting plant growth, thereby influencing ecosystem dynamics and agricultural productivity. A widespread but molecularly much less understood group of plant symbiotic and endophytic fungi are the Mucoromycotina fungi, a sister lineage of the Glomeromycotina.

To characterise and compare these diverse fungal-plant interactions we have identified and established the axenic cultivation of two Mucoromycotina 'fine root endophyte' (MFRE) isolates from plant tissues, studied their hyphal structures formed during the colonisation in vitro and obtained their genome sequences. A comparison of AM and MFRE fungal genomes reveals common and contrasting signatures underpinning their plant-associated lifestyles and revealed the presence of MFRE saprotrophic traits.

Our genomic and molecular findings support a classification of the interaction between plants and MFRE as a distinctive type of mycorrhizae.

705B **Tracing host-specificity in *Magnaporthe oryzae* pathotype *Triticum*: Loss of a lineage-specific gene affects virulence rather than defines host range** Florencia Casanova[1], Lucia I Trepp Salinas[1], Judith Eberle[2], Louisa Wirtz[1], Alex Wegner[1], Ulrich Schaffrath[1] [1]Dept of Molecular Plant Physiology, RWTH Aachen University, [2]RWTH Aachen University

The fungus *Magnaporthe oryzae* is a threat to global food security, causing high yield losses in rice and wheat. Molecular analyses have identified multiple divergent lineages and sub-divisions within the population of the pathogen, each associated with a specific host genus. Understanding the molecular basis for host preferences is critical to improving disease management and preventing future spread to new hosts. Comparative genomic analyses have highlighted specific differences in the genomes of the aforementioned lineages. However, there is currently no experimental proof confirming whether genes in *M. oryzae* exclusively associated with certain hosts delimitate host ranges.

This study focuses on elucidating the role of *MoT6098*, which is only absent in rice-infecting isolates but present in isolates of all other lineages. Comparison of protein structures suggests a function as an acid trehalase. We challenged the hypothesis that *MoT6098* is related to host-specificity by generating a gene deletion mutant in the wheat-infecting isolate BR32 using a CRISPR/Cas9-mediated approach. The mutants exhibit significantly reduced virulence, indicating that *MoT6098* is necessary for the entire progression of infection. Despite the resemblance to rice infecting isolates, due to the absence of *MoT6098,* these deletion mutants are unable to successfully infect rice. Similarly, *MoT6098* gene insertion in rice-infecting isolates does not alter the aggressiveness of the fungi. Next, we focused on a second gene *TRE1* that also encodes a secreted protein with trehalase activity. It has been previously published that deletion of *TRE1* in rice-infecting isolates has no impact on virulence, and we obtained the same results for a *TRE1*-deletion mutant generated in the wheat-infecting BR32. A double gene deletion mutant in BR32 lacking both, *MoT6098* and *TRE1,* did also show no altered virulence on wheat. Constitutive expression of *MoT6098* in isolates infecting wheat or rice did not affect virulence in their respective hosts. Overall, we have demonstrated the contribution of *MoT6098* to full expression of virulence in the wheat-infecting isolate. An involvement of this gene, i.e. the absence of *MoT6098* in rice-infecting isolates, with host-specificity was not evident.

Our results underscore the need for further experimental approaches to elucidate host specificity in the *Magnaporthe* species complex, which may enable anticipation of future host jumps.

706B **Unconventional suppression of plant defence responses by the signal peptide peptidase Spp1 in the *Ustilago maydis* - maize interaction** Nora Marie Kühne, Niko Pinter, Anja Poehlein, Rolf Daniel, Kai Heimel Georg-August-University Goettingen

Biotrophic fungi use effector proteins for communication with their host plants to manipulate the host immune system and establish a plant environment supportive of fungal colonization. During the interaction between the corn smut fungus *Ustilago maydis* and its host, the unfolded protein response (UPR) is specifically activated after successful plant penetration. This generates an optimised intracellular infrastructure securing efficient processing and secretion of effectors. To assess the virulence contribution of UPR regulated genes, we utilised a combined RNAseq/ChIPseq approach. The identified UPR core genes were deleted individually and tested for altered virulence and endoplasmic reticulum (ER) stress resistance. Screening of more than 40 deletion strains identified the signal peptide peptidase Spp1 as a novel key factor essential for virulence. SPPs are ER-membrane localised aspartic proteases that cleave type II oriented transmembrane domains, including remnant signal peptides, which have previously been processed by the signal peptidase complex. While Spp1 is dispensable for vegetative growth, filament formation and ER stress resistance, it is required to supress plant defence responses upon infection of the host plant maize. Dual RNAseq based transcriptome analysis reveals an early and broad induction of defence related marker genes in the plant suppressing growth of Δ*spp1* mutant strains prior and subsequent to plant penetration. The essential virulence function of Spp1 requires catalytic activity but cannot be attributed to known physiological roles of SPPs, such as ER-associated degradation (ERAD), hypoxia adaptation or ER homeostasis. Since we were not able to detect alterations in effector secretion, Spp1 generated cleavage products establish an additional layer of fungal-plant communication that is crucial for plant defence suppression. We are currently combining proteomics and metabolomic approaches to identify relevant substrates and elucidate the underlying mechanism(s).

707B **A putative UDP-galactose transporter is a direct UPR-target crucial for stress resistance and virulence of *Ustilago maydis*** Anja Katharina Sieven, Niko Pinter, Kai Heimel Georg-August-University Goettingen

The unfolded protein response (UPR) is a conserved signaling pathway of eukaryotes to ensure endoplasmic reticulum (ER) homeostasis under stress conditions. UPR activity restructures the secretory pathway to increase the ER folding capacity and thereby reduce the amount of un- or misfolded proteins. Fungal pathogens rely on a functional UPR as it is connected to various virulence contributing traits, such as thermotolerance, antifungal drug resistance and efficient protein secretion. However, the

contribution of individual components within the UPR regulon that are important for virulence is still poorly understood. In the corn smut fungus *Ustilago maydis* the UPR is intricately connected to its biotrophic lifestyle. During plant penetration the direct interaction between the developmental regulator Clp1 (Clampless1) and the central UPR regulator Cib1 (Clp1 interacting bZIP) promotes enhanced ER stress tolerance which is required for successful proliferation within the plant. In a screening approach to systematically investigate the virulence and ER stress function of UPR regulated genes, we identified a UDP-galactose transporter that is required for virulence and stress resistance. The respective gene is a direct UPR target that is consistently upregulated during the fungal-plant interaction. Deletion mutants exhibit reduced ER and cell wall stress resistance. While filamentous growth on the leaf surface and appressoria formation appear not to be affected, fungal proliferation within the plant is almost completely blocked. We are currently investigating the subcellular localization, the relevance of protein interactions with ER and/or Golgi resident proteins, as well as the requirement of the predicted UDP-galactose transporter during defined developmental stages using our conditional expression system. In summary, our data further expands the repertoire of UPR-regulated virulence factors in *U. maydis* and the role of nucleotide-sugar transporters in fungal virulence.

708B ***Colletotrichum scovillei*** **orchestrates LysM effectors CsLysM1 and CsLysM2 to suppress defense response of chili pepper** Yu-Nung Yen, Chen-Lin Chiang, Tai-Keng Hsieh, Chi-Kuan Tu, Miin-Huey Lee Plant Pathology, National Chung Hsing University

Chili pepper anthracnose disease caused by *Colletotrichum scovillei* has severe impact on chili pepper production. This pathogen infects chili pepper fruit with a hemibiotrophic lifestyle. Genomic and transcriptomic analysis reveal a large number of candidate effector proteins during the interaction of *C. scovillei* and chili pepper fruit. We identified 17 LysM-containing proteins in *Colletotrichum scovillei* strain Coll-524, among them, two LysM proteins, CsLysm1 and CsLysm2, classified to fungal/bacterial group, were specifically and highly expressed during pathogenesis. CsLysm1 selectively expressed at the pre-penetration stage, while CsLysm2 highly expressed during the biotrophic growth. The gene deletion strains of CsLysm1 and CsLysm2, respectively, caused smaller lesions than the wild-type. In addition, plant defense genes were significantly up-regulated early in biotrophic infection by CsLysm1 gene deletion strains, while late-stage up-regulation occurred with CsLysm2 gene deletion strains. Except acting as virulence factors, both CsLysm1 and CsLysm2 protected fungal cells from hydrolase digestion. However, the two proteins localized differently. Using GFP-fusion protein for localization assay, CsLysm1 was found to highly accumulated at spore cell wall on plant surface but to be hardly detected after penetration. In contrast, CsLysm2 could be detected on all analyzed fungal structures before and after penetration. Polysaccharide binding affinity assay reveals that both recombinant CsLysm1 and CsLysm2 proteins could bind chitin, but in addition to chitin, recombinant CsLysm2 could bind chitosan and cellulose. Using WGA-AF488 staining, chitin was found to present on all fungal structures before and after penetration, except the germ tube formed on plant surface. We propose that *C. scovillei* orchestrated the secretion of CsLysm1 and CsLysm2 at pre-penetration and post-penetration to overcome the plant defense for successful infection.

709B **Diversity and phenotypes of fungi recovered from animals at a large veterinary diagnostic laboratory** Steven D Harris, Sydney Marks Iowa State University

With increasing attention focused on the significant threat that fungi pose to the health and well-being of humans, it is equally important to consider the potential risks that fungi represent to the health of our animals (i.e., livestock and domesticated pets). The global burden of livestock and poultry disease is likely in excess of billions of dollars. In addition, the control and management of disease in our domesticated pets imposes a significant cost on their owners. Beyond the threat to animal health, our livestock and pets also serve as vehicles for the transmission of pathogens to humans. Although the role of animals in transmitting bacterial and viral diseases is well-studied, relatively less is known about fungal diseases whose transmission is mediated by animals. The purpose of this study is two-fold; (i) to investigate the diversity of fungi associated with livestock and pets, and (ii) determine the extent to which these animal-associated fungi possess traits that might enable human pathogenesis. The Veterinary Diagnostic Laboratory (VDL) at Iowa State University is a fully accredited facility that handles >120,000 cases and processes >1.5 million diagnostic tests on an annual basis. Between 2010 and 2022, the VDL performed 4920 tests for potential fungal pathogens of a range of livestock, pets, and wild animals. Analysis of archived data from these tests provides some insight into the types of fungi that are recovered. For example, 152 cases of *Aspergillus fumigatus* were diagnosed, with the majority consisting of internal/systemic cases in cows, birds, or dogs. More recently, 169 fungal isolates collected from the VDL during the past year have been identified and subjected to preliminary phenotypic characterization. Notably, the origins of these isolates range from internal organs (37%) to body surfaces (45%) and the proximal environment (18%). The majority of the 169 isolates (40%) belong to the Eurotiomycetes, including 15 isolates of *A. fumigatus* primarily from avian air sacs. Other prominent genera represented in the collection include *Penicillium*, *Microsporum*, *Fusarium*, *Cladosporium*, *Alternaria*, *Candida*, *Trichosporon*, *Mucor*, and *Lichtheimia*. Preliminary phenotypic characterization of 31 Ascomycete and Basidiomycete yeasts present in the collection has identified several (i.e., *Candida*, *Saccharomyces*, *Lodderomyces*, *Magnusiomyces*, *Trichosporon*) capable of robust growth at temperatures ≥37^{o}C.

710B **Exploring host compatibility in *Fusarium oxysporum*-cucurbit interactions through ECC1 effector analysis** Babette V. Vlieger, Frank L. W. Takken, Martijn Rep Molecular Plant Pathology, University of Amsterdam

Fusarium wilt disease, caused by the fungus *Fusarium oxysporum* (Fo), affects over one hundred plant species, resulting in significant crop losses globally. Pathogenic Fo strains are often host specific, only able to infect one or a few related plant species. These strains are grouped into *formae speciales* (ff.spp.) based on their defined host specificity. For example, Fo f. sp *melonis* (Fom) only causes disease in melon. In contrast, Fo f. sp. *radicis-cucumerinum* (Forc) can infect several different hosts within the cucurbits, among which cucumber and melon. We aim to determine how Fo evolved compatibility towards different cucurbit species. In previous research, the first 'non-host' avirulence gene was found in Fom, termed *Effector Candidate for Cucurbits* (*ECC1a*^{Fom}). Transferring this gene, encoding a small secreted protein with unknown function, into a Forc strain compromised its ability to infect cucumber. Interestingly, ECC1a homologs are present in both Fom and Forc, with ECC1a differing markedly in sequence (15 amino acids) between the two *formae speciales*. To identify the role of *ECC1a* and its homologs in host compatibility, knock-out strains of Fom and Forc were generated using a CRISPR/Cas9-mediated genome editing approach. The mutants showed differential loss of virulence towards cucumber and melon, suggesting a role in host compatibility. This is currently investigated through mutant complementation and exchange of the *ECC1a* homologs between Fom and Forc.

711B **Regulators and downstream genes involved in the defense of the mushroom-forming fungus *Schizophyllum commune* against its competitors** Erik P. W. Beijen, Marieke H Van Maanen, Robin A. Ohm Utrecht University

Mushroom-forming fungi interact with numerous organisms during their lifecycle and some of these can cause infections of the mycelium and fruiting bodies. Little is known about how mushroom-forming fungi defend themselves against their competitors.

We performed RNA-Seq during interaction of the mushroom-forming fungus *Schizophyllum commune* with the ascomycete fungal competitors *Trichoderma harzianum*, *T. aggressivum* and *Purpureocillium lilacinum*, as well as the bacterium *Serratia quinivorans*. The expression response to competitors was predominantly local, but to a lesser extent there was also a systemic response.

Three transcription factor genes (*tf21, tf22* and *tf23*) were up-regulated during interaction. Knockout strains were more sensitive to infection and were more easily overgrown, showing that these transcription factors indeed play an important role in regulating the defense against competitors. Subsequent RNA-Seq identified putative target genes that are regulated by these transcription factors.

Numerous other genes were up-regulated during interaction, including genes encoding effector proteins, transporters and other putative defense-related proteins. We created fluorescent reporter strains to visualize the activation of promoter activity of six of these genes, allowing us to screen for signals that activate defense. Most reporter strains responded to the presence of a living competitor, while some also responded to a heat-killed competitor, spent medium, damage and/or reactive oxygen species. This shows that there are multiple signals of competitors that may lead to a defense response. Moreover, we showed that while most responses are local to the interaction zone, some genes are activated only on the opposite side of the colony, indicating that there is a (limited) systemic response.

Several ABC transporter genes are strongly up-regulated during interaction. Individual knockouts of four of those genes were significantly more sensitive to antifungals, and the quadruple knockout was even more sensitive. This suggests that these transporters play a role in interaction by pumping out secondary metabolites secreted by the competitors.

A dark line of pigment is formed by *S. commune* during interaction with some competitors. We knocked out the gene *pig1*, which resulted in an absence of this dark pigmentation, showing that *pig1* is involved in this process.

Combined, we have identified regulators and downstream genes that play important roles during the defense response.

712B **Functional characterization of the Target of Rapamycin signalling pathway during *Magnaporthe oryzae* infection-related development** Matthew Wengler, Neftaly Cruz-Mireles, Iris Eisermann, Frank Menke, Nicholas J Talbot The Sainsbury Laboratory

The Target of Rapamycin (Tor) signalling pathway plays a pivotal role in regulating eukaryotic growth and homeostasis through the activity of two functionally distinct multiprotein complexes, TORC1 and TORC2. In comparison with yeast and mammalian systems,

our understanding of the orchestration of these signalling events during the pathogenicity of filamentous fungal plant pathogens remains poorly understood. While spores of the rice blast fungus *Magnaporthe oryzae* treated with Tor activators fail to initiate appressorium development, spores treated with Tor inhibitors undergo constitutive autophagy and produce immature infection structures. Previous reports have suggested that Tor cycles between states of inactivation and autophagy-dependent reactivation during appressorium morphogenesis. To further investigate the precise role of TORC complexes, the regulation of Tor activity was assessed by monitoring the global phosphorylation of Rps6, a standardized readout for TORC1 activity. Phosphorylated Rps6 is detected by 1 h post germination and remains phosphorylated throughout appressorium development. We therefore set out to identify components of the Tor signalling complexes in *M. oryzae.* A yeast two-hybrid assay identified interactions between Tor and homologous components of both yeast TORC1 and TORC2 complexes. These interactions are currently being studied by co-immunoprecipitation in *M. oryzae.* Subsequently, we aim to further substantiate TORC complexes by performing large-scale temporal proximity labelling-mass spectrometry studies. Progress in understanding the global transcriptomic and phosphoproteomic responses downstream of each TORC complex during vegetative growth and appressorium development will be presented. These studies aim to lead to a deeper understanding of the role of Tor kinase during fungal pathogenesis.

713B **Identification and characterization of effectors VR1 and VR2 in the *Pyrenophora teres* f. *teres*-barley interaction** Michele C Malvestiti[1,2], Gayan Kariyawasam[1], Jinling Li[1], Nathan A Wyatt[3], Ashley C Nelson[2], Ryan M Skiba[1], Jason Fiedler[1], Karl Effertz[4], Zhaohui Liu[2], Simon Williams[5], Robert S Brueggeman[4], Timothy L Friesen[1] [1]Northern Crop Science Lab, USDA-ARS Edward T. Schafer Agri Res Ctr, [2]Plant Pathology, NDSU, [3]Sugarbeet and Potato Research Unit, Edward T. Schafer Agricultural Research Center, USDA-ARS Edward T. Schafer Agri Res Ctr, [4]Dept of Crop and Soil Science, Washington State University, [5]Research School of Biology, Australian National University

The necrotrophic Ascomycete *Pyrenophora teres* f. *teres* is the causal agent of net form net blotch in barley. A previous study used a biparental population of *P. teres* f. *teres* isolates 15A and 6A to identify two quantitative trait loci (QTL), namely, *VR1* and *VR2*. Each QTL contained a gene which contributed to virulence in Rika barley. In this study, we cloned and functionally validated both *VR1* and *VR2* and investigated their role in fungal virulence. We used CRISPR-Cas9-based gene disruption and gene editing, QTL analysis, haplotype and isoform diversity analysis, protein structure prediction, quantitative PCR, and laser confocal microscopy to validate and functionally characterize *VR1* and *VR2* and the corresponding proteins. Both *VR1* and *VR2* were present in a global *P. teres* f. *teres* collection and isolates possessing different VR1 and VR2 protein isoforms quantitatively varied in virulence. Protein structure prediction revealed that *VR1* encodes for a secreted prolyl-endopeptidase, whereas *VR2* encodes for a small secreted protein with unknown domains. Inoculation of the *VR1* and *VR2* edited isolates onto the Rika × Kombar barley population showed that both *VR1* and *VR2* were likely targeting the same susceptibility locus *Spt1* in Rika barley chromosome 6H. The *VR1* and *VR2* gene-edited isolates showed that virulent alleles of *VR1* and *VR2*, derived from *P. teres* f. *teres* isolate 6A, were sufficient to cause disease on Rika barley alone. However, increased symptom severity was observed on Rika barley when both VR1 and VR2 virulent alleles were present in the *VR1* and *VR2* gene-edited isolates. Analogously, confocal microscopy data showed that the fungal isolates possessing the virulent *VR1* and *VR2* allele displayed more rapid host tissue colonization and increased fungal biomass. Isogenic isolates carrying both *VR1* and *VR2* showed an additional increase in symptom severity and biomass relative to isolates carrying *VR1* or *VR2* alone. Taken together, these observations suggest that *VR1* and *VR2* may act in a synergistic manner. To validate this hypothesis, single and double *VR1* and *VR2* gene disruption mutants in *P. teres* f. *teres* isolate 6A were generated to independently assess the contribution of each effector gene to fungal virulence. Infection assays on Rika barley with the wild type *P. teres* f. *teres* isolate 6A and single and double *VR1* and *VR2* mutant isolates are currently ongoing. We expect to present the outcome of the infection assays at the time of the conference.

714B **Toward understanding of the biosynthetic pathway of Ptr ToxC in *Pyrenophora tritici-repentis*** Gongjun Shi[1,1], Carly George[1], Paula Moolhuijzen[2], Pao Theen See[2], Zhaohui Liu[1] [1]Plant Pathology, North Dakota State University, [2]School of Molecular and Life Sciences, Curtin University

Pyrenophora tritici-repentis, the causal agent of tan spot of wheat, is known to produce at least three necrotrophic effectors (NEs), namely Ptr ToxA, Ptr ToxB and Ptr ToxC to induce disease in wheat. Both Ptr ToxA and Ptr ToxB are proteins and their encoding genes have been cloned. In contrast, Ptr ToxC has been partially characterized as a secondary metabolite and its production in the fungus remains largely unknown. We recently identified a genetic locus (*ToxC*) and cloned a gene (*ToxC1*) in the locus that is required but not sufficient for the Ptr ToxC production. To identify additional genes, we performed a transcriptomics study using a Ptr ToxC-producing isolate and its *ToxC1* mutant. Three genes within the locus were identified as candidates because they had a similar expression pattern as *ToxC1*. Among them, one is a homolog of *ToxC1*, and was shown again to be required for the Ptr ToxC production, which was designated as *ToxC2*. The other two genes are a hypothetical and a hydroxylase which are being functionally characterized. A polyketide synthase gene (*PKS1*) that is adjacent to *ToxC* was shown to control melanin production. However, the

PKS1 mutant was still fully virulent and produces Ptr ToxC as wild type suggesting PKS1 is not involved in the Ptr ToxC production. This work provides a better understanding of the biosynthetic pathway of Ptr ToxC in wheat tan spot pathogen.

715B **Nutrient transport upregulation across five clades of *Candida auris* in the novel thermo-relevant Arabian Killifish embryo infection mode** Hugh E. C. Gifford[1], Nicolas Helmstetter[1], Tina Bedekovic[1], Jack Gregory[1], Alexandra Brand[1], Mark Ramsdale[1], Johanna Rhodes[2], Duncan Wilson[1], Tetsu Kudoh[3], Rhys A. Farrer[1] [1]MRC CMM, University of Exeter Center for Medical Mycology, [2]Dept of Medical Microbiology, Radboudumc, [3]MRC CMM, University of Exeter Biosciences

Candida auris is an emerging human fungal pathogen constituting at least five highly clonal clades. Many isolates have been typified by rising antifungal resistance, nosocomial outbreaks, and high associated mortality. The transcriptional response of *C. auris* to host infection *in vivo* remains poorly described. We aimed to establish an embryonic yolk sac infection model in the thermotolerant teleost fish *Aphanius dispar* (Arabian Killifish, AK) to study gene expression programmes at mammalian relevant temperatures. We found that representative strains from clades I-V were lethal (91.7 – 100 % 7-day mortality, n = 34-37 per condition) vs control injection (5.6 %, $p < 1.5E^{-10}$). RNA from infected embryos was extracted at 24 and 48 h post infection (HPI) and sequenced with Illumina. Of 52.9 million reads from each infected embryo, 3.83 (0.81-13.1) % aligned to the *C. auris* reference genome and 61.1 (50.0-67.4) % to the host transcriptome assembly. We discovered 936 differentially expressed genes (DEGs) across the 2 time points compared to growth in YPD, 32 of which were shared by all *C. auris* clades. Notably, two orthologues of iron-acquisition related siderophore transporter *SIT1* – a greatly expanded gene family in *C. auris* – were upregulated at 48 h; as were nicotinic acid transporter *TNA1* and sugar transporter *HGT2; HGT19* was upregulated at 24 h, and *HGT12* at both time points. Presumed virulence factor ortholog, secreted aspartyl protease *SAP8*, was downregulated in infection. Major facilitator superfamily siderophore and sugar transporters were enriched across all clades. There were 451 and 251 DEGS for AK at 24 and 48 h, including *HSP70* and several haem oxygenases (*HMOX*), likely to be involved in inflammatory responses and nutritional immunity *via* iron restriction from pathogens. These results provide evidence for a critical role of nutrient transport in *C. auris via* multiple sugar and siderophore transporters expressed in the first 48 h of survival within host tissue. Shared differential gene expression across all clades point towards conserved infection strategies with potential relevance to mammalian infection and therapeutic targeting.

716B E**stablishing laboratory model systems for ectomycorrhizal symbiosis** Ines Teichert Forest Botany and Tree Physiology, University of Göttingen

Mycorrhizal symbiosis is a mutualistic interaction between plants and fungi. Most trees in temperate forests show colonization of fine roots by ectomycorrhizal fungi. The trees benefit from this symbiosis by easier access to water and mineral nutrients, and the fungi acquire carbohydrates from the trees. Many studies have focused on ectomycorrhizal interaction, and for example transcriptomic analysis has identified a huge number of genes as differentially regulated during the onset and / or maintenance of symbiosis. Yet, molecular analysis of mycorrhizal interaction partners in laboratory model systems as well as functional analysis of genes remains scarce.

To establish such models, we morphologically and genetically analyze mycorrhizal fungi from different roots, concentrating on beech and pine. Microscopic analysis is complemented by DNA isolation and sequencing of different morphotypes. We aim to establish lab cultivation methods for selected fungal interaction partners to use these partners in further studies, including mycorrhization of trees. Furthermore, we intend to establish transformation methods for ectomycorrhizal fungi to be able to analyze the molecular basis for initiation and maintenance of the symbiosis.

717B **Biparental and natural population genetics identify *Pyrenophora teres* f*. teres* loci associated with a broadly effective barley resistance** Ryan M Skiba[1], Nathan A Wyatt[1], Jinling Li[2], Gayan K Kariyawasam[2], Jonathan K Richards[3], Karl Effertz[4], Sajid Rehman[5], Robert S Brueggeman[4], Timothy L Friesen[1] [1]USDA-ARS, [2]NDSU, [3]LSU, [4]WSU, [5]Olds College

Pyrenophora teres f. *teres*, the fungal pathogen responsible for the foliar barley disease net form net blotch (NFNB), is an increasingly significant pathogen of barley worldwide. Though many genetic sources of resistance to NFNB have been identified, few are as broadly resistant as barley line CIho5791. To identify the genetic factors underlying virulence/avirulence on CIho5791, we created a biparental mapping population using a Moroccan *P. teres* f. *teres* isolate MorSM40-3 displaying substantial virulence crossed with the CIho5791-avirulent Canadian reference isolate 0-1. Whole-genome sequencing was performed for 103 MorSM40-3 × 0-1 progeny and used to create a saturated genetic map that was used alongside reaction-type data on CIho5791 to identify major quantitative trait loci (QTL) associated with virulence/avirulence. Major QTL were identified on chromosomes (Ch) 1 and 8 accounting for 27% and 15% of disease reaction type variation, respectively. Interestingly, while MorSM40-3 contributed the virulent allele at the Ch1 locus, the avirulent parent 0-1 contributed the virulent allele at the Ch8 locus, indicating an epistatic

interaction between the two loci, as this Ch8 virulent allele appeared to be functional only in the presence of the MorSM40-3 Ch1 allele. We sequenced a natural *P. teres* f. *teres* population made up of 165 geographically diverse isolates and screened this population on CIho5791. The resulting data from the natural population as well as a subset of this population representing only the Moroccan and North American isolates was used to perform genome wide association studies (GWAS), which also indicated a strong association with virulence/avirulence at the Ch1 and Ch8 loci we had previously identified using the biparental population. Additionally, long and short-read sequencing were used to assemble and polish a reference quality genome for isolate MorSM40-3, and RNA-seq data from multiple infection timepoints was used to generate gene annotations indicating multiple candidate effector genes in the Ch1 and Ch8 loci.

718B **Mechanisms of Infection and Response of the Fungal Wheat Pathogen *Zymoseptoria tritici* during Compatible, Incompatible and Non-Host Interactions** Sandra V. Gomez[1], Cassidy R. Million[2], Namrata Jaiswal[2], Michael Gribskov[3], Matthew Helm[2], Stephen B. Goodwin[2] [1]Botany and Plant Pathology, Purdue University, [2]Crop Production and Pest Control Research Unit, USDA–Agricultural Research Service, [3]Biological Sciences, Purdue University

Zymoseptoria tritici causes Septoria tritici blotch (STB) on wheat. Despite the importance of this disease, our understanding of the infection strategy and the arsenal of candidate effectors that are activated by the pathogen, is currently limited. To investigate the infection phase-specific gene expression in *Z. tritici*, we analyzed the transcriptome activation response during infection of susceptible (Taichung 29) and resistant (Veranopolis and Israel 493) wheat cultivars, plus the non-host species barley at 1, 3, 6, 10, 17 and 23 days post-inoculation (DPI). We observed dramatic differences in pathogen gene expression at 10 DPI in the compatible compared to both incompatible interactions. A total of 275 and 226 genes in *Z. tritici* were expressed at 10 DPI during the compatible interaction compared to the incompatible interactions with Veranopolis and Israel 493, respectively. This correlates with the initiation of the necrotrophic lifestyle of the pathogen. We observed a significant up-regulation of genes at 10 DPI that encode carbohydrate-active enzymes (CAZymes). The largest differences in pathogen gene expression occurred at 3 DPI in both compatible and incompatible interactions compared to the non-host interaction. Of the *Z. tritici* genes that were significantly expressed at 1 and 3 DAI in the compatible interaction, we identified thirty-one putative effectors. Subsequent subcellular localization studies using *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana* revealed two candidate effectors that localize to mobile cytosolic bodies, suggesting involvement in intracellular signaling or host gene regulation. Mycgr3109710, which localized to cytosolic bodies, belongs to the non-plant PR-1-like protein family implicated in virulence in other pathogens. We found that the genome of *Z. tritici* encodes four CAP-domain-containing PR-1-like proteins. Two of them contain a predicted signal peptide. However, only Mycgr3109710 is predicted as an effector. Mycgr3109710 contains three CAP signature motifs and a conserved CNY motif, which is important for the immunity activity of PR-1s in plants and is present in PR-1-like proteins with confirmed virulence activity in other fungal pathogens. We are investigating the evolution of PR-1-like proteins in the genomes of 19 *Z. tritici* isolates, four sister *Zymoseptoria* species, and selected Dothideomycetes species through comparative genomics and phylogenetic reconstruction.

719B **The plant hormone, strigolactone, inhibits the yeast phosphate transporter, Pho84, by regulating transporter localisation** James M Bradley[1], Michael Bunsick[2], George Ly[1], Bruno Aquino[1], Dario Bonetta[3], Peter McCourt[1], Shelley Lumba[1] [1]Cell and Systems Biology, The University of Toronto, [2]Dept of Molecular Biology, Princeton University, [3]Cell and Systems Biology, Ontario Tech University

When phosphate is scarce in the soil, plants synthesise a collection of small molecules termed strigolactones (SLs) and exude these signals into the soil. One role for SLs in the soil is to recruit beneficial fungi to plant roots, which provides plants with access to additional phosphate for growth. Yet, despite documented phenotypic responses to SLs in a range of filamentous fungi, the mechanism of fungal perception to SLs remains unknown. As many filamentous fungi are experimentally intractable, our lab chose to address this question using the model fungus, *Saccharomyces cerevisiae* (yeast). Using genetic, cell biology and physiological experiments we demonstrated that (1) SLs induce a strong Pi-starvation response in yeast, even in Pi-replete media, and (2) this response is due to the inhibition of the high affinity phosphate transporter, Pho84.

To further our understanding of the mechanism by which SLs inhibit Pho84, I will first present experiments in which I tracked Pho84-Gfp localisation in yeast during exposure to SL. These data revealed the importance of transporter internalisation from the plasma membrane during the SL response. I will then present the genetic approaches I have taken to discover yeast mutants showing altered SL responses. In particular, I have isolated a collection of SL-hyposensitive mutants, some of which are explained by mutations in PHO84, whilst others appear to map outside of PHO84. I will discuss how these mutants are helping to further probe the mechanism of SL perception in yeast.

Overall, these data provide mechanistic insights into how a plant-derived small molecule can inhibit a fungal Pi transporter, which has exciting implications for our understanding of plant-fungal interactions.

720C **Mycorrhiza-driven mechanisms shaping the niche of pathogenic plant-interacting fungi** Stephanie Heupel, David Figueira-Galán, Ruben Betz, Natalia Requena Karlsruhe Institute of Technology

Microbes living on plants often have to compete for space and resources to succeed in niche occupation and maintenance. In natural ecosystems, the most widespread fungal association with plants is the arbuscular mycorrhizal (AM) symbiosis with fungi from the Glomeromycotina subphylum. These mutualistic fungi provide plants with mineral nutrients in exchange for fixed carbon. Root colonization by AM fungi substantially reprograms plants in a systemic way, and thus, impacting on their microbiome. Mycorrhizal plants have often been shown to have a reduced susceptibility towards microbes occupying the same niche, in particular fungal and oomycete root pathogens. But also, to induce systemic defense responses thereby conferring increased resistance to the shoot bacterial pathogen *Xanthomonas campestris*, to the root bacterial pathogen *Ralstonia solanacearum* or to the fungal pathogens *Botrytis cinerea, Alternaria solani or Magnaporthe oryzae*. To investigate how AM fungi modulate the plant response to other fungi, we have chosen the system barley - *Rhizophagus irregularis* - *Magnaporthe oryzae*. Plants colonized by *R. irregularis* showed a decreased number of lesions in leaves and a lower intracellular shoot colonization by *M. oryzae*. This result suggests that mycorrhizal colonization induces a systemic response that hinders *M. oryzae* growth *in planta*. Furthermore, using mycorrhizal mutants in which arbuscule-development is impaired, we could show that not only mycorrhizal colonization is necessary for the protective effect against *M. oryzae*, but also that a functional symbiosis is required. But surprisingly, *M. oryzae* also negatively impacted on the colonization by *R. irregularis* in roots, reduced the number of arbuscules and increased the number of septated hyphae. These results were corroborated by the analysis of key mycorrhizal markers genes that were deregulated in response to *M. oryzae* after only four days of infection, indicating that even a well-established symbiosis can be rapidly turned down in response to a pathogenic invasion in the leaves. Altogether, these results suggest that both fungi are able to impact on the niche establishment of the other in a systemic manner through the plant. However, it is also possible that they might also employ antimicrobial effectors that might help them to defend their niche in a more direct manner. We are currently analyzing these possibilities using transcriptomic studies.

721C **Alternative splicing regulation in plants by effectors of symbiotic arbuscular mycorrhizal fungi** Ruben Betz[1], Sven Heidt[1], David Figueira-Galan[1], Anna Miucci[1], Thorsten Langner[2], Natalia Requena[1] [1]Karlsruhe Institute of Technology KIT, Joseph Gottlieb Kölreuter Institute for Plant Scienes JKIP, [2]Max Planck Institute for Biology Tuebingen

Most plants in natural ecosystems live in association with beneficial AM (arbuscular mycorrhizal) fungi to survive under poor nutrient conditions and to cope with other abiotic and biotic stresses. To engage in symbiosis, AM fungi secrete effector molecules that, similar to pathogenic effectors, reprogram plant cells. Despite numerous effectors being predicted in the genome of AM fungi, only a few have been functionally characterized. Here we show that the SP7-like family impacts on the alternative splicing program of their hosts. We identified 13 members of this effector family within the genome of the model organism *Rhizophagus irregularis*, including the presence of several effector paralogs. In addition, SP7-like effector sequences were detected in various other symbiotic fungi of the Glomeromycotina phylum, but were absent from fungal species outside this clade. *In planta* expression of SP7-like members revealed their localization at cellular condensates within the plant nucleus and cytoplasm. Biomolecular condensates are often described as the result of phase separation to form micro-compartments in which functional relevant molecules are concentrated, such as mRNA processing related nuclear splicing speckles or P-bodies that serve as cytoplasmic mRNA degradation centers. Indeed, we found multiple components of the plant mRNA processing machinery that physically interacted with the SP7-effector family, most prominently with the splicing factor SR45. Co-expression of SP7-like members and SR45 led to re-localization of the effectors to SR45 occupied nuclear condensates, while co-expression with the plant P-body marker DCP2 additionally demonstrated P-bodies as effector localization target. Furthermore, ectopic expression of two of these effectors in the crop plant potato changed the alternative splicing pattern of a specific subset of SR45 related genes. Cell-type specific expression of SR45 in arbuscule containing cells negatively impacts symbiosis progression, indicating the need for a fine balanced SR45 activity during plant-fungal association. Together our data suggest a scenario where the SP7-like effector family targets the plant mRNA processing machinery, engages in mRNA processing related phase separation events and influences the activity of SR45, ultimately enabling full establishment of symbiosis. Future approaches aim towards identifying specific plant mRNA effector targets using *in planta* RNA immunoprecipitation as well as studying the effector ability to directly bind to RNA molecules.

722C **Finding function through form: predicting effector function in the blast fungus through structural homology** Angus H Bucknell, Adam R Bentham, Xia Yan, Nicholas J Talbot The Sainsbury Laboratory

Magnaporthe oryzae is the causal agent of blast disease, which results in major yield losses across agriculturally significant crops such as rice, wheat, and millet. Susceptibility or resistance of rice to *M. oryzae* is predominantly dependent on presence/absence polymorphisms of *Magnaporthe* effector protein-encoding (*MEP*) genes within the fungus, which are recognised by intracellular NLR immune receptors. *M. oryzae* secretes hundreds of lineage-specific MEP effectors during infection to facilitate pathogenesis through interaction with host target proteins to induce susceptibility, but these can also lead to effector-triggered immunity if detected by a cognate NLR. The wild type Guy11 strain of *M. oryzae* has at least 558 *MEP* genes, however, their low sequence similarity to known proteins makes identifying the function of individual effectors challenging. Using AlphaFold2 and DALI, we have performed structural predictions of 32 MEP effectors and identified structurally homologous proteins with known functions. Identification of structural homologs allows for prediction of MEP effector function and can be used to guide further experimental characterisation. Subsequent *in-silico* investigation is being used to predict the quality of possible binding interfaces between effectors and putative host targets. These predictions can then be validated using immunoprecipitation coupled with mass spectrometry, as well as structural and biophysical analyses. We aim to identify novel host target proteins and thereby determine effector function in *M. oryzae*. We will report the development of a proof-of-concept 'effector discovery' structure-guided pipeline to facilitate *in-vitro* investigations into 32 MEP effectors and subsequent experimental validation of their predicted biological functions.

723C **Exploring strain-specific differential gene essentiality in *Candida albicans* through an innovative inducible CRISPRi system** Lauren Wensing[1], Meea Fogal[1], Abdul-Rahman Adamu Bukari[2], Anthony Hendriks[1], Nicholas Gervais[1], Clara Fikry[3], Aleeza Gerstein[2], Rebecca Shapiro[1] [1]Molecular and Cellular Biology, University of Guelph, [2]Depts of Microbiology & Statistics, University of Manitoba, [3]Microbiology, University of Waterloo

With the emergence of antifungal-resistant *Candida albicans* strains, the need for new antifungal drugs is critical in combating this fungal pathogen. Investigating essential genes in *C. albicans* is a vital step in characterizing putative antifungal drug targets. As some of these essential genes are conserved between fungal organisms, developed therapies targeting these genes have the potential to be broad-range antifungals. In order to study these essential genes, classical genetic knockout or CRISPR-based approaches cannot be used as disrupting these genes leads to lethality in the organism. Here, we describe the generation and application of a novel, inducible CRISPR interference (CRISPRi) system for precise transcriptional repression of genes of interest in *C. albicans*, without introducing genetic mutations. CRISPRi utilizes an endonuclease dead Cas9 protein which can be targeted to a precise promoter location where it prevents the binding of RNA polymerase to the genomic through steric hindrance. We demonstrate the use of CRISPRi to efficiently and reversibly repress genes of interest in *C. albicans*, and further expand the use of this technology to generate large-scale libraries repressing essential genes. We use an efficient, high-throughput cloning strategy to generate a CRISPRi pooled plasmid library of ~554 plasmids with unique guide RNAs targeting 130 putative essential genes in *C. albicans*. We use this plasmid library to generate *C. albicans* gene repression pooled libraries across three unique strain backgrounds (a laboratory strain, and two drug-resistant clinical isolates). We further characterize these library pools in competitive growth assays, where we monitor fitness differences between mutants by assessing shifts in their relative abundance via a guide RNA-barcode sequencing strategy. Through the construction of these essential gene CRISPRi libraries, we can begin to study the function of essential genes across different strain backgrounds, and under different conditions and identify genes that are involved in survival and critical processes such as drug tolerance in antifungal-resistant background strains. These genes can ultimately be characterized as putative targets for novel antifungal drug development, or targeted as a means to sensitize drug-resistant strains to antifungal treatment.

724C**Mining the *Penicillium expansum* genome for virulence genes: using forward and reverse genetics approaches to identify novel loci mediating blue mold decay of apple fruit** Dianiris Luciano-Rosario[1], Hui Peng[2], Verneta L. Gaskins[1], Jorge M. Fonseca[1], Nancy P. Keller[3], Wayne M. Jurick[1] [1]USDA-ARS, [2]Everglades Research and Education Center, University of Florida, [3]University of Wisconsin-Madison

Penicillium expansum is the causative agent of blue mold disease of pome fruits. This postharvest pathogen produces patulin and other harmful mycotoxins that threaten food safety. While there are many studies describing the molecular biology of virulence and patulin regulation in *P. expansum*, these mostly focus on genetic regulators. In this study, forward and reverse genetics approaches were used to identify genes involved in blue mold infection biology in apple fruit. To discover new loci that have a role in *P. expansum* virulence, we generated a library of 448 random T-DNA insertional mutants using *Agrobacterium*-mediated transformation (AMT). These mutants were then screened for reduced decay phenotypes on inoculated apple fruits. We selected six T-DNA single-insertion mutants (T-193, T-275, T-434, T-588, T-625, and T-711) that showed reduced lesion diameter on inoculated apples when compared to the wild type strain. Five unique genes of interest were identified using TAIL-PCR. To further characterize these mutants, we generated two deletion mutants (Δ*t-625* and Δ*t-588*) and one knock-down strain (t-434^{KD}). Data showed that Δ*t-588* phenocopied the T-DNA mutant T-588 in Honeycrisp apples and had reduced colony diameter upon cultivation with exogenous methylgyoxal (MG). Based on bioinformatic predictions and the observed reduction in colony diameter during growth in methylglyoxal, we hypothesize that this locus functions as a glyoxalase. This research unveils previously unknown

members of signaling networks and additional genetic factors governing fungal virulence in the blue mold fungus during the decay of apple fruit.

725C **A novel broad range effector from *Fusarium oxysporum* is able to induce cell death hijacking plant immune system** Andrea Doddi[1,2], Giulia Lancia[1], Wessel Groot[3], Matteo Amadei[4], Maria Carmela Bonaccorsi[4], Giorgio Giard ina[4], Martijn Rep[3], Bart P.H.J. Thomma[5], Massimo Reverberi[1], Luigi Faino[1,6] [1]Dept of environmental biology, University of Rome "La Sapienza", [2]Institute for Plant Sciences, University of Cologne, [3]Molecular Plant Pathology, Swammerdam Institute for Life Science, University of Amsterdam, [4]Dept of Biochemistry, University of Rome "La Sapienza", [5]Cluster of Excellence on Plant Sciences, Institute for Plant Sciences, University of Cologne, [6]SARA EnviMob S.R.L.

Effector molecules are used by microbes to manipulate plant immunity and colonize the host. Pathogenic fungi adopt different strategies to manipulate the plant immune system aiming to interfere and manipulate the immunity response produced by cell surface receptors. *Verticillium dahliae* (*Vd*) and *Fusarium oxysporum* (*Fo*) are two very well studied soil-borne pathogenic fungi with a similar lifestyle. Although they share the same ecological niche, *Vd* can infect a wide variety of plant species, while each *Fo* strain is able to infect only one or few related species and for this they are sub-categorized in *formae speciales*. Functional studies characterized few effector proteins that are shared by these two plant pathogens and play a role in pathogen-host interaction.

One of them is the d gene isolated in *Vd* (d-*Vd*) strains belonging to *Vd* strains that defoliate cotton plants after infection. BLAST analysis showed that many *Fo* strains have a homolog gene to the *d-Vd* located on the dispensable chromosome. Surprisingly, we also found an homolog of the d gene in many non-defoliating *Vd*. To test the functionality of the different d-homolog proteins, we produced and purified different version of the proteins from *Fo f.sp. vasinfectum* (*Fov*), *Fo f.sp. radices-cucumerinum* (*Forc*), *Vd* non-defoliating an compared to the *Vd* defoliating strain. An In planta assays using different d-protein homologs showed that all the protein variants can induce wilting on cotton suggesting a conserved functionality including the d version from non-defoliating *Vd* strains. Furthermore, we tested the d-Fov homolog on many dicotyledon plants, and we found a wilting symptoms on all tested plants. Confocal experiment by *Agrobacterium* transient expression in *N.benthamiana* using PR1::RFP::DFOV protein showed that the protein localize at the plasma membrane borders. In order to investigate the plant pathway involved in the wilting, we investigate the activation of plant hormones using LC/MS and GC/MS. We found that salicylic acid and ethylene are induced after treatment. Similarly, we found a production of ROS after treatment with d-*Fov*. Ultimately, we resolved the 3D structure of the d-*Fov* and we found a burrel-like structure that resemble other fungal toxins. All collected data suggest that the d-*Fov* protein might bebe a toxin acting at the apoplast level on a conserved plant structure yet unknow.

726C **A matter of life and death: characterizing the innate immune response of the mucoromycete *Rhizopus microsporus* to the antagonistically perceived *Mycetohabitans* bacterium** Delia Tota[1], Maria Laura Gaspar[2], Carlos Lax[3], Victoriano Garre[3], Teresa Pawlowska[2] [1]Field of Microbiology, Cornell University, [2]Plant Pathology & Plant-Microbiology, Cornell University, [3]Genetics, University of Murcia

Like plants and animals, fungi employ innate immunity mechanisms to perceive and regulate interactions with microbes, including bacteria. Regulated cell death (RCD) is one of the many innate immune mechanisms with functional similarities shared by plants and animals. However, the surveillance system and means of innate immune defense in fungi, including the use of RCD, is not well understood. The symbiosis between the mucoromycete *Rhizopus microsporus* and the bacterial endosymbiont *Mycetohabitans* sp. presents a valuable system in which to investigate the innate immune response of early-divergent fungi. In contrast to the accommodations for bacteria made by host *R. microsporus* strains, nonhost strains, which do not naturally harbor endosymbionts, perceive *Mycetohabitans* antagonistically upon interaction. The fungus launches a reactive oxygen species (ROS) response accompanied by elevated lipid peroxidation, decreased levels of the antioxidant glutathione, and death of a subpopulation of fungal cells, as indicated by microscopy, glutathione quantification, and flow cytometry assays. Application of exogenous antioxidants and the iron chelator deferoxamine alleviates lipid peroxidation. We also have preliminary evidence that RCD as a defense response against *Mycetohabitans* is initiated, at least in part, by the fungal adenylyl cyclase 1 (Cyr1) receptor sensing bacterial signals. Ongoing experiments will be aimed at quantifying fungal RCD through differential staining in flow cytometry assays and using fluorescent in situ hybridization (FISH) to discern whether the RCD is mediated by bacterial penetration into fungal cells.

727C **The role of cell wall remodeling in innate immunity of early divergent Mucoromycotina fungi** Hana Barrett[1], Maria Laura Gaspar[1], Carlos Lax[2], Victoriano Garre[2], Teresa E Pawlowska[1] [1]Plant Pathology and Plant Microbe Biology, Cornell University, [2]University of Murcia

The fungal cell wall plays a crucial role in the maintenance of cell integrity and homeostasis, including immune defense. Since the mechanisms of innate immunity in early divergent fungi are not well understood, our research focus es on elucidating the role of

the cell wall in immune defense. To examine this process, we use the antagonistic interaction between the fungus Rhizopus microsporus (Mucoromycotina) and the bacterium Mycetohabitans spp, since certain fungal strains harbor his bacteria as an endosymbiont while other strains exhibit an ROS response to the same bacteria. Previous work from our lab has found significant differential gene expression in non-host fungi in coculture with bacteria vs fungi alone. Notably, genes associated with cell wall components chitin, fucose, glucan, mannose, and galactose were differentially expressed in the antagonistic interaction. We are currently quantifying and visualizing cell wall remodeling in the antagonistic interaction between nonhost fungal germlings and Mycetohabitans bacteria by fluorescently probing cell wall components and using microscopy and flow cytometry for visualization of the stains. We have found an increase in the amount of exposed chitin on the surface of the wildtype fungal cell wall in the antagonistic interaction vs the fungus grown alone. No change in mannose content between the two conditions has been seen. We also generated CRISPR/Cas9 disruption mutants of two adenylyl cyclase (cyr1 and cyr2) genes that encode PGN receptors in other fungi to examine their role in our study system. Disruption of the adenylyl cyclase 1 (Δcyr1) led to increased exposed chitin in the fungus alone vs antagonistic interaction and disruption of the adenylyl cyclase 2 (Δcyr2) had decrease chitin in fungus alone vs antagonistic interaction. The increase in chitin in wildtype may indicate that the fungus is reinforcing the cell wall as a defense response. The decreased chitin in the coculture in Δcyr1 may be due to the activity of bacterial chitinases. In Δcyr2 the cell wall remodeling response seems to be heightened. These results suggest that the change in chitin content is an immune defense response that is influenced by the activity of adenylyl cyclase proteins.

728C **Screening for *Fusarium* effectors that play a role during root rot infection on dry bean** Rubylyn M Infante[1], Michael F Seidl[2], Malaika K Ebert[1] [1]Plant Pathology, North Dakota State University, [2]Dept of Biology, Theoretical Biology and Bioinformatics, Utrecht University

Dry bean production is continuously challenged by various bacterial and fungal diseases. While some diseases like white mold and rust are caused by pathogens of a single genus, root rot is caused by a pathogen complex. Besides *Rhizoctonia solani* and *Pythium*, many major causal agents within this root rot complex belong to the genus *Fusarium*. In 2022, we conducted an extensive survey on dry bean to sample for root-rot causing *Fusarium* spp. in North Dakota and Minnesota. Using outgrowth assays followed by single spore selection, we isolated 43 *Fusarium* spp. from the root rot symptoms displaying dry bean plants collected during the survey. The individual *Fusarium* isolates were characterized to a species level using a translation elongation factor 1 alpha (TEF1-α) primer set. Moreover, we assessed the virulence of all isolated *Fusarium* strains in greenhouse infection assays and identified strain '10-1', a member of the *Fusarium solani* species complex, as the most virulent isolate when compared to all other *Fusarium* spp. strains tested. Previous studies in the *F. oxysporum*-tomato pathosystem used xylem sap analyses to identify SIX effectors that are secreted by the fungus during host infection. To determine whether isolate 10-1 also secretes effectors during dry bean infection, our first step was to sequence strain 10-1 using the PacBio sequencing technology followed by genome assembly and annotation. Furthermore, we collected xylem sap from plants inoculated with *Fusarium* isolate '10-1' at 13 days post-inoculation. Mass spectrometry analyses will allow us to screen for proteins that are secreted by the pathogen during dry bean infection. These data will aim provide a basis for the identification of effectors that play a role in promoting *Fusarium* root rot. Understanding the molecular mechanisms that take place during *Fusarium*-dry bean interaction is crucial for the identification of disease resistance genes and development of resistant crop varieties.

729C **Training a pathogen: uncovering the evolutionary mechanisms of host adaptation in *Cryptococcus neoformans*** Zoe A Hilbert[1,2,3], Joseph M Bednarek[4], Mara JW Schwiesow[2,3], Krystal Y Chung[4], Christian T Moreau[4], Jessica CS Brown[4], Nels C Elde[2,3] [1]Dept of Biology, Boston College, [2]Dept of Human Genetics, University of Utah, [3]HHMI, [4]School of Biological Sciences, University of Utah

For many fungal pathogens, the range of conditions under which they are able to survive and replicate is impressively large. And yet, our understanding of how adaptation to environmental conditions, or wide-ranging hosts, occurs on a molecular level and contributes to pathogenicity in these species is still limited. In this study we use experimental evolution approaches to watch adaptation of the human fungal pathogen *Cryptococcus neoformans* unfold in real time, exploring how interactions with different host species and changing environments shape the evolution of this model fungal pathogen. Using *C. neoformans* environmental isolates, we performed serial passaging experiments exposing fungal cells to relevant environmental and mammalian host cells—amoeba and mouse macrophages, respectively—for many generations to observe how these interactions select for the emergence of fungal populations with an enhanced ability to thrive within host environments. Through this approach, we identify several independent populations that rapidly adapted to their respective hosts, and—via whole genome sequencing—reveal key genetic changes in the populations that underlie these adaptive phenotypes. We find that each evolved *C. neoformans* population acquired a unique set of mutations and took a distinct evolutionary trajectory during the experiment, with some populations being swept early by beneficial mutations and others maintaining more genetic heterogeneity. This suggests that there are many possible

routes to host adaptation in this species. We further perform molecular and genetic dissection of these adaptive phenotypes to reveal differences in the function of key signaling pathways across divergent strains of *C. neoformans* and their roles in regulating pathogenicity. Together, this work provides new insight into the evolutionary strategies used by fungi to adapt to different host species and environments, and how these adaptations contribute to the transformation of some fungi, like *C. neoformans*, from environmental microbe to potent pathogen.

730C ***Macrophomina phaseolina*** **expresses distinct waves of effectors and carbohydrate active enzymes during different stages of infection under high temperature** Christine Jade D. Ermita[1], Kayla K. Pennerman[2], Olivia Gutierrez[2], Zoey Jimenez[2], Gerardo Ramos[2], Polly H. Goldman[2], Peter M. Henry[2] [1]Plant Sciences, University of California, Davis, [2]Crop Improvement and Protection Research, USDA-ARS

Macrophomina crown rot disease in strawberry, caused by *Macrophomina phaseolina,* has become more destructive in recent years as climate change-induced heat stress has exacerbated the disease. Several studies show that higher soil and air temperatures can increase disease severity by *Macrophomina phaseolina*, but the molecular mechanisms of this phenomenon are unknown. We hypothesized that higher temperatures accelerate the transition to necrotrophy, leading to upregulation of necrotrophy- and pathogenicity-related genes. To test our hypothesis, we conducted two growth chamber experiments with low (23°C day/18°C night) and high (30°C day/25°C night) temperature treatments. Plants were either inoculated by submersion in an 8% *M. phaseolina* mycelial suspension or sterile V8 broth. Disease severity was scored on a 1 to 5 scale and root tissues from five plants per treatment were harvested at 1-, 5-, 12-, and 21-days post inoculation (dpi) for RNA sequencing and microscopy. Root and crown necrosis, wilting, and plant death was accelerated and more severe in inoculated plants at high temperature compared to those at low temperature. A total of 2,330 differentially expressed genes were identified in the *M. phaseolina* transcriptome between different temperatures, timepoints, and disease scores. Analysis of weighted gene co-expression networks of 7,827 genes with transcriptomic evidence revealed 23 distinct gene modules. Gene modules with upregulated gene expression during early infection, late infection stages, and during saprotrophic growth on dead root tissues were observed. The early to mid-infection modules were enriched with genes encoding glycoside hydrolases, pectinesterases, and effectors. The profile of carbohydrate active enzyme (cazyme) and effector gene expression was distinct at later infection stages and the transition to these expression patterns occurred earlier for plants that were incubated at higher temperatures. Additionally, there were differentially expressed effectors and cazymes during plant death that resembled the gene expression of *in vitro* control. Our study demonstrated that the switch to necrotrophy occurred earlier at higher temperatures, as evidenced by the transition to necrotrophy-associated patterns of gene expression. More research is needed into whether the higher disease severity at increased temperatures is due to fungal growth rates or factors related to plant susceptibility.

731C **One signal, two kingdoms: Decoding interkingdom plant signals in fungi** James M. Bradley[1], Michael Bunsick[1], George Ly[1], Bruno Aquino[1], Dario Bonetta[2], Peter McCourt[1], Shelley Lumba[1] [1]University of Toronto, [2]Ontario Tech University

Plants and fungi have been interacting for billions of years and must have evolved an extensive molecular dialogue to coordinate an exchange of nutrients and carbon. This dialogue consists of small molecule signals secreted by plants into the soil to mediate interactions with fungi. Despite the importance of plant-fungal interkingdom communication in agri- and ecosystems, very little is known about the mechanisms by which fungi perceive small molecule signals from plants. To address this knowledge gap, we have developed a novel pipeline centered on transcript profiling of the fungal model, *Saccharomyces cerevisiae* (yeast), treated with a plant small molecule, followed by mutational and structure-function analysis to identify a target. As a proof-of-concept, we put strigolactones (SLs), which act as both hormones in plants and as environmental communication cues for plants and fungi in the rhizosphere, through our pipeline. When plants are starving for phosphate, they produce and exude more SLs into the soil to facilitate symbiotic interactions with fungi. Suprisingly, we discovered that SL causes phosphate depletion in a variety of fungi by inhibiting the high-affinity phosphate transporter, Pho84. SL-regulated phosphate responses are conserved in an endophytic fungus called *Serendipita indica* and the pathogen, *Fusarium graminearum*. Through genetic and structure-function analyses, we have identified a potential binding pocket for SL in the phosphate transporter. Intriguingly, this binding pocket is ubiquitous in phosphate transporters across the fungal kingdom. Our results address longstanding evolutionary questions about the molecular dialogue between plants and fungi.

732C **Investigating mitochondrial targeted proteins (MTPs) and their potential contribution in the recent outbreak of *Fusarium* wilt of banana** Joni Rey H Campilan[1], Yong Zhang[2], Li-Jun Ma[3] [1]Plant Biology, University of Massachusetts, Amherst, [2]Organismic and Evolutionary Biology, University of Massachusetts, Amherst, [3]Biochemistry and Molecular Biology, University of Massachusetts, Amherst

Mitochondria fuels fungal pathogens during host infection. A recent study comparing *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (FocTR4) to Foc Race 1 shows over production of reactive nitrogen species nitric oxide in fungal mitochondria during TR4-banana infection (Zhang et al., 2023). The nuclear genome encodes the majority of mitochondrial proteins, which are then delivered to the mitochondria. In Foc TR4 reference genome II5, these mitochondrial targeted proteins (MTPs) remain unknown. The goal of this study is to identify and characterize FocTR4 MTPs using the reference genome II5 to elucidate key mechanisms associated with fungal virulence.

Using known mitochondrial proteins in the model genome *Saccharomyces cerevisiae*, we documented that the prediction tool DeepLoc 2.0 has higher sensitivity in predicting MTPs compared to TargetP 2.0. Using this sequence based predictive tool, we identified a total of 1,039 II5 MTPs with 28.68% of them form orthogroups with human and yeast mitochondrial proteins. RNA-seq and MitoPathway analysis showed that 49.63% of expressed MTPs were upregulated and span all seven functional categories of MitoCarta 3.0-MitoPathways. Protein-protein interaction analysis of upregulated MTPs shows that top hub genes are involved in oxidative phosphorylation. Succinate dehydrogenase (CII), Cytochrome bc1 complex (CIII), Cytochrome c (CIV), and ATP Synthase (CV) were found to be enriched in the oxidative phosphorylation pathway. These enriched upregulated complexes might be associated to ATP synthesis, fueling pathogen machinery in banana Fusarium wilt. Future studies will discuss further the potential contribution of these MTPs in the mechanisms of banana Fusarium wilt.

733C **Symbiotic stress response: Ectomycorrhizal fungi change seedling drought physiology and gene expression** Laura M. Bogar[1], Demorie Ayanna Galarza[2] [1]Plant Biology, University of California, Davis, [2]Plant Sciences, University of California, Davis
As forests around the world face increasingly frequent and severe droughts, understanding how fungal symbiosis impacts tree drought response is essential. Symbiotic relationships between tree roots and soil fungi, known as ectomycorrhizal mutualism, are essential to the survival of most trees, especially at the seedling stage, and are known to improve drought tolerance in many circumstances. The mechanisms by which this occurs, however, are likely complex, and may include direct hydraulic support, nutritional improvements, and immunological impacts. Complicating matters, an individual ectomycorrhizal plant will often associate with dozens of fungal partners simultaneously, each of which may interact with the plant and the soil environment in different ways. To investigate how these diverse ectomycorrhizal fungi affect tree seedling response to drought, we conducted a growth chamber experiment with Douglas fir seedlings (*Pseudotsuga menziesii*) and three fungal partners: *Suillus lakei*, *Truncocolumella citrina*, and *Rhizopogon parksii*. Prior to harvest, watering was stopped for five days for half of the plants. Using lysimetry, we determined that fungal colonization subtly influenced hourly plant water use in ways that varied with species, often decreasing water use in well watered plants and increasing water use in the dried down conditions (especially for plants colonized by *Rhizopogon* or *Truncocolumella*). To examine the molecular underpinnings of these effects, we are performing a differential gene expression analysis for plants and fungi to contrast transcriptional behavior of uncolonized fine roots, symbiotic roots with Truncocolumella, and free-living Truncocolumella hyphae in the soil across well-watered and dried-down conditions. These data link plant and fungal drought physiology to symbiotic gene expression to shed light on how fungal symbiosis impacts plant drought response.

734C **The role of purine metabolism in the *C. elegans* Intracellular Pathogen Response to microsporidia and Orsay Virus infection** Nicole Wernet[1], Eillen Tecle[2], Mario Bardan Sarmiento[1], Crystal Chhan[3], Cheng-Ju Kuo[4], Ian Baick[1], Wendy Hanna-Rose[5], Latisha Franklin[5], Emily Troemel[1] [1]UC San Diego, [2]University of Wisconsin-Eau Claire, [3]University of Washington, [4]Sanford Burnham Prebys, [5]Pennsylvania State University

The intestinal-infecting obligate intracellular pathogen *Nematocida parisii* is a species of microsporidia (fungi), and the most common natural pathogen of the model nematode *Caenorhabditis elegans.* Infection with *N. parisii* induces a similar transcriptional response in *C. elegans* as infection with a molecularly distinct natural pathogen called the Orsay Virus, a single-stranded RNA virus. This shared transcriptional response, called the Intracellular Pathogen Response (IPR), is mostly distinct from responses induced by other pathogens and stressors and promotes resistance against microsporidia and virus infection.

Previously, a forward genetic screen identified the *C. elegans* ortholog of purine nucleoside phosphorylase PNP-1 as a negative IPR regulator. PNP-1 is an enzyme of the purine salvage pathway, and we showed that it acts in intestinal epithelial cells to regulate

defense against intra- and extracellular pathogens (Tecle et al. 2021 PLOS Pathogens). It remained unclear which purine metabolites regulate IPR gene expression in *pnp-1* mutants.

In unpublished work, we have performed a targeted RNAi analysis on other enzymes of the purine salvage pathway and identified adenosine deaminase ADAH-1 as another negative regulator of the IPR. Loss of ADAH-1 leads to increased expression of IPR genes and resistance to intracellular pathogens. Metabolomic analyses confirmed similar enzymatic activity of *C. elegans* ADAH-1 as the human ortholog: RNAi against *adah-1* led to increased levels of adenosine and decreased levels of inosine and hypoxanthine. In supplementation studies, we found that deoxyadenosine induced the IPR and increased resistance to *N. parisii* and Orsay Virus, suggesting increased levels of this purine metabolite triggers IPR expression and resistance in animals defective with either PNP-1 or ADAH-1. In contrast to PNP-1, we found a broader expression pattern of ADAH-1, as it is expressed in epidermis, muscle, neurons, and the intestine. These new findings have similarities with data about type-I interferon signaling in humans, where humans with PNP and ADA deficiencies have increased interferon levels and inflammatory syndromes (Dhanwani et al. 2020 Science Advances). Thus, our findings may have evolutionary implications for human innate immunity as well. Overall, our data suggest that *C. elegans* senses perturbations in purine metabolism as a cue to induce defense mechanisms upon epithelial infections as part of surveillance immunity.

735C **The Cell Wall Glucan-glycogen Complex: A Novel Determinant of the *Candida albicans* Host-pathogen interaction** Jian Miao[1], Zuchao Ma[2], Alex Hopke[2], Michael Kruppa[2], David Williams[2], Kristiana Avad[3], Krik Hevener[3], Brian Peters[3] [1]Clinical Pharmacy and Translational Science, The University of Tennessee Health Science Center, [2]East Tennessee State University, [3]The University of Tennessee Health Science Center

Complex carbohydrates are major components of the fungal cell wall and serve as important pathogen associated molecular patterns to potentiate innate immune responses. We recently reported that glycogen and β-(1→3)-glucan form a covalently linked macromolecular complex associated with the cell wall of *C. albicans*. A recombinant glycogen probe was engineered to visualize the distribution of glycogen content and confirm its cell wall incorporation. Challenge of human PBMCs with isolated glucan-glycogen complexes led to strong increases in pro-inflammatory cytokine signaling as measured by ELISA and transcriptional profiling. Using a combination of biochemical and genetic techniques, we confirmed that loss of *GSY1* (encoding for glycogen synthase) ablated cell wall glycogen content. Challenge of human macrophages with formalin-fixed *gsy1*Δ/Δ led to exacerbated secretion of IL-1β as compared to WT or a revertant strain. Neutralization experiments demonstrated this to be partially mediated by the hDectin-1 receptor. Moreover, loss of glycogen also led to enhanced human neutrophil swarming. Lastly, a collection of vaginal clinical isolates was screened for glycogen content. Remarkably, overexpression of *GSY1* in a subset of reduced glycogen accumulation isolates reversed their hyperinflammatory phenotype, and deletion of *GSY1* in a subset of WT-like glycogen accumulation isolates induced a hyperinflammatory phenotype. Further analysis of cell wall components by fluorescence staining and flow cytometry revealed that levels of total glucan, total mannan and total chitin remained similar, while reduced glycogen significantly correlated with increased β-(1→3)-glucan exposure. Collectively, our data demonstrate that the glucan-glycogen macromolecular complex may be a novel cell wall determinant important for governing the host-*Candida* interaction.

736C **The *Candida albicans* quinone reductase Zta1 promotes resistance to oxidative stress** Rafael M Gandra[1], Chad J Johnson[2], Jeniel Nett[2], James B Konopka[1] [1]Stony Brook University, [2]University of Wisconsin-Madison

Candida albicans is an effective pathogen because it can adapt to dynamic environmental conditions and resist vario us types of stress in the host, including oxidative stress. Previous investigations by our group identified four Flavodoxin-like proteins (FLPs) localized in the eisosome domains of the plasma membrane, functioning as NAD(P)H:quinone reductases. These proteins play a pivotal role in oxidative resistance and contribute significantly to *C. albicans* virulence. We now investigate the functional role of Zta1, a member of a distinct family of quinone reductases named medium-chain dehydrogenase/reductases (MDR). Zta1 fused to GFP localizes to the cytoplasm and is rapidly induced by quinones and other types of oxidants. Deletion of *ZTA1* leads to an accumulation of reactive oxygen species in *C. albicans* upon exposure to quinones and other oxidants, consistent with a role for Zta1 in protection against oxidative stress. Deletion of *ZTA1* in a mutant lacking all four FLP genes (*zta1*D *pst1*D *pst2*D *pst3*D *ycp4*D) increases *C. albicans* sensitivity to 2-tert-Butyl-1,4-benzoquinone, indicating that these quinone reductases work in combination. This *ZTA1* deletion also correlates with increased susceptibility to neutrophil-mediated killing and a reduced kidney fungal burden in mice, indicating a possible partial contribution to *C. albicans* virulence. Altogether, these data indicate that Zta1 contributes to the *C. albicans* capacity to resist the stressful conditions imposed by oxidative attacks by the host immune system.

737C **Combating emerging *Aspergillus fumigatus* triazole resistance by targeting the fungal hypoxia response** Cecilia Gutierrez-Perez[1], Charles Puerner[1], Elisa M Vesely[1], Sandeep Vellanki[1], Jane T Jones[1], Mark A Xaste[2], Andre F.C. Vieira[2], Carissa Perez Olsen[2], Sourabh Dhingra[3], Neveen Sidhom[4], Steven Cardinale[4], Steven M Kwasny[4], Narendran G-Dayanandan[4], Scott Nolan[4], Timothy J Opperman[4], Robert A Cramer[1] [1]Microbiology and Immunology, Geisel School of Medicine at Dartmouth, [2]Chemistry and Biochemistry, Worcester Polytechnic Institute, [3]Clemson University, [4]Microbiotix Inc.

There are currently only three contemporary antifungal therapies to treat aspergillosis in all its manifestations. Antifungal therapy efficacy in established infections is sub-optimal. Moreover, rapidly increasing drug resistance to first line triazole therapies highlights a significant need to develop novel antifungals with innovative mechanisms of action. Research from our and other laboratories has observed that the fungal hypoxia response, mediated by the transcriptional regulator SrbA, is a promising antifungal target necessary for virulence and azole resistance in *Aspergillus fumigatus* and other human pathogenic fungi. To identify inhibitors of the SrbA mediated hypoxia response pathway and overcome triazole resistance, we developed a novel cell-based antifungal assay strain and used it to screen over 200,000 small molecule compounds for antifungal activity in the presence of fluconazole or hypoxic conditions. Using this high-throughput screen we identified MBX-7591: a novel small molecule with minimal mammalian toxicity that has exhibited proof-of-principle in vivo efficacy in a mouse model of invasive pulmonary aspergillosis. MBX-7591 has increased activity in hypoxic conditions, against fungal biofilms, and in the presence of triazoles. MBX-7591 is active against triazole resistant *A. fumigatus* strains and potentiates the activity of triazoles in the setting of known *cyp51A* mutations. Preliminary mechanism of action data suggest that MBX-7591 is acting through the SrbA-dependent hypoxia response pathway to disrupt cell membrane lipid homeostasis . Taken together, these data suggest MBX-7591 is a promising antifungal agent with a novel mechanism of action that can combat the emergence of triazole resistance.

738C **Dispersal and biotic filtering structure Mucoromycota fungal communities and their associated bacteria across two different biomes** Nicole Reynolds[1], Kevin Amses[2], Jessie Uehling[3], Rasheed Adeleke[4], Margaret Branine[5], Teresa E. Pawlowska[6] [1]Integrative Plant Science, Cornell University, [2]Perelman School of Medicine, University of Pennsylvania, [3]Dept of Botany and Plant Pathology, Oregon State University, [4]Unit for Environmental Sciences and Management, North-West University, [5]Graduate Field of Microbiology, Cornell University, [6]School of Integrative Plant Science, Cornell University
Despite the ecological importance of Mucoromycota fungi as mycorrhizal symbionts, opportunistic human and plant pathogens, and post-harvest spoilage agents, they remain understudied compared to Dikarya. Fundamental aspects such as geographical distribution, dispersal patterns, and community structure remain unclear. Furthermore, recent and ongoing discoveries about the endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions regarding their effects on fungal host evolution. EB have different effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. Our investigations of Mucoromycota and their associated bacteria communities are focused on testing three main community filtering hypotheses: dispersal (based on geographic distance), biotic (influenced by plant communities), and environmental (incorporating abiotic variables). We collected rhizosphere soils from four total locations in California representing two biomes (Desert and Mediterranean scrub) with three transects and two different plant species sampled from each site. These samples are being analyzed using both culture dependent and culture independent (metabarcoding) methods. Metabarcoding data were generated using bacterial (16S rDNA) and fungal (28S) primers and show desert communities had higher proportions of Zoopagomycota taxa, whereas the coastal samples had more mycorrhizal taxa (Glomerales and Endogonales). Both biotic filtering and dispersal filtering significantly affected fungal and bacterial communities; however, dispersal filtering was only significant over larger distances (km rather than m scale). In addition, network analyses show differential structural characteristics as well as unidentified OTUs as potentially influential hub organisms. Ongoing culture-based screening results have yielded lower diversity than amplicon data, but potentially new fungal/EB combinations. Phylogenetic analyses indicate several distantly related species from each biome with varying EB associations.

739C **Integrative multi-omics analyses of host and pathogen signaling during Fusarium Head Blight disease of cereals** Lovepreet Singh[1,2], Yadong Huang[1], Gerit Bethke[1], Erin Schwister[3], Gary Muehlbauer[1], Mitch Elmore[3,4] [1]Agronomy and Plant Genetics, University of Minnesota, [2]USDA-ARS Cereal Disease Lab, [3]Cereal Disease Lab, USDA-ARS, [4]Plant Pathology, University of Minnesota

Plant-pathogen interactions are shaped by complex, multi-level signaling networks that ultimately control disease outcomes. By reconstructing the topology of these networks, we can begin to understand how signals are propagated and predict the genes that have the largest influence on plant phenotypes. To this end, we are using integrative multi-omics coupled with network biology to dissect the molecular basis for Fusarium Head Blight (FHB) disease of barley and wheat. We performed time course infection experiments in barley leaves and spikes to analyze host and pathogen signaling during disease progression. Tissue was collected and multi-omics profiling revealed differentially regulated transcripts, proteins, and phosphorylated peptides at specific stages of

infection. Proteomics experiments quantified a total of 12,000 protein groups and 24,000 phospho-peptides, including 3,000 proteins and 6,000 phospho-peptides from *F. graminearum*. We use a machine learning approach to integrate these orthogonal datasets into multi-level models of cellular signaling. Gene regulatory networks predict the transcription factors that regulate modules of differentially expressed transcripts. Phospho-signaling networks identify activated kinases and predict their phosphorylated targets. Proteins with high centrality in the reconstructed networks will be prioritized for functional validation. We are especially interested in host genes that contribute to susceptibility, as well as genes in *Fusarium* that regulate pathogenicity. This dual-organism, multi-omics strategy represents a powerful approach to predict and test the genes that regulate FHB and serves as a foundation for understanding and engineering disease resistance in cereal crops.

740C **Suppression of host immune processes by effectors from *Zymoseptoria tritici*** Graeme J Kettles, Haider Ali School of Biosciences, University of Birmingham

The wheat-infecting fungus *Zymoseptoria tritici* is a significant constraint on crop productivity and a concern for food security. Following spore germination on wheat leaf surfaces, the fungus grows epiphytically before invading leaves through open stomata. Following penetration, the fungus colonises the leaf mesophyll. On susceptible plants, this extensive level of growth happens without the appearance of macroscopic disease symptoms or induction of a host immune response. This phase is commonly referred to as the symptomless, latent or biotrophic phase of this fungus' life cycle. Suppression of host immunity likely plays an essential role during this phase of host colonisation and secreted proteins (effectors) containing LysM-domains are known to contribute to suppression of chitin-triggered immunity. However, *Z. tritici* mutants lacking LysM effectors remain partially virulent. We hypothesised that *Z. tritici* secretes other effectors that have immune suppressive activity that are important for colonisation. To test this hypothesis, we investigated 49 candidate effectors that were previously identified as being highly expressed during the symptomless colonisation phase. We used Agrobacterium-mediated transient expression to express these genes in leaves of the model plant *Nicotiana benthamiana*. We assessed these effectors for ability to (i) suppress host cell death, (ii) suppress the pathogen-associated molecular pattern (PAMP)-induced ROS burst, and (iii) interfere with normal stomatal dynamics. In these experiments, we found that seven effectors suppressed cell death induced by one or more of the *Z. tritici* elicitors Zt9, Zt11 or Zt12. None of the effectors were able to suppress cell death induced by the ribonuclease toxin Zt6. We continued to demonstrate that some *Z. tritici* effectors were able to suppress the ROS burst induced by the PAMPs flg22, chitin or the β-glucan laminarin. Intriguingly, some effectors were able to suppress both induction of cell death and ROS production, suggesting that these proteins may be multifunctional. We are currently investigating whether any of these candidate effectors are able to interfere with stomatal dynamics using thermal imaging. Together, these results suggest that *Z. tritici* relies on a battery of effector proteins to suppress host immunity during the extended symptomless phase of wheat leaf colonisation.

741C **Transcriptional Profiling and Functional Analysis of *Candida auris* Biofilm Regulators During Infection** Tristan W Wang[1], Dimitrios Sofrax[2], Daniel Montelongo-Jauregui[3], Hans Carolus[2], Vincent Bruno[3], Patrick Van Dijck[2], Mary Ann Jabra-Rizk[3] [1]Dept of Oncology and Diagnostic Sciences, University of Maryland Baltimore, [2]KU Leuven, [3]University of Maryland Baltimore

Candida auris is a newly emerged species associated with exponential rise in life-threatening invasive disease due to its high level of transmissibility and multi-drug resistance. We have previously demonstrated aggregative and non-aggregative growth phenotypes for *C. auris* strains with different biofilm forming abilities. Additionally, using clinically-relevant murine models of infection we demonstrated host niche-specific pathogenic traits for *C. auris*. In this follow-up study, we aimed to identify unique transcriptional profiles associated with the two phenotypes during *in vitro* and *in vivo* biofilm growth. Comprehensive RNAseq analysis was performed on aggregative and non-aggregative *C. auris* strains recovered from *in vitro* and *in vivo* grown biofilms using a mouse model of catheter infection. Comparative analysis identified a set of genes consistently differentially regulated in the aggregative strain under *in vitro* and *in vivo* conditions with key roles in adhesion, biofilm formation, stress resistance and white-opaque transition among others. Notably, the gene exhibiting the highest upregulation in the aggregative phenotype encodes a protein displaying partial similarity to the *C. albicans* Rbt1 cell wall adhesin involved in cell-cell adhesion, specifically in the Flo11 domain. To investigate the role of this adhesin, we generated 3 independent mutant strains in the aggregative strain; phenotypic evaluation of the mutants demonstrated significantly reduced adhesion and biofilm formation compared to wild type parental strain. Most interestingly, in an aggregation assay, the strains lacking the adhesin lost aggregative capability. These phenotypic qualities were confirmed using scanning electron microscopy and confocal laser scanning microscopy analysis of biofilms. In summary, our presented findings demonstrated significant transcriptional changes associated with the aggregative form with a key cell wall adhesin being the central mediator of cell aggregation, and observed differences in biofilm formation among the *C. auris* strains. Functional diversity of cell surface antigens is crucial for rapid adaptation to the environment. Therefore, it is plausible to speculate that the aggregative phenotype may possess a form of regulation in cell wall proteins that provide it with flexibility to control its surface qualities, potentially impacting virulence. Evaluation of the mutant strain in our mouse model of catheter infection is currently underway.

742C **Single-cell profiling of *Magnaporthe oryzae* infections on rice plants** Timothy Johnson[1], Christopher Dervinis[2], Wendell Pereira[2], Matias Kirst[2], Jessie Fernandez[1] [1]Microbiology and Cell Science, University of Florida, [2]School of Forestry, Fisheries and Geomatics Sciences, University of Florida

Rice blast disease remains one of the most important diseases threatening global food security. This disease is caused by the filamentous fungus, *Magnaporthe oryzae*. *M. oryzae* is directly responsible for the loss of more than 30% of the rice harvested annually. Although we have a broad understanding of *M. oryzae* morphogenic process, little work has been done to characterize transcriptional changes during M. oryzae infection in both fungal and rice cells. To date, we are in the infancy of understanding what triggers the fungus to differentiate into invasive structures or how plant responses vary in different cell types upon pathogen attacks. This project aims to explore the intimate relationship between *M. oryzae* and rice by looking at the global transcription levels during infection through a single-cell RNA sequencing (scRNA-seq) technique. This technique provides a novel approach to capturing transcriptional changes at the single-cell level to study cell behaviors at a higher resolution during infection. Here, we applied an alternative to generating high-quality protoplasts from plant tissue infected with *M. oryzae* by characterizing the mRNA extracted from individual nuclei instead of whole cells. We initially performed a time course of *M. oryzae* infections on susceptible rice cultivars. After infections, infected leaves were collected, trimmed, and used for nuclei isolation. Nuclei were stained with DAPI and analyzed to assess their integrity with confocal microscopy. Nuclei concentration was estimated using the BD FACSAria™ II fluorescence-activated nuclei sorter (FANS). We obtained approximately 50,000 nuclei at a final concentration of 400 nuclei/µl. Approximately 10,000 nuclei from each sample were subjected to the 10X Genomics Chromium platform to create 3' single-cell libraries. Overall, this work will lead to uncovering novel genes and cellular pathways that are essential for maintaining the intimate association between *M. oryzae* and rice cells. By simultaneously looking at the transcriptional changes in *M. oryzae* and rice, this study will uncover cellular heterogeneity within an infected rice leaf. Moreover, this data will reveal previously unknown perspectives on plant responses to *M. oryzae* infection at the single-cell level.

743C **Unraveling the genetic determinants of virulence in the pathogen *Cryptococcus neoformans*** Katrina Jackson[1], Kirsten Nielsen[2] [1]Pathogen and Microbiome Institute, Northern Arizona University, [2]University of Minnesota

In the last decade it has become clear that the genotype of *Cryptococcus neoformans* is associated with cryptococcal meningitis patient outcome, but the etiology of this link remains unknown. Several studies have shown that Sequence Types (STs), or Multilocus Sequence Typed (MLST) clades of closely related isolates, associate with patient outcome. ST93 is a common sequence type isolated from patients globally and is the most common clinical isolate found in Uganda. In a previous study, we performed whole genome sequencing on 38 ST93 clinical isolates, identified Single Nucleotide Polymorphisms (SNPs) across the population, and performed a Genome-Wide Association Study (GWAS) to link those SNPs to patient outcome and immune response. To further explore the relationship between strain virulence and isolate genotype, we used the mouse inhalation model of cryptococcosis to define disease phenotypes of each isolate. We identified four disease manifestations – typical Central Nervous System (CNS) disease, typical non-CNS disease, latency, and hypervirulence. While most infected mice had a type-2 (CNS and non-CNS disease) or undetectable (latent) immune response, mice infected with hypervirulent isolates caused increased IFNγ, a response previously linked to reduced virulence. Next, we found that disease manifestation corresponded with sub-clades in the ST93 population. We performed a GWAS to identify SNPs related to disease and identified 32 genes associated with mouse survival and immune response. When mice were infected with deletion strains of genes associated with changes in IFNγ, we found that the *itr4Δ* deletion mutant recapitulated the hypervirulent phenotype, and many of the other genes had capsule size defects. These data suggest that a network of mutations in key virulence related genes impact cryptococcal meningitis outcome by altering the host immune response to *C. neoformans*. Finally, we leveraged previous genomic studies of *C. neoformans* and identified genes and gene regions associated with virulence across multiple genomic studies and diverse clinical isolates, indicating the universal function of these regions in cryptococcal disease.

744C **Investigating the prevalence and influence of endohyphal bacteria on Mucorales** Elizabeth R Ballou[1], Dora Corzo-Leon[1], Isabel Dickie[1], Jack Gregory[1], Dan Stark[2], Jessie K Uehling[3], Jason S King[2] [1]MRC Centre for Medical Mycology, University of Exeter, [2]University of Sheffield, [3]Oregon State

Mucorales fungi are the causative agents of destructive and lethal mucormycosis. These soil-associated fungi can engage in intimate and successful bacterial endosymbioses that are critical to plant disease via bacterial-produced endotoxins, and ~50% of clinical mucormycosis isolates host endofungal bacteria. Recent clinical data from our group and others raise the hypothesis that dynamic bacterial-fungal partnerships might also contribute to human disease. This project uses the model clinical isolate *R. arrhizus* var. *delemar* to test the hypothesis that diverse endohyphal bacteria can form stable interactions with clinically relevant Mucorales species.

While the contribution of bacterial endosymbionts to plant disease is well established, their contribution to mammalian disease remains poorly understood. We previously showed that, in an isolate from a wounded-soldier, an endosymbiotic association between *R. microsporus* and the nosocomial pathogen *Ralstonia pickettii* contributed to fungal pathogenesis. The canonical bacterial endosymbiont *Mycetohabitans rhizoxinica* was also identified to cause bacteriemia in a deeply immunocompromised individual suffering from pulmonary *R. microsporus* mucormycosis. Finally, our analysis of more than 60 clinical and environmental Mucorales isolates reveals ~50% prevalence and high levels of diversity among endofungal bacteria.

To better understand the contribution of endofungal bacteria to fungal phenotypes, we modeled bacterial uptake rates in mono- and poly-microbial cultures using high-throughput approaches. Newly established 'holobiont' pairs were evaluated for changes in cell wall characteristics, antifungal susceptibility, stable passage to the next fungal generation, and during co-culture with phagocytic cells. Overall, these observations begin to reveal the potential role of endofungal bacteria as modifiers of Mucormycosis pathogenesis.

745C **The sugar beet (Beta vulgaris subsp. vulgaris) phyllosphere harbors bacteria capable of inhibiting Cercospora beticola, the causal agent of Cercospora leaf spot (CLS)** Madison Christenson[1], Lorena Rangel[2], Mari Natwick[1], Melvin Bolton[3] [1]North Dakota State University, [2]James Hutton Institute, [3]United States Dept of Agriculture

The phyllosphere harbors a large habitat of microbes, with bacteria being the most abundant despite the revolving door of abiotic and biotic stressors. Colonized communities on the foliage can have properties to ward off plant pathogens as a mechanism of self-preservation. Cercospora beticola is a destructive foliar pathogen of sugar beet (Beta vulgaris) causing severe yield loss when left untreated. Fungicides and tolerant cultivars are currently the only defense against this widespread fungal pathogen. However, a functional biocontrol agent could help alleviate the pressure on these disease management methods. Recently, Pantoea ananatis was recovered from a sugar beet leaf infected with C. beticola. This bacterium was able to inhibit fungal growth in vitro without direct contact with the mycelia. Strain specific sequences were acquired, and a genome was assembled to a model P. ananatis genome. A total of 311 type I secreted proteins and secreted bacterial effectors were discovered. Further analysis of the bacterial transcriptome in the presence and absence of C. beticola will be used to narrow down the potential genes responsible for the antagonistic phenotype of P. ananatis.

746C **Intermicrobial carbon substrate metabolism contributes to the pathogenesis of fungal-bacterial intra-abdominal co-infection** Saikat Paul[1], Olivia A Todd[1], Mairi C Noverr[2], Brian M Peters[1] [1]Clinical Pharmacy and Translational Science, The University of Tennessee Health Science Center, [2]Microbiology and Immunology, Tulane University

Fungal-bacterial intra-abdominal infection (IAI) is associated with worse clinical outcomes than monomicrobial or mixed bacterial infection. A murine model of polymicrobial IAI using the prototypical organisms *Candida albicans*(fungus) and *Staphylococcus aureus* (bacterium) results in synergistic lethality that is driven by *Candida*-induced upregulation of *S. aureus* α-toxin leading to increased organ damage. To identify candidal effector(s) of enhanced *agr*activity, we screened a *C. albicans* transcription factor deletion library for reduced capacity to augment *S. aureus agr*activation during polymicrobial growth. We identified several mutants that displayed defects in augmenting *S. aureus agr* activity, but after ruling out confounding variables, only a single mutant (*zcf13*Δ/Δ) failed to cause lethality, organ damage, or elevated α-toxin levels during IAI. Transcriptional profiling was used to initially characterize the function of *ZCF13*. Differential gene expression analysis revealed that the *zcf13*Δ/Δ mutant displayed significant down-regulation of glycolysis-associated genes and *RBK1* (encoding for ribokinase) involved in the conversion of D-ribose in the pentose phosphate pathway (PPP). Phenotypic microarray screening of > 150 carbon substrates further revealed that, unlike wild-type, the *zcf13*Δ/Δ failed to grow on pentose sugars, including D-ribose. *P3*-GFP reporter assays demonstrated that D-ribose significantly inhibited quorum sensing activity and α-toxin production in vitro during co-culture. Quantitation of ribose levels by LC-MS revealed reduced ribose catabolism by *zcf13*Δ/Δ during co-culture and coinfection. Transcript abundance of *RBK1* and *HGT7* (a homologous, low-affinity ribose importer) as measured by qRT-PCR was significantly decreased in the *zcf13*Δ/Δ mutant. Overexpression of HGT7 and RBK1 in the zcf13Δ/Δ mutant restored WT-level pathogenicity during murine IAI. Therefore, we present a model by which the metabolism and import of ribose (and potentially other PPP intermediates) by *C. albicans* derepresses staphylococcal *agr*activation to drive exacerbated toxin secretion. Strategies to reshape the metabolic landscape during fungal-bacterial co-infection may yield new therapeutic approaches to improve the unacceptably high mortality rates associated with polymicrobial IAI.

747C **Effects of Synonymous and Nonsynonymous Mutations on Cyp51 Expression and DMI Resistance in *Cercospora beticola*** Isaac Courneya[1], Lorena Rangel[2], Nathan Wyatt[3], Mari Natwick[1], Gary Secor[1], Viviana Rivera-Varas[1], Melvin Bolton[4] [1]North Dakota State University, [2]James Hutton Institute, [3]United State Dept of Agriculture, [4]United States Dept of Agriculture

Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola*, is the most economically important disease of sugar beet worldwide. One of the primary means of combatting this disease is the timely application of fungicides. Demethylation inhibitors (DMIs) are a class of fungicide that target Cyp51, a key enzyme in the synthesis of an essential fungal cell membrane component called ergosterol. While DMIs are important for managing CLS, resistance to these fungicides has been observed. Previous work in our lab has shown that resistance to DMIs is highly correlated with synonymous and non-synonymous mutations in the Cyp51 gene. One such mutation, L144F, is found in two codon variants TTC and TTT, where TTC is associated with resistance and TTT is associated with sensitivity even though both codons encode phenylalanine. Notably, resistance is also strongly associated with the synonymous mutation E170. We have identified five Cyp51 haplotypes exhibiting different combinations of these mutations. To improve our understanding of the effects of silent mutations on DMI resistance, we have recently undertaken two studies involving these different haplotypes. First, we have analyzed differential expression of Cyp51 across haplotypes in liquid cultures grown in the presence or absence of two different DMIs to assess whether these mutations differentially enhance Cyp51 expression. Significant differences in Cyp51 expression were observed between haplotypes in both unamended media and after difenoconazole treatment. Secondly, we have created mutant strains of *C. beticola* where the native Cyp51 gene is swapped with each of the different haplotypes to assess whether the exchange of this gene affects DMI sensitivity. The results and potential implications of the Cyp51 expression and DMI sensitivity studies will be presented.

748C **Characterizing the *Magnaporthe oryzae* acyl-CoA-binding protein-encoding gene *ACB1* reveals critical roles in homeoviscous adaptation during rice infection** Michael Richter, Richard Wilson, Wayne Riekhof University of Nebraska-Lincoln

Rice (*Oryza sativa*) is of global importance and production is limited by devastating diseases such as rice blast, caused by *Magnaporthe oryzae*. The initial phase of biotrophic growth is facilitated by the translocation of effectors into the host cytoplasm following secretion via an unconventional pathway into the biotrophic interfacial complex (BIC), a plant membrane-rich compartment formed outside of the invasive hyphae (IH). Apoplastic effectors follow the conventional endoplasmic reticulum (ER)-Golgi-dependent secretion pathway; both types of effectors lead to the suppression or evasion of plant immunity. Our molecular understanding of fungal growth during biotrophy is limited and closing this knowledge gap may aid in the discovery of novel determinants of virulence that could be exploited to restrict the spread of rice blast. Here, to determine how fungal lipid metabolism contributes to biotrophy, we focused on characterizing the *M. oryzae ACB1* gene encoding a putative acyl-carrier protein involved in lipid biosynthesis. Using targeted gene deletions, plate tests and live-cell imaging, we discovered that *ACB1* was required during biotrophy for effector trafficking through the ER-Golgi pathway, and for BIC integrity, in a temperature-dependent manner. Loss of *ACB1* resulted in mutant strains that accumulated Pwl2-mCherry:NLS in two or more BICs in the first infected host cell and retained the apoplastic effector Bas4 in IH, suggesting membrane trafficking through the ER-Golgi pathway was perturbed which we confirmed using the FM4-64 dye. On plates, D*acb1* strains were defective, compared to wild type (WT), for growth at suboptimal (22˚C) and optimal (26˚C) temperatures, but grew like WT at supraoptimal (29˚C) temperatures. LC-MS/MS analyses showed perturbations in the homeostasis of saturated and unsaturated fatty acids at all temperatures in D*acb1* compared to WT, most noticeably in the relative abundance of palmitic acid (16:0; reduced in D*acb1*) and alpha-linolenic acid (18:3; increased in D*acb1*). In detached rice leaf sheath assays, BIC formation and apoplastic effector secretion were remediated when D*acb1* colonized rice at 29˚C (following a temperature shift from 26˚C). On whole plants, after 5 days of inoculation, D*acb1* virulence was compromised at temperatures lower than 29˚C, but the mutant strain was fully virulent compared to WT at 29˚C (following a switch from 26˚C). Thus, *ACB1* is required for permitting the fungus to infect rice at low- and optimal temperatures, but is dispensable for infection at supraoptimal temperatures, likely due to its temperature-dependent role in homeoviscous adaptation. This work thus provides the first insights into the molecular processes required for thermal adaptation during host infection, which may be relevant to understanding pathogen strategies for coping with the fluctuating temperature extremes expected under climate change.

749C **Fungi with diverse lifestyles employ antimicrobial proteins to mediate niche establishment** Anton Kraege, Fantin Mesny, Valentina Wolf, Bart Thomma Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne

Recently, several plant pathogenic fungi were shown to use antimicrobial proteins to manipulate the plant microbiota and promote host colonisation. For example, during various stages of the infection cycle, the soil-borne fungus *Verticillium dahliae* secretes various proteins with antimicrobial activity to supress the growth of diverse antagonistic microbes. However, it is unclear how many antimicrobials are encoded in the *V. dahliae* genome to mediate host colonization, nor how widespread the use of antimicrobial proteins among other fungal plant pathogens is to promote host colonisation. To discover novel antimicrobial proteins encoded in fungal genomes we developed a machine-learning based predictor that can recognize antimicrobial activity based on protein sequence and (predicted) structural properties. Surprisingly, thirty percent of the predicted secretome of *V. dahliae* is a predicted antimicrobial. Intriguingly, a similar proportion is not only encoded by other plant pathogens, but also by

fungi with other lifestyles. To investigate how conserved such antimicrobial proteins are, we predicted the antimicrobial activity of the most conserved secreted protein families in a set of 150 phylogenetically diverse fungi. Remarkably, many protein families that are widely conserved in fungi have predicted antimicrobial activity, suggesting that they are used for microbiota manipulation in diverse microbial ecosystems. We propose that fungi with diverse lifestyles have co-opted antimicrobial proteins that evolved in ancestor fungi to act in current-day niche establishment by targeting microbial niche competitors.

750C **An Analysis of Cryptococcal Dissemination and Organ Seeding** Joseph M Bednarek[1], Payton Compton[2], Jessica Brown[2] [1]Biology, University of Utah, [2]University of Utah

BACKGROUND: *Cryptococcus neoformans* is an opportunistic fungal pathogen with global distribution. Infections begin in the lungs, where fungi can lie dormant or proliferate under immunosuppression. Proliferation is followed by escape to the bloodstream and dissemination to extra-pulmonary organs. The dynamics of dissemination remain poorly understood. There are three hypotheses describing this process: 1) the 'free fungi' model, in which fungi cross barriers without assistance from phagocytes, 2) the 'Trojan horse' model, where fungi cross barriers and disseminate inside phagocytes, and 3) a hybrid model, in which fungi exploit phagocytes for some steps (i.e. endothelial barrier crossing), but not others (i.e. delivery to barriers). Previous work has demonstrated that *C. neoformans* is capable of crossing an *in vitro* blood-brain barrier (BBB) when internalized by macrophages. In contrast, murine intravital and zebrafish imaging have demonstrated that *C. neoformans* can cross the BBB without host phagocytes. These conflicting findings indicate a need for further study of *C. neoformans* infections, particularly at the blood-organ interface where dissemination occurs. **OBJECTIVE:** With this study, we intend to answer two fundamental questions regarding *C. neoformans* in the blood. How many fungi persist in the blood throughout infection? Do blood fungi disseminate as free yeast or inside phagocytes? **METHODS:** Blood dissemination is studied using the murine model of cryptococcosis and a hybrid flow cytometer/fluorescent microscope called an Amnis ImageStream. The latter provides the first opportunity to resolve whether fungi are internalized by or associated with phagocytes in the blood, while simultaneously identifying blood phagocytes by surface markers. **RESULTS:** The number of fungi in the blood increases steadily from 3 to 21 days post infection. Free fungi also increase steadily, whereas those associated with phagocytes remain constant. Therefore, fungi predominantly disseminate as free cells in the bloodstream. This observation is consistent throughout the course of infection. **CONCLUSIONS:** This finding provides new insight into the state of fungi in the bloodstream and supports the hybrid model of dissemination. It could represent a shift in how we think about *C. neoformans* infections.

751C **Waterways are hotspots for *Coccidioides* in arid, urban environments** Robert Wagner[1], Liliam Montoya[1], Justin V. Remais[2], John W. Taylor[1] [1]Plant and Microbial Biology, University of California, [2]Division of Environmental Health Sciences, University of California

Recent ecological studies focused on the fungal agent of Valley Fever (*Coccidioides* spp., coccidioidomycosis) have targeted undisturbed land, highly disturbed agricultural land, air, and lungs of native rodents in the San Joaquin Valley of California and much of Arizona. Results of these studies are consistent with the hypothesis that *Coccidioides* behaves, following inhalation, like an endophyte, lurking not in plants but imprisoned in granulomas in the lungs of native rodents. As an endozoan, it waits until the rodent dies to be released from the granuloma and use the carcass as a substrate to grow and reproduce. Here we sample disturbed, urban landscapes, by taking soil from rodent burrows in the riparian environments formed by waterways that flow in or near two California cities, Coalinga and Bakersfield, each with high annual incidences of Valley Fever. From a total of 52 sites, we surveyed 1030 rodent burrow soil samples for *Coccidioides* presence comprising 825 samples taken along the Kern River in Kern County and Los Gatos Creek in Fresno County and 205 samples from in and around Bakersfield. We surveyed an additional 100 urban, surface samples from Coalinga. A subset of Kern River sites was sampled over one year at four timepoints. Presence of *Coccidioides* was determined for all samples using the CocciEnv, qPCR test that detects a multicopy transposon unique to *Coccidioides*. The total fungal community was characterized using the fungal barcode (internal transcribed spacer, ITS2) for 68 rodent burrow soil samples (28 from the Kern River and 40 from Los Gatos Creek). *Coccidioides* was found abundantly and consistently in rodent burrow soils situated in urban and urban-adjacent riparian habitats in the San Joaquin Valley. *Coccidioides* was more commonly found closer to population centers and not found along the Kern River at higher elevations. *Coccidioides* was found at 12 of 52 sites and its presence was temporally consistent with no seasonal pattern. Few associations were present between *Coccidioides* and the greater soil fungal community. The lack of seasonality in *Coccidioides* detection and its sporadic geographic distribution raise questions about the persistence of infection in rodent subpopulations and how the fungus spreads within and among rodent subpopulations. Long-term surveys of *Coccidioides* in rodent populations will be needed to address these questions.

752C **Exploring the role of carbon catabolite repression in *Aspergillus fumigatus* virulence in the cornea** Becca Wells[1], Emily Adams[2], Kevin Fuller[1,2] [1]Microbiology and Immunology, University of Oklahoma Health Sciences Center Center, [2]Ophthalmology, Dean McGee Eye Institute, University of Oklahoma Health Sciences Center

Fungal keratitis (FK) is an invasive infection of the cornea that, despite antifungal intervention, results in vision loss or a need for corneal transplantation in up to 50% of cases. The development of better therapeutics first requires an understanding of fungal pathways that promote adaptation and growth within the corneal tissue. The transcription factor CreA acts as the major regulator of carbon catabolite repression (CCR) for the common FK pathogen *Aspergillus fumigatus*, repressing genes associated with alternative carbon source utilization in the presence of glucose. CreA has been shown to contribute to *A. fumigatus* virulence in the lung, particularly at stages of infection that correlate with the development of tissue hypoxia, and a subsequent metabolic shift of the fungus toward glycolysis. As our group has recently demonstrated that the cornea becomes hypoxic shortly after fungal inoculation, we hypothesized that CreA similarly contributes to *A. fumigatus* virulence in the setting of FK. To test this, we generated a knockout strain in which the gene encoding CreA was deleted using Cas9-mediated homologous recombination. This deletant displayed a moderate growth defect on both carbon catabolite repressive and de-repressive substrates. The *creA* knockout was also hypersensitive to the cell wall targeting agents Congo red, calcofluor white, and caspofungin, in agreement with previous findings that suggest CreA regulates cell wall homeostasis. To further probe the influence of CreA on the cell wall, we stained WT and *creA* knockout hyphae with calcofluor white and wheat germ agglutinin. Decreased total as well as exposed chitin was observed in the *creA* knockout strain, indicating that the transcription factor modulates cell wall composition and antigenicity. Virulence of the mutant was assessed in 6-8 week old male C57BL/6 mice using an epithelial damage model of FK. Strikingly, the *creA* knockout was unable to establish infection in the cornea. No fungus was recovered from *creA* knockout inoculated corneas at 72h p.i., and these eyes showed no clinical signs of FK, demonstrating that the carbon catabolite repressor is required for the establishment of corneal infection. Beyond regulating nutrient metabolism, CreA's influence on the cell wall could impact fungal interaction with the host immune system during FK, leading to rapid clearance of the mutant from the cornea. Future work will focus on the exploration of this possibility.

753C **Comparative proteomic analysis of *Solanum lycopersicum* in response to endophytic and pathogenic strains of *Fusarium oxysporum*** Madison Newman[1], Shira Milo Cochavi[2], Gengtan Li[3], Minjae Ko[3], Abraham Meyerson[3], Domingo Martinez Soto[4], Li-Jun Ma[3] [1]Biochemistry and Molecular Biology, University of Massachusetts Amherst, [2]The Open University of Israel, [3]University of Massachusetts Amherst, [4]Ensenada Center for Scientific Research and Higher Education

Plant-fungal interactions involves molecular communication between the fungus and the plant host; however, little is known about how host plants differentiate between beneficial versus pathogenic fungi. Identifying fungal secreted proteins during these interactions can give insights into mechanisms involved in the different interactions. This study employs *Fusarium oxysporum* species complex (FOSC) that includes both pathogenic and endophytic strains that share a highly conserved core genome but also contain differing genetic material on their respective accessory chromosomes that contribute to distinct plant responses after colonization. To explore pathogenic versus endophytic cross-talks and identify secreted proteins contributing to these differential interactions, the apoplast of the host plant *Solanum lycopersicum* was extracted after inoculation with pathogenic strain Fo4287 and endophytic strain Fo47, respectively, for protein identification using mass spectrometry. We identified a total of 189 proteins in the apoplastic fluids of tomato plants inoculated with the endophytic strain Fo47. Of those, 62 were predicted to be candidate effectors. These secreted proteins have roles in different pathways, including biosynthesis of secondary metabolites, amino acid metabolism, and lipid metabolism. For Fo4287, the host-specific pathogen for *S. lycopersicum*, there were 121 predicted proteins with 41 being predicted as effectors. These proteins play roles in amino acid metabolism, lipid metabolism, metabolism of cofactors of vitamins, and more. We will further discuss how secreted proteins contribute to the distinct beneficial versus pathogenic interactions.

754C **Functional characterization of the Avr4 effector in the banana pathogen *Pseudocercospora fijiensis* using CRISPR-Cas9-mediated transformation** Maikel B.F. Steentjes, Rahim Mehrabi, Gert H.J. Kema Laboratory of Phytopathology, Wageningen University

Pseudocercospora fijiensis is the causal agent of Black Leaf Streak Disease (BLSD), or black Sigatoka, of banana. The disease affects many banana varieties, including the highly susceptible Cavendish banana that dominates the global export trade, and several cooking bananas that are a staple food for hundreds of millions of people worldwide. Currently, the disease is controlled using preventative fungicide treatments with up to 70 applications per year in Cavendish plantations, which accounts for approximately 30% of the production costs. Resistant cultivars are required for more sustainable production, but not a single resistance gene to

BLSD has been identified. This is partly due to the poor genetic amenability of *P. fijiensis* and lacking methods for functional gene analysis.

To address these limitations, we developed an optimized CRISPR-Cas9-mediated transformation system for *P. fijiensis*. First, we established a protocol for the production of *P. fijiensis* protoplasts, evaluated their capacity to regenerate into new colonies, and assessed their sensitivity to hygromycin and other antibiotics. Secondly, a PEG-mediated transformation was performed using these protoplasts to assess the delivery and integration of foreign DNA including resistance markers into the fungal genome. This resulted in the generation of *P. fijiensis* mutant lines expressing mCherry and GFP, which can be useful tools to study infection biology.

Thirdly, following the successful generation of these ectopic mutants, we pursued targeted transformation using CRISPR-Cas9. We knocked out the renowned effector Avr4 with a notable transformation efficiency of 55%. The knockout mutants were not reduced in their virulence on susceptible Cavendish plants, indicating that Avr4 is not essential for virulence. In addition to susceptible plants, we also tested the Avr4 mutants on the wild diploid banana accession Calcutta 4, which is resistant to *P. fijiensis*. It was reported that this is likely due to the recognition of Avr4. Our results, however, show that Calcutta 4 remains resistant upon inoculation with the *P. fijiensis* Avr4 knockout mutants, suggesting that the resistance is based on the recognition of alternative effectors. The established gene disruption system is efficient and enables functional characterization of any gene, particularly virulence factors, to provide insights into the pathogenesis of *P. fijiensis* and the identification of resistance genes against BLSD.

755C ***Trichoderma atroviride*** **small RNA1 targets the Arabidopsis *PRIM2* gene to establish a mutulaistic relationship** Sergio Casas-Flores, Eyra Judith Hernández-Hernández, Mitzuko Dautt-Castro Molecular Biology, Institute for Scientific and Technological Research of San Luis Potosi

In their natural settings, plants interact with both, pathogens and beneficial microorganisms. To defend from pathogens, plants have evolved several layers of defense, including basal chemical defenses, structural barriers and innate immunity. Once pathogens surpass the first defense barriers, plants trigger sophisticated mechanisms to neutralize pathogen attack, which initiates with the detection of pathogen-associated molecular patterns (PAMP) to activate PAMP-triggered immunity (PTI), that limits the pathogen spreading from the original site of infection. However, some pathogens have developed effector molecules to suppress PTI. It has been shown that small RNAs (sRNAs) produced by pathogens can act as effectors molecules to suppress plant immunity. *Trichoderma* spp. are plant beneficial fungi that colonize plant roots, conferring beneficial effects to plants by promoting their growth and inducing the systemic disease resistance. Here, we show that the *Arabidopsis* DNA primase large subunit encoding gene, *PRIM2* is targeted by the *T. atroviride* small sRNA1 (*Ta*_sRNA1). *Ta*_sRNA1 is accumulated in the presence of Arabidopsis, which anticorrelates with the downregulation of *PRIM2*. Arabidopsis overexpressing lines of *Ta*_sRNA1 (*At*_OE_sRNA1) showed differential accumulation levels of *Ta*_sRNA1, which agree with low levels of *PRIM2*. Furthermore, co-expression assays of *Ta*_sRNA1 and *PRIM2* wild-type (wt) gene in tobacco, showed decreased accumulation of *PRIM2* transcript, whereas a *PRIM2* version bearing synonymous mutations on the *Ta*_sRNA1 target site was resistant to mRNA slicing. Arabidopsis transgenic lines bearing a short tandem target mimic (STTM) to interfere with the activity of *Ta*_sRNA1 showed increased accumulation of *PRIM2* during its interaction with *T. atroviride*. In addition, both *prim2* mutants and *At*_OE_sRNA1 plants presented enhanced resistance to the fungal pathogen *Botrytis cinerea*. Intriguingly, *At*_OE_sRNA1 and Col-0 (wt) plants inoculated with a *T. atroviride* strain overexpressing the *Ta*_sRNA1, manifested enhanced susceptibility to *B. cinerea*. Together, our results indicate a role of *Ta*_sRNA1 in stablishing of mutualistic relationship of *T. atroviride* with plants

756C **Exploring Ectomycorrhizal Fungal Diversity of three Taiwan Endemic Pinaceae Trees in Mountain Forest Ecosystems** Ren-Cheng Liu[1], Wan-Rou Lin[2], Alija Bajro Mujic[3], Pi-Han Wang[1] [1]Dept of Life Science, Tunghai University, [2]Bioresource Collection and Research Center, Food Industry Research and Development Institute, [3]Dept of Biology, California State University, Fresno

Global warming impacts on species diversity and distribution in forest ecosystem has become a topic attracting wide attention. Taiwan is a small island (area ca. 36,000 km2), spanning both tropics and subtropics, and has more than 300 mountains greater than 3,000 meters. The high mountain forests predominantly consist of three Taiwan endemic Pinaceae plant species, Taiwan fir (*Abies kawakamii* (Hayata) T. Itô), Taiwan hemlock (*Tsuga chinensis* var. *formosana* (Hayata) H.L.Li & H.Keng), and Taiwan spruce (*Picea morrisonicola* Hayata). These trees are ectomycorrhizal (ECM) plants. The ECM symbiosis is a crucial for maintaining functions and stability of the forest ecosystem. This study describes ECM fungal communities of bulk soil (background) and roots (host associated) of these three Pinaceae plant species from high elevation mountain ecosystems using Illumina MiSeq sequencing. The main results showed Taiwan fir and Taiwan hemlock share a high proportion of ECM fungi within their ECM community, whereas Taiwan spruce has a significantly different ECM fungal community with certain ECM fungi not detected in Taiwan fir and

Taiwan hemlock. These results demonstrate that host effect is the main determination of the ECM fungal community in both roots and soil. Furthermore, the dominant ECM fungi associated with each tree species were core species of the ECM community rather than satellite species of the ECM community. Based on these findings, it is predicted that Taiwan hemlock could migrate into forests of Taiwan fir and encounter compatible ECM fungi and *vice versa*. Thus, the availability of ECM fungi might not limit the migration of these two endemic Pinaceae trees. For Taiwan spruce, it is necessary to identify potential habitats for *ex situ* conservation to protect this vulnerable plant and sustain forest development. Additionally, the core ECM fungi of these three endemic tree species could potentially serve as inoculum for aiding in plant seedling cultivation and conservation.

757C **Exploring Microbiome Stability and Biocontrol Agents in Rice Blast Mitigation: A Gnotobiotic Approach with Oryza Sativa** Tim Johnson Microbiology and Cell Science, University of Florida

The microbiome of eukaryotic species impacts a diverse range of systems within the host organism and has the potential to cause significant alterations in host fitness. In the context of plants, there is an interplay between microbiome stability and host immune function. Using the model organism Oryzae sativa we plan to explore the interaction between the microbiome and rice blast infection using a gnotobiotic growth system. Simultaneously we used the growth system to test potential biocontrol agents (BCAs) against rice blast infection. By simultaneously elucidating the impact on the microbiome by rice blast infection and identifying novel BCAs, we aim to generate a robust new method of rice blast mitigation.

758C **CbCyp51 mediated DMI resistance is modulated by codon bias** Lorena Rangel[1], Nathan Wyatt[2], Micah Curran[3], Isaac Courneya[3], Mari Natwick[3], Gary Secor[3], Viviana Rivera-Varas[3], Melvin Bolton[2] [1]James Hutton Institute, [2]United States Dept of Agriculture, [3]North Dakota State University

Cercospora leaf spot (CLS) is the most damaging foliar disease of sugar beet globally. To combat CLS, multifaceted efforts are widely employed, including breeding for resistance, cultural practices, and the application of fungicides. However, populations of Cercospora beticola have become resistant to most fungicides used for CLS management, including those in the sterol demethylation inhibitor (DMI) class of fungicides. In this study, we sampled nearly 600 isolates of Cercospora beticola from MN and ND during the 2021 sugar beet growing season. For each isolate, EC50 values were determined for DMIs tetraconazole (Eminent), prothioconazole (Proline), difenoconazole (Inspire), and mefentrifluconazole (Revysol). Using the CYP51 gene sequence for each isolate, we determined that the synonymous E170 mutation and the synonymous/nonsynonymous L144(F) can be used to predict resistance to these four DMIs. The prevalence and accuracy of the six mutation combinations were calculated and specific combinations can predict resistance with greater than 90% accuracy. Interestingly, one prevalent mutation combination resulted identified cross-resistance to difenoconazole and mefentrifluconazole, but sensitivity to tetraconazole and prothioconazole. This data reveals the importance of codon bias in fungicide resistance and is the first demonstration of the use of synonymous mutations to predict cross-resistance.

759C **The effect of *Candida albicans* ENA family P-type ATPases on pH maintenance and virulence** Jennifer L Tenor[1], Dena L Toffaletti[2], Wiley A Schell[2], John R Perfect[1] [1]Medicine, Duke University School Of Medicine, [2]Duke University School Of Medicine

The ENA family of P-type ATPases have gained interest as potential antifungal targets. These proteins are distinct from human P-type ATPases and can play a critical role in maintenance of the fungal cytosolic pH. We and others have previously established that *ENA1*, a P-type ATPase, is necessary for *Cryptococcus* to cause disease in the mouse and rabbit animal models. Yet, little is known about the importance of the ENA family P-type ATPases, *ENA21* and its paralogue, *ENA22*, in another human fungal pathogen, *Candida albicans*. To further our investigation of ENA family P-type ATPases and their relevance as an antifungal target, we constructed single and double deletion strains of *ENA21* and *ENA22*. The results from in vitro phenotypic analyses demonstrated that *ENA21* and *ENA22* are compensatory as loss of these genes individually resulted in growth like wildtype. However, loss of *ENA21* and *ENA22* together resulted in a strain unable to grow at alkaline pH. Importantly, this double deletion strain failed to establish disease in our systemic candidiasis mouse model. Our results have demonstrated that ENA family P-type ATPases in the invasive fungal pathogen, *C. albicans*, like *Cryptococcus* is required for in vivo survival and indicate that ENA P-type ATPases are necessary for yeast cell viability in vivo. This work further supports the building evidence that the ENA family of P-type ATPases are viable targets for the development of antifungals.

760C **Discovery of plant- and algal-derived plastids in diverse fungi** Julia Kelliher[1], Aaron Robinson[1], Demosthenes Morales[1], La Verne Gallegos-Graves[1], Karen Davenport[1], Guillaume Cailleau[2], Saskia Bindschedler[2], Gregory Bonito[3], Pilar Junier[2], Patrick Chain[1] [1]Los Alamos National Laboratory, [2]University of Neuchatel, [3]Michigan State University

Fungi can form complex and close associations with plants and algae as well as plant- and algal-associated chloroplasts and other plastids. These interactions can affect organismal functioning in a variety of ways ranging from fungal modulation of photosynthesis to the stimulation of chloroplast-derived defenses against fungal pathogen invasion. However, little is known regarding the nature and extent of more intimate associations between fungi and plastids, nor what these relationships may mean in the broader context of fungal interactions. In filamentous fungi, new endohyphal relationships with bacteria are being continuously discovered, but the broader endohyphal microbiome and its potential inhabitants (e.g. archaea, viruses, other fungal cells, etc.) remain largely underexplored. Herein, we present the discovery of a new form of kleptoplasty, where we demonstrate that phylogenetically diverse fungi can internalize plant and algal-derived plastids that can then persist inside fungal cells. Plastome sequences were initially identified when screening a fungal culture collection for bacterial associates. Further investigations utilizing fluorescence *in situ* hybridization imaging, phylogenetic analyses, probe-based chloroplast enrichment sequencing from fungi, screens of tens of thousands of publicly available fungal sequencing datasets, and chloroplast internalization experiments confirmed our findings, and supported the notion that phylogenetically diverse fungi harbor plastids derived from various plants and algae. Supported by a Joint Genome Institute (JGI) Community Science Program (CSP), internalization experiments coupled with time-course transcriptome sequencing on both the fungal and plastid sides provide insights into the mechanisms associated with fungal internalization of endohyphal microbiome components. While the functional implications of such interactions are not yet known, our discovery of fungal kleptoplasty has far reaching implications for fungal biology and fungal evolution, plant-fungal and algal-fungal interactions, and bioengineering.

761C **Transcriptional Responses During Early Fungal-Algal Symbiotic Interactions in the lichen-forming fungus *Umbilicaria muhlenbergii*** Diwen Wang[1], Yanyan Wang[2], Jin-Rong Xu[1] [1]Purdue University, [2]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences

Lichens are the mutualistic symbiotic association of a heterotrophic fungal partner with the photobiont. The ascomycete fungus *Umbilicaria muhlenbergii* is the only lichenized fungus that exhibits a yeast-to-hypha/pseudohyphal dimorphic transition during symbiotic interactions with algal cells, which is regulated by the *UMP1* MAP kinase and cAMP signal pathways. To better understand symbiotic interactions between fungal-algal cells and the role of *UMP1*, we conducted RNA-seq analysis of the wild type and *ump1* mutant strains of *U. muhlenbergii* co-incubated with algal cells of *Trebouxia jamesii* for 10 days. Among the 326 differentially expressed genes (DEGs) upregulated by algal cells, we identified genes that are important for capsule formation and pseudohyphal/hyphal growth, which are associated with the development of primitive lichen thalli. The promoter sequences of the upregulated DEGs were found to be enriched for an element equivalent to the Tec1-binding site and other transcription factors known to be involved in dimorphic transition in *Candida albicans*. In addition, our analysis revealed that upregulated DEGs tend to have more introns and are enriched for genes related to RNA splicing or processing. 109 putative fungal effectors were identified from the whole genome and 6 of them is upregulated during the symbiotic interactions. Further analysis and functional characterization of putative regulators and fungal effectors involved in dimorphic transition are in progress.

762C **Mechanisms of bacterial-fungal interactions and their environmental roles** Leah Johnson, Reid Longley, Julia Kelliher, Buck Hanson, La Verne Gallegos-Graves, Aaron Robinson, Patrick Chain Los Alamos National Laboratory

Soil microbes perform important functions in their environmental niche, such as nutrient cycling, carbon sequestration, and contributing to overall ecosystem health and resilience. As two dominant constituents of the soil microbiome, bacteria and fungi play important roles in these ecosystems, and interactions between these organisms can shape the function of the microbial community overall. While there is some knowledge on how bacteria and fungi impact each other, it is a burgeoning field and the impacts of bacterial-fungal interactions (BFIs) on their communities and ecosystems are not well understood, particularly in the face of a changing climate. Our group aims to systematically characterize interactions between bacteria and fungi and their underlying genetic and functional mechanisms to understand how BFIs impact their roles in their environments and hosts under changing climate conditions. To this end, we have isolated bacteria and fungi from the stress-tolerant plant, Bouteloua gracilis (blue grama), across three desert grassland sites in New Mexico. These field sites undergo climate change-relevant stresses such as drought and warming, and provide a valuable model for predicting how these stresses will impact the resident microbes. We have screened bacterial-fungal interaction phenotypes, such as pigmentation and growth inhibition, of blue grama endophytic bacteria (e.g. Pseudomonas, Bacillus, Streptomyces) and fungi (e.g. Darksidea, Fusarium, Monosporascus), under a relevant soil warming stress temperature for our field sites. These isolates have been sequenced to enable a multi-omics approach towards characterizing the underlying molecular mechanisms of these BFI phenotypes. We will additionally characterize how these BFIs

impact their blue grama host, such as impacts on root colonization by these endophytes and plant stress responses. Together, this work will elucidate how BFIs impact their environments and plant hosts under changing climate conditions.

763C **The fungal virulence factor cardiolipin synthase MoGep4 acts as a fungicide target** Peng Sun, Guotian Li Dept of Plant Pathology, Huazhong Agricultural University

Crop diseases cause serious losses to agriculture worldwide. To investigate the mechanisms of pathogenesis of plant pathogens lays the foundation for the development of fungicides. Phospholipids are important components of biological membranes, including that of autophagosomes, and enzymes involved in phospholipid biosynthesis also serve as fungicide targets. Mitophagy is specialized autophagy that degrades impaired or excessive mitochondria. Although multiple proteins involved in mitophagy have been identified, the role of phospholipids in mitophagy and pathogenesis is largely unknown in pathogenic fungi. Deletion of the cardiolipin biosynthesis related gene *MoGEP4* in the rice blast fungus *Magnaporthe oryzae* resulted in defects in growth, conidiation and virulence. Functionally, MoGep4 is involved in cardiolipin biosynthesis, mitochondrial function and mitophagy. Alexidine dihydrochloride, a MoGep4-interacting chemical shown by different techniques, inhibited cardiolipin biosynthesis and mitophagy. In plant infection assays, alexidine dihydrochloride efficiently inhibited 10 plant pathogens under the laboratory conditions and controlled rice blast disease and Fusarium head blight in the field. Our study demonstrated that MoGep4 regulates mitophagy-mediated pathogenesis in *M. oryzae*. Meanwhile, we show that the MoGep4-interacting chemical, alexidine dihydrochloride, displays broad-spectrum antifungal activity.

764C **Endophyte fungi of the *Talaromyces* genus help *Typha latifolia* plants to tolerate contamination by heavy metals** Amauri Ponce-Hernández[1], Javier A. Gómez-Rubio[1], Candy Carranza-Álvarez[1], Domingo Martinez Soto[2] [1]Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, [2]Dept of Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada

Typha latifolia plants grow naturally in contaminated sites. They can tolerate high concentrations of heavy metals and other toxic contaminants. The plant roots are the tissue establishing more interactions with soil microorganisms. For example, roots can be colonized by endophytic fungi establishing beneficial interactions. The goal of this work was to evaluate the capacity of tolerance of the root endophytic fungi of *T. latifolia* to the stress caused by heavy metal contamination and understand how these fungi help plants to tolerate toxic concentrations of metals. First, we determined the concentrations and diversity of heavy metals in the leaf, stem, rhizome, and root of *T. latifolia* plants collected from highly contaminated sites. Interestingly, the roots showed a higher concentration and diversity of heavy metals. Afterwards, 26 endophytic fungi were isolated from the roots, and ten of them showed a significant tolerance to toxic concentrations of heavy metals and agrochemical compounds. The ten best metal-tolerant endophytic fungi belong to the *Talaromyces* genus. Finally, we also evaluated how the colonization of *T. latifolia* roots by the endophytic *Talaromyces* fungi helps the plant to tolerate toxic concentrations of metals and improve its capacity for metal bioaccumulation.

765C **Horizontal transfers between fungal *Fusarium* species contributed to successive outbreaks of coffee wilt disease** Lily D Peck[1], Timothy Barraclough[2] [1]Ecology and Evolutionary Biology, University of California Los Angeles, [2]University of Oxford

Outbreaks of fungal disease have devastated plants and animals throughout history. Over the past century, the repeated emergence of coffee wilt disease caused by the fungal pathogen *Fusarium xylarioides* severely impacted coffee production across sub-Saharan Africa. To improve disease management of such pathogens, it is crucial to understand their genetic structure and evolutionary potential.

We compared the genomes of 13 historic strains spanning six decades and multiple disease outbreaks to investigate population structure and host specialisation. We found *F. xylarioides* comprises at least four distinct lineages: one host-specific to *Coffea arabica*, one to *C. canephora* var. *robusta*, and two historic lineages isolated from various *Coffea* species. Mapping variation onto a new long-read reference genome showed that host-specificity appears to be acquired through horizontal transfer of effector genes from members of the *F. oxysporum* species complex. This species complex is known to cause wilt disease in over 100 plant species. Multiple transfers into the *F. xylarioides* populations matched to different parts of the *F. oxysporum* mobile pathogenicity chromosome and were enriched in effector genes and transposons. Effector genes in this region and other horizontally transferred carbohydrate-active enzymes important in the breakdown of plant cell walls were shown by transcriptomics to be highly expressed during infection of *C. arabica* by the fungal arabica strains. Widespread sharing of specific transposons between *F. xylarioides* and *F. oxysporum*, and the presence of large *Starship* elements, indicate that transposons were involved in horizontal transfers.

Our results support the hypothesis that horizontal gene transfers contributed to the repeated emergence of this fungal disease.

766C **Communication between *Fusarium* and its microbial partners: the role of microRNAs** Marine MN Navarro[1], Nadia Ponts[2] [1]INRAE, INRAE, [2]INRAE

Fusarium graminearum is the main agent of fusarium head blight (FHB) of small grain cereals and producer of mycotoxins resistant to agricultural practices and food processes. This fungal disease is caused by a complex of species of phytopathogenic fungi, including the genus *Fusarium*. Worldwide, FHB represents an important economic loss due to a loss of crop yield and a sanitary problem with the presence of mycotoxins dangerous for health. Recently, intra-microbiota interactions in plants have been shown to play a key role in infection and mycotoxin production. These interactions are orchestrated in part by microRNAs, which are small non-coding RNAs that induce post-transcriptional silencing of genes. These miRNAs are differentially expressed according to environmental conditions and interactions with other organisms present in the flora. Increasing evidence indicates that the molecular dialogue between Fusarium species during Fusarium head blight is essential for the outcome of the disease in the pathological system. The aim of this research is to investigate the role of fungal small RNAs in this dialogue. To achieve this, confrontation tests were set up with different species of *Fusarium*. Based on these tests, we performed transcriptomic analyses (RNAs and miRNAs) to identify the miRNAs produced during inter-species communication. Then, the transcriptomic data will be studied using an *in-silico* approach. This approach will enable us to catalog the miRNAs expressed during *Fusarium* interactions and to identify the putative targets of these miRNAs. Finally, these results will provide us with keys to the diagnosis and prevention of FHB and mycotoxin accumulation in the plant system.

767C **Mechanism of niche adaptation and defence: beneficial endophytes deploy host-protective antimicrobial effectors** Laura Armbruster[1,2], Ruben Eichfeld[1,2], Lisa K. Mahdi[1], Concetta K. De Quattro[1,2], Asmamaw B. Endeshaw[1], Shingo Miyauchi[3], Margareta J. Hellmann[4], Stefan J. Cord-Landwehr[4], Igor Grigoriev[5], Daniel Peterson[5], Vasanth Singan[5], Kathleen Lail[5], Emily Savage[5], Vivian Ng[5], Gregor Langen[1], Bruno M. Moerschbacher[4], Alga Zuccaro[1,2] [1]Institute for Plant Sciences, University of Cologne, [2]Cluster of Excellence on Plant Sciences, [3]Okinawa Institute of Science and Technology Graduate University, [4]University of Münster, [5]Joint Genome Institute

Associations between plants and beneficial root-endophytic fungi enhance plant performance by improving nutrient uptake, abiotic stress tolerance, and disease resistance. To colonize diverse plant hosts and protect their niche against competing microbes, root endophytes secrete a multitude of effectors. However, the functions, specificity, and transcriptional regulation of effectors remain poorly understood. We analysed the gene expression profiles of two closely related Sebacinales fungi, *Serendipita indica* (*Si*) and *Serendipita vermifera* (*Sv*), in the presence of monocot and dicot hosts and competing microbes. All three host plants triggered extensive transcriptional reprogramming in *Si* and *Sv*, which largely overlapped with their response to the fungal competitor *Bipolaris sorokiniana* (*Bs*). This suggests there are common underlying principles in the interaction of Sebacinales with eukaryotic organisms, including the activation of genes encoding for multifunctional core effectors involved in cell wall degradation and nutrient acquisition, such as carbohydrate-active enzymes (CAZymes). In addition, Sebacinales expressed distinct effectors in response to host plants (phytosymbiotic effectors) or microbial competitors (antimicrobial effectors). We functionally characterized one of these antimicrobial effectors, a GH18-CBM5 chitinase exclusive to Basidiomycota, and demonstrated that this enzyme hampers the growth of *Bs*, thereby reducing the disease symptoms caused by the plant pathogen in Arabidopsis and barley.

While more fungal effectors are functionally characterized every year, it is unclear how endophytes control effector gene expression. To uncover the regulatory networks underlying the induction of effector gene expression, we screened *Si* transcriptional maps for clusters of co-expressed - and hence likely functionally related - effector genes and transcription factors. Currently, we are generating transcription factor null-mutants and overexpression lines to characterize the biological relevance of promising candidates *in vivo*. In addition, we collaborate with the JGI to perform DNA-affinity purification sequencing on roughly 300 *Si* transcription factors. Understanding the regulatory landscape governing effector gene expression in beneficial fungi might pave the way for sustainable agricultural practices.

768C **An environmental isolate of *Pseudomonas* reduces *Aspergillus flavus* growth in an iron-dependent manner affecting the expression of numerous genes and mycotoxin production** Elizabeth M Wyman[1], Scott Grayburn[2], Matthew K Gilbert[3], Matthew Lebar[3], Jeffery W Cary[3], Ana M Calvo[1] [1]Biological Sciences, Northern Illinois University, [2]Northern Illinois University, [3]Food and Feed Safety Research Unit, USDA/ARS, Southern Regional Research Center

Aspergillus flavus is an opportunistic pathogenic fungus that infects oilseed crops worldwide. When colonizing plants, it produces mycotoxins, including carcinogenic compounds such as aflatoxins. The design of new strategies to control *A. flavus* colonization and mycotoxin contamination is paramount. In this study, we identified a promising new isolate of *Pseudomonas*, 20EI1, and observed that it is able to reduce the growth of *A. flavus*. SEM analysis revealed strong adhesion of the bacterial cells to the hyphal surface. Interestingly, this anti-fungal activity by the 20EI1 isolate was dependent on iron availability. Through transcriptomics analysis we have gained insight into 20EI1's potential to prevent fungal colonization and mycotoxin production. Metabolomics analysis revealed that both aflatoxin and cyclopiazonic acid production by *A. flavus* were significantly reduced in the presence of the bacteria.

769C **Comparative transcriptomics identifies secreted protein associated with virulence of Seiridium cardinale** Edoardo Scali[1], Takao Kasuga[2], Giovanni Emiliani[3], Gianni Della Rocca[3,3], Roberto Danti[3], Matteo Garbelotto[1] [1]ESPM, UC Berkeley, [2]UC Davis, [3]National Research Council

Cypress Canker Disease (CCD) is caused by Ascomycetes belonging to the genus Seiridium. CCD occurs mainly on Cupressaceae and causes bark necrotic lesions. Nevertheless, some Seiridium species can cause bark cankers on Angiosperms. Given the wide geographical distribution of CCD, it is considered a remarkable example of a tree pandemic and it offers an excellent case study for the understanding of host-pathogen interactions. Despite the successful selection of resistance in Cupressus sempervirens genotypes, genomics and host-pathogens molecular interactions have never been investigated. In this study, we focused on the genomics and gene expression of the pathogen Seiridium cardinale. We hypothesized that the effectors produced by S. cardinale undergo a differential regulation during the infection cycle.

We further hypothesized that effectors and secreted proteins of S. cardinale may have unique features associated with high pathogenicity when compared to mildly pathogenic and saprophytic phylogenetically related taxa. In order to test these hypotheses, we first developed a reference genome for S. cardinale, and produced dual-RNA interaction data of C. sempervirens infected with S. cardinale. We also isolated in vitro RNA of S. cardinale and compared it with in vivo gene expression. In addition, we conducted full-length cDNA sequencing of inoculated plant tissues, and identified alternative splicing in S. cardinale. Together, our data support the hypothesis that S. cardinale undergoes a differential gene expression and possibly differential alternative splicing when it is interacting with the host C. sempervirens. We then compared effectors and secreted proteins of S. cardinale with those of other plant-pathogenic fungi and of saprobic fungal species. Finally, we evaluated the phylogenetic distribution of orthologous proteins at genus and class level. Comparative genomics showed that the majority of Seiridium effectors and secreted proteins are conserved amongst Sordariomycetes plant pathogens. We, nevertheless, found a set of proteins unique to S. cardinale. The results presented represent the basis for our future work comparing dual host-pathogen transcriptomes in resistant and susceptible cypress genotypes.

770A **Global genomic analyses of wheat powdery mildew reveal association of pathogen spread with historical human migration and trade and more** Alexandros Georgios Sotiropoulos[1,2], Epifanía Arango-Isaza[3], Tomohiro Ban[4], Chiara Barbieri[3,5], Salim Bourras[2,6], Christina Cowger[7], Paweł C. Czembor[8], Roi Ben-David[9], Amos Dinoor[10], Simon R. Ellwood[11], Johannes Graf[2], Koichi Hatta[12], Marcelo Helguera[13], Javier Sánchez-Martín[14], Bruce A. McDonald[15], Alexey I Morgounov[16], Marion C. Müller[2], Vladimir Shamanin[17], Kentaro K. Shimizu[3,4], Taiki Yoshihira[18], Helen Zbinden[2], Beat Keller[2], Thomas Wicker[2] [1]Centre for Crop Health, University of Southern Queensland, [2]Dept of Plant and Microbial Biology, University of Zurich, [3]Dept of Evolutionary Biology and Environmental Studies, University of Zurich, [4]Yokohama City University, [5]Max Planck Institute for Evolutionary Anthropology, [6]Swedish University of Agricultural Sciences, [7]North Carolina State University, [8]Plant Breeding and Acclimatization Institute - National Research Institute, [9]Institute of Plant Sciences, ARO-Volcani Center, [10]The Robert H. Smith Faculty of Agriculture, Food & Environment, The Hebrew University of Jerusalem, [11]Curtin University, [12]Hokkaido Agricultural Research Center Field Crop Research and Development, National Agricultural Research Organization, [13]Centro de Investigaciones Agropecuarias (CIAP), INTA, [14]University of Zurich, [15]Institute of Integrative Biology, ETH Zurich, [16]Food and Agriculture Organization of the United Nations, [17]Omsk State Agrarian University, [18]Rakuno Gakuen University

The fungus *Blumeria graminis* f. sp. *tritici* (*B.g. tritici*) causes hexaploid wheat powdery mildew disease. We have studied its spread and evolution by analyzing a global sample of 172 mildew genomes. Our population genomics analyses (including admixture, diversity and coalescence analyses etc.) show that *B.g. tritici* emerged in the Fertile Crescent during wheat domestication, while we also identified a candidate region of selection that includes an effector family differentiated in the tetraploid wheat powdery

mildew (*B.g. dicocci*), which could associate with host specificity. After hexaploid wheat mildew spread throughout Eurasia, colonization brought it to America, where it hybridized with unknown grass mildew species. Recent trade brought USA powdery mildew strains to Japan, and European strains to China, where in both places, they hybridized with local ancestral strains. Thus, although mildew spreads by wind regionally, our results indicate that humans drove its global spread throughout history and that wheat powdery mildew rapidly evolved through hybridization.

771A **Role of gene flow in dictating adaptation and evolution of reproductive barriers.** Supreet Saini, Prachitha Nagendra Indian Institute of Technology Bombay

Gene flow is a conundrum as a variable which influences adaptation of populations. Very few studies have directly studied its role in dictating adaptation and evolution of reproductive barriers. In this work, we perform evolution experiments, with yeast as a model system where we control the timing and quantum of gene flow between otherwise allopatric populations. Specifically, we evolve 6 lines in 0.2% glucose and 6 lines in 0.2% galactose. At every 'g' generations (g = 50, 100, 150), we introduce gene flow between the two environments. Each line was evolved for 600 generations. Our results show that gene flow, via introduction of maladaptive mutations, slows down adaptation. Moreover, gene flow also changes the kinetics of mating, which introduces a decrease in mating efficiency. Over study provides a framework to study how gene flow impacts adaptive trajectories.

772A **Assembly of *Alternaria solani* reference whole genome sequence to elucidate single nucleotide polymorphism based phylogenetic relationships among the isolates in US.** Ipsita Mallik[1], Sunil Shrestha[2], Binod Pandey[3], Julie S Pasche[2] [1]Plant Pathology, North Dakota State University, [2]Plant Pathology, NDSU, [3]NDSU

Alternaria solani is an ascomycete fungus which causes early blight, one of the most globally destructive diseases of potato. We used MinION Nanopore technology to assemble a 32.9 Mb reference genome from A. *solani* isolate AS13-1 collected in 1998. The assembled genome consists of 45 contigs with an average size of 0.7Mb and an N50 value of over 1.5Mb. The reference genome assembly predicted 11,798 putative genes, 55,414 open reading frames, and 22,866 introns. The assembly aligned with 100% coverage to the published *A. solani* reference genome ASM295215v1. Whole genome sequences from additional *A. solani* isolates generated using the Illumina MiSeq were mapped to the reference genome AS13-1. The phylogenetic tree constructed based on these sequences revealed diversity among *A. solani* isolates. The whole genome sequences from *A. solani* isolates from several potato growing regions in US over the span of two decades will provide insight about the genetic diversity among the isolates which will aid in better disease management.

773A **Increased genetic diversity of clonal rice blast fungus lineages through multiple mini-chromosome transfers** Cristina Barragan[1], Sergio M. Latorre[2], Angus Malmgren[1], Adeline Harant[1], Joe Win[1], Yu Sugihara[1], Hernan A. Burbano[2], Sophien Kamoun[1], Thorsten Langner[1,3] [1]The Sainsbury Laboratory, [2]Centre for Life's Origins and Evolution, Dept of Genetics, Evolution and Environment, University College London, [3]Max Planck Institute for Biology

Crop disease pandemics are often driven by clonal lineages of plant pathogens that reproduce asexually. How these clonal pathogens adapt to their hosts despite harboring limited genetic variation is poorly understood. Here, we show multiple instances of horizontal chromosome transfer involving clonal lineages of the rice blast fungus Magnaporthe (Syn. Pyricularia) oryzae. We identified the horizontally transferred chromosome as a 1.2Mb supernumerary mini-chromosome, mChrA, which is remarkably conserved across blast fungus isolates from lineages infecting the wild grass Eleusine indica (Indian goosegrass) and rice. Further analyses revealed mChrA was acquired by clonal rice blast fungi through parasexual-mediated horizontal transfer, with evidence of at least eight distinct transfer events over the past four centuries. These findings establish horizontal mini-chromosome transfer as a mechanism facilitating genetic exchange among blast fungi infecting different hosts. We propose that blast fungus populations infecting wild grasses act as genetic reservoirs that contribute to the evolvability of pandemic clonal lineages that afflict crops.

774A **The emerging Eucalyptus scab and shoot malformation epidemic in North Sumatra defined by panmictic populations of *Elsinoe necatrix*** Nam Q Pham[1], Tuan A Duong[1], Hiroyuki Suzuki[1,2], Brenda D Wingfield[1], Irene Barnes[1], Alvaro Durán[3], Michael J Wingfield[1] [1]Forestry and Agricultural Biotechnology Institute (FABI), [2]Niigata Agro-Food University, [3]Asia Pacific Resources International Holdings Ltd. (APRIL)

In the early 2010s, a seriously damaging leaf and shoot disease of unknown aetiology was discovered in *Eucalyptus* plantations of North Sumatra, Indonesia. The symptoms were unlike any other leaf or shoot diseases known on these trees elsewhere in the world. After intensive studies for approximately 10 years, the causal agent was recently discovered and described as a novel species, *Elsinoe necatrix*. Nothing is known regarding its possible origin or why it emerged so rapidly to cause a serious local epidemic.

To gain insights into the biology of *E. necatrix*, we generated whole genome sequence data for two isolates. Using these data, we characterized the structure of the mating-type locus so as to understand its reproductive biology. Polymorphic microsatellite markers were developed to study the population biology of the pathogen.

Populations of *E. necatrix* from North Sumatra had a high level of haplotypic diversity. Discriminant analysis of principal components, haplotype networks and analysis of molecular variance revealed a lack of population structure related to geographic locations and a high level of gene flow among sampled regions. *Elsinoe necatrix* was shown to have a typical heterothallic mating system characterized by having either the MAT1-1 or MAT1-2 idiomorph present in an isolate. Mating type ratios and linkage disequilibrium analyses suggest that sexual recombination is likely occurring, even though a sexual state of the fungus has not been found.

The results of this study highlight the fact that new genotypes of *E. necatrix,* likely arising from cryptic sexual recombination will challenge efforts to manage the disease, and that breeding and selection for tolerance will require substantial host genetic diversity. In this regard, it should be considered as a high-risk pathogen and efforts should be made to prevent its spread to new environments.

775A **Observing Histoplasma across the globe, Elucidating Transposable Elements and Synteny** Tania Kurbessoian[1], Victoria Sepulveda[2], Daniel R. Matute[2], Marcus M. Teixeira[3], Bridget M. Barker[4] [1]Biology, University of North Caorlina, Chapel Hill, [2]Biology, University of North Carolina, Chapel Hill, [3]Biology, University of Brasília, [4]Biological Sciences, Northern Arizona University

A known soil-inhabiting pulmonary pathogen, Histoplasma spp, causes the disease histoplasmosis with a crude mortality rate of 5% for adults and 8% for children. Individuals who live near the Mississippi and Ohio river valleys have an estimated 60-90% infection rate. While worldwide, a 30% rate of histoplasmosis is present among individuals with a positive HIV/AIDS diagnosis. Four known Histoplasma species have been described since 2022, H. capsulatum sensu stricto, H. ohiense, H. mississippiense, and H. suramericanum, where H. ohiense and H. mississippiense were obtained from the U.S. specifically (Sepulveda et al., 2017; Jofre, Gaston I., et al., 2022). As species diverge over time, self-replicating mobile elements like transposable elements (TEs) are an important contributor to genome size expansion and genetic diversity. As a result of TE transposition and abiotic stressors, the genome experiences alteration of regulatory networks, new chromosomal variants, and genome rearrangement. Yet no systematic effort has evaluated the role of TEs in Histoplasma genetic diversity. Through collaborators' efforts and the Matute lab, a large number (about 300) of Histoplasma clinical isolates were collected worldwide, then sequenced, assembled, annotated, and assessed for transposable elements using tools to understand genomic arrangements, phylogeny, and synteny. A great diversity of TEs are present in the isolates we evaluated but the majority consists of ancestral LTR/Gypsy and LTR/Copia, with recent expansions in LINE/Tad transposons. We confirm the identity of multiple regional species and observe a variety of genome expansions and contractions that could advance our understanding of how genetic differentiation in the Histoplasma genus is taking place.

776A **Do spore killing genes maintain accessory chromosomes in plant pathogenic fungi?** Linnea Sandell[1], Anna Mirandola[1], Samuel Jorayev[1], Andrew Urquhart[1], Alexandra Granger Farbos[2], Corinne Clavé[2], Sven Saupe[2], Aaron Vogan[1] [1]Uppsala University, [2]IBGC, UMR 5095, CNRS Université de Bordeaux

The genomic diversity of many fungal species are augmented by the presence of accessory chromosomes that are variably present or absent in individual strains across a given species. These genomic regions often have high evolutionary rates, accumulating genes important in fast-paced lifestyles like pathogenicity, but also become burdened with significant amounts of transposable elements (TEs). It is thus assumed in many cases that accessory chromosomes represent a trade-off, providing benefits through their roles in infection, but being deleterious due to their highly repetitive nature and contributions to genomic instability. However, it has been difficult to ascribe adaptive benefits to many accessory chromosomes, and they often appear to be stably maintained even when the fungi are grown as benign saprotrophs. Previously, we had observed that genes with homology to meiotic drive toxin/antidote proteins from the fungus *Podospora anserina* (*Spoks*) are found in high abundance on accessory chromosomes in various *Fusarium* species. By investigating their functionality in yeast, we have demonstrated that some of these homologs have active toxin and antidote properties. This leads us to propose that this selfish class of genes acts to maintain accessory chromosomes during vegetative growth and may influence their spread via parasexual cycles. Finally, *Spok* genes are mobilized by the newly described TE superfamily, the *Starships*, and may play crucial roles in the formation of accessory chromosomes and accessory genomic regions more broadly. These results illuminate a mysterious facet of fungal biology, a key step towards describing the origin, spread, and maintenance of pathogenicity in many fungal species.

777A **Spontaneous chlorate resistance mutations in *Fusarium verticillioides*** Maninder Kaur, Tamara Krska, Christoper Toomajian, John Leslie Plant Pathology, Kansas State University

Mutations are the driving force for the adaptation of pathogenic species to changing or toxic environments, creating crucial genetic diversity for species evolution. Understanding how mutations occur in terms of rate and patterns, furthers our understanding of how these pathogens adapt to environmental changes. In this study, we evaluated spontaneous mutations that inactivate the nitrate reductase (*nit1*) gene when *Fusarium verticillioides* is grown in the presence of chlorate. Spontaneous mutants (115) in the *nit1* gene of *F. verticillioides* were selected following growth on media containing 1.5% chlorate. The mutant *nit1* genes were sequenced and 68.6% had single nucleotide polymorphisms (SNPs), 12.7% had insertions, and 27.1% had deletions. There was atleast one changed site per mutant and 118 sites were mutated in total with no more than four mutants having a mutation at the same nucleotide. All insertion and deletion events resulted in a frame-shifted translation pattern and presumptive premature termination of protein biosynthesis. Of the 81 SNPs, 38.2% generated a premature stop codon, 25.9% resulted in an amino acid change near a site known to be essential for enzyme activity, and 28.3% resulted in an amino acid change at sites not previously known to be essential for enzyme activity. As a control, we sequenced 1 kb of the *fum1* gene, which encodes the first step in fumonisin biosynthesis and was not under selection in this experiment, in 92 of the *nit1* mutants. No mutations were detected in any of the *fum1* sequences. Although chlorate is not mutagenic *per se*, *F. verticillioides* responds to this toxic compound by mutating proteins involved in its metabolism. The distribution of the mutations within the *nit1* gene and the limitation of mutations to SNPs or to insertions or deletions of no more than a few nucleotides in length are consistent with errors in DNA replication and/or repair serving as the causal agent. Such errors could enable *F. verticillioides* to quickly and permanently respond to environmental stresses and help explain how these plant pathogenic fungi can evolve, adapt and survive in rapidly changing and challenging environments.

778A **Divergence of TORC1-mediated stress response leads to novel acquired stress resistance in a pathogenic yeast** Bin He[1], Jinye Liang[1], Hanxi Tang[1], Lindsey F. Snyder[1,2], Christopher E. Youngstrom[1] [1]Biology, University of Iowa, [2]Interdisciplinary Graduate Program in Genetics, the University of Iowa

Acquired stress resistance (ASR) enables organisms to prepare for environmental changes that occur after an initial stressor. However, the genetic basis for ASR and how the underlying network evolved remain poorly understood. In this study, we discovered that a short phosphate starvation induces oxidative stress response (OSR) genes in the pathogenic yeast *C. glabrata* and protects it against a severe H2O2 stress; the same treatment, however, provides little benefit in the low pathogenic-potential relative, *S. cerevisiae*. This ASR involves the same transcription factors (TFs) as the OSR, but with different combinatorial logics. We show that Target-of-Rapamycin Complex 1 (TORC1) is differentially inhibited by phosphate starvation in the two species and contributes to the ASR via its proximal effector, Sch9. Therefore, evolution of the phosphate starvation-induced ASR involves the rewiring of TORC1's response to phosphate limitation and the repurposing of TF-target gene networks for the OSR using new regulatory logics.

779A **Parallel expansion and divergence of an adhesin family in pathogenic yeasts** Bin Z. He[1], Rachel A. Smoak[2], Lindsey F. Snyder[1,3], Jan S. Fassler[1] [1]Biology, University of Iowa, [2]Civil and Environmental Engineering, University of Iowa, [3]Interdisciplinary Graduate Program in Genetics, University of Iowa

Opportunistic yeast pathogens arose multiple times in the *Saccharomycetes* class, including the recently emerged, multidrug-resistant (MDR) *Candida auris*. We show that homologs of a known yeast adhesin family in *Candida albicans*, the Hyr/Iff-like (Hil) family, are enriched in distinct clades of *Candida* species as a result of multiple, independent expansions. Following gene duplication, the tandem repeat–rich region in these proteins diverged extremely rapidly and generated large variations in length and β-aggregation potential, both of which are known to directly affect adhesion. The conserved N-terminal effector domain was predicted to adopt a β-helical fold followed by an α-crystallin domain, making it structurally similar to a group of unrelated bacterial adhesins. Evolutionary analyses of the effector domain in *C. auris* revealed relaxed selective constraint combined with signatures of positive selection, suggesting functional diversification after gene duplication. Lastly, we found the Hil family genes to be enriched at chromosomal ends, which likely contributed to their expansion via ectopic recombination and break-induced replication. Combined, these results suggest that the expansion and diversification of adhesin families generate variation in adhesion and virulence within and between species and are a key step toward the emergence of fungal pathogens.

780A **Extensive and independent evolution of secondary metabolism genes across the early diverging fungal genus *Basidiobolus*** Jasper Carleton, Liam P Cleary, Emily Newman, Madison Hincher, Javier F Tabima Biology, Clark University

Secondary metabolism is a hallmark of fungal species and plays fundamental roles in fitness like survival, competition, and resource acquisition. Most fungal secondary metabolytes have been reported in Dikarian fungi. The paucity of secondary metabolism has

been reported in early divergent fungi such as zygomycetes. However, recent genomic and functional approaches show that secondary metabolism is present in the zoopagomycete genus *Basidiobolus.*

Basidiobolus is a microfungus predominantly found in the intestinal tracts of amphibians. Previous studies have shown high rates of secondary metabolites in *Basidiobolus* with predicted functions of antibiosis, metal acquisition in anoxic environments and other functions. In addition, these secondary metabolism genes are apparently derived from horizontal gene transfer (HGT) from bacteria that coinhabit the amphibian gut tract. There are limitations with these hypotheses, as only three published genomes are available. More information is needed to determine the secondary metabolite richness in this early diverging fungal species.

Here, we present the result of the genome sequences of 35 samples of *Basidiobolus* across different locations, hosts and environmental sources to test the hypothesis of prevalent secondary metabolite genes as a hallmark of the genus. Our sequences show a high richness of genic families such as non-ribosomal peptide synthetases, polyketide synthase and terpene cyclase genes. We confirm the hypothesis of bacterial HGT as the source of these genes, as well as finding genes with dikaryan origins. Interestingly, our results suggest that each SM acquisition may have occurred independently, and further investigation in the sources of SM transfer needs to be studied across these early diverging fungi.

781A **Interspecific hybridisation as a new evolutionary fungicide resistance mechanism in the fungal pathogen *Pyrenophora teres*** Chala Turo[1], Wesley Mair[2], Anke Martin[3], Simon Ellwood[1], Richard Oliver[4], Francisco Lopez-Ruiz[1] [1]MLS, Curtin University, [2]Curtin University, [3]University of Southern Queensland, [4]University of Nottingham

The barley net blotch diseases are caused by two fungal species of the Pyrenophora genus. Specifically, spot form net blotch is caused by *P. teres f. sp. maculata* (Ptm) whereas net form net blotch is caused by *P. teres f. sp. teres* (Ptt). Ptt and Ptm show high genetic diversity in the field due to intraspecific sexual recombination and interspecific hybridisation of the two species, although the latter is considered rare. Here we describe the detection of Ptt × Ptm hybrids with demethylase inhibitor (DMI) fungicide resistance ("HR Ptm") and discuss the implications for barley disease management. The genomes of one putative hybrid, three Ptm, and ten Ptt isolates were sequenced, and recombination analyses performed in the intergenic and whole genome level. Of the 12 chromosomes, 11 showed significant ($P < 0.05$) recombination events in the intergenic regions while variable recombination rate showed significant recombination across all the chromosomes. Further genotyping using Diversity Arrays Technology markers of fourteen Ptt, fifteen Ptm, 48 HR Ptm, and two *P. teres* isolates from barley grass, showed that all HR Ptm isolates were clonal and not clustered with Ptt or Ptm. The Nei's genetic differentiation among the observed clusters accounted for over 99% of the total variation. Interestingly, lower genetic distance was found between HR Ptm and Ptm isolates (0.189) than HR Ptm and Ptt (0.807) isolates, indicating HR Ptm are more closely related to Ptm. Further locus specific analyses of the DMI target Cyp51A gene showed at least four recombination breakpoints, including the F489L point mutation that is correlated with DMI resistance. The result confirms occurrence of natural recombination between Ptt and Ptm and indicates that the HR Ptm likely acquired DMI resistance through interspecific recombination, followed by clonal expansion of this genotype in barley-growing areas of Western Australia. The use of effective fungicides in integrated disease management tactics will be crucial to minimise and restrict further dissemination of these adaptive HR Ptm isolates.

782A **Second Alternative Oxidase Genes in Aspergillaceae: Genesis, Loss and Mutations** Levente Karaffa, Michel Flipphi, Alexandra Márton, Vivien Bíró, István Bakondi-Kovács, Viktória Ág-Rácz, Norbert Ág, Erzsébet Fekete University of Debrecen

Alternative oxidase (Aox) is a terminal oxidase in branched mitochondrial electron transport that provides a non-electrogenic alternative to canonical cytochrome-mediated electron flow, bypassing the proton-pumping complexes III and IV. The consequence of the direct transfer of electrons from ubiquinol to oxygen without concomitant build up of proton motive force is the uncoupling of ATP synthesis via oxidative phosporylation from NADH reoxidation, to allow carbon catabolism to continue unabated even when ATP demand is low or when non-carbon nutrients become limiting. Thus, Aox plays an important role in the energetics of overflow metabolism-based bioprocesses such as *Aspergillus niger* citric acid fermentation and *Aspergillus terreus* itaconate production.

Aox (*aoxA* gene) is near ubiquitous in the fungal kingdom, but coexistence of multiple *aox* genes is rare. However, a second *aox* gene (*aoxB*) is present in some taxa of *Aspergillaceae*. Paralogous genes generally originate from duplication and inherit vertically; we provide evidence for four independent duplication events at different points in evolution that resulted in *aoxB* paralogs in contemporary Aspergilli and Penicillia. The paralog in *A. niger* has a different origin than the paralog in *A. terreus*, while a third independently formed paralog is found in *A. wentii*. All paralogous clades arise from original *aoxA* parent genes but never replace them. Few species have accumulated three co-expressed *aox* genes. Therefore, loss of once acquired paralogs co-determines contemporary *aox* gene content in individual species. For instance, section *Fumigati* has lost all its transient paralogs. In the subgenus *Nidulantes*, we identified seven independent occasions of *aoxB* gene loss and two gains. In *A.*

calidoustus, both more ancient *aoxB* paralogs present in the last common ancestor of the subgenus have been substituted by two other *aoxB* genes of completely distinct origins.

We found that the paralogous *aoxB* gene in some 75 genome-sequenced *A. niger* strains features variation at a level not detected for the ubiquitous *aoxA* gene. Five mutations were identified that plausibly affect transcription, function, or terminally modify the gene product. A full-length AoxB is encoded in the acid producer ATCC 1015. Hence, the *A. niger sensu stricto* complex can be subdivided into six taxa according to the resident *aoxB* allele. To date, confident separation could only be accomplished after comparative analyses of whole genome sequences.

783A **Population structure is linked to host vernalization requirement in the barley net blotch fungal pathogen** Julie Ramirez Martinez[1,1], Sonia Guillou[1], Pauline Di Vittorio[2], Florelle Bonal[3], Demetris Taliadoros[4,5], Elise Guéret[6], Elisabeth Fournier[1], Eva Stukenbrock[4,5], Romain Valade[7], Pierre Gladieux[8] [1]PHIM, INRAe, [2]University of Montpellier, [3]UMR AGAP (Amélioration génétique et adaptation des plantes), [4]Max Planck Institute for Evolutionary Biology, [5]Christian-Albrechts University of Kiel, [6]MGX-Montpellier GenomiX, [7]ARVALIS, [8]INRAe

Invasive fungal pathogens pose an important threat to widely cultivated crops due to their ability to adapt to new hosts and environmental conditions. It is crucial to gain insights into the demographic history of these pathogens and understand the mechanisms driving coevolutionary processes for effective disease management. *Pyrenophora teres*, a major barley fungal pathogen, consists of two lineages, *P. teres* f. *teres* (Ptt) and *P. teres* f. *maculata* (Ptm), with global distributions reflecting barley domestication and spread. Despite the heterogeneous nature of barley agrosystems in terms of varietal diversity and environmental conditions, the factors influencing the population structure of *P. teres* are poorly understood. We explored the population genomic structure of *P. teres* in France and globally. Genotyping-by-sequencing revealed that Ptt and Ptm coexist in barley fields in France, with Ptt being predominant. Additionally, we also observed that differences in the vernalization requirement of barley varieties were linked to population differentiation in France and globally, with one population cluster found on spring barley, and another cluster found on winter barley. Our findings highlight how cultivation conditions, potentially linked to genetic differences among host populations, contribute to maintaining divergent invasive pathogen populations across large geographic areas. This research advances our understanding of the coevolutionary dynamics within the Pt-barley pathosystem and prompts further investigation into the relative contributions of host adaptation versus adaptation to abiotic conditions in shaping the structure of Ptt populations.

784A **Mystery of virulence gene duplication unravels - the *ToxB* effector gene in *Pyrenophora tritici-repentis* was likely captured and copied by a Helitron** Ryan Gourlie[1], Megan C McDonald[2], Mohamed Hafez[1], Reem Aboukhaddour[1] [1]Agriculture and Agri-food Canada, [2]University of Birmingham

Copy-number variation is a major driver of genome evolution, correlating with increased virulence and effector development in fungal plant pathogens. In the wheat tan spot pathogen *Pyrenophora tritici-repentis* (Ptr), the *ToxB* gene, encoding the chlorosis inducing effector, exhibits varying copy numbers across isolates ranging from 0 to 10 copies, as previously reported. In this study, we utilized 20 long-read (PacBio RS II) assemblies (Hi-CANU) to understand the replication mechanism of *ToxB* within the Ptr genome. Our results revealed that in multi-copy isolates, *ToxB*, along with variable segments of surrounding sequences, exists as tandem unidirectional copies. Distinctive features strongly support the involvement of a Helitron class of transposable elements in *ToxB* replication, although there is limited evidence for a non-LTR retrotransposon or potentially multiple rounds of unequal crossing-over. Additionally, our analysis showed that *ToxB* resides within a repeat-dense region rich with transposon activity, including evidence for two different Copia-like transposons disrupting and inactivating the *ToxB* reading-frame (i.e. *toxb*). The region containing *ToxB* is completely absent in isolates lacking the *ToxB* gene. The size of the semi-conserved region may support the presence of a supernumerary chromosome arm, or perhaps an ancient, now defunct, large mobile element. Our study provides a comprehensive look at virulence gene duplication in a fungal pathogen, utilizing the tan spot genome as a case study.

785B **Comparative genomics reveals intra and inter species variation in the pathogenic fungus *Batrachochytrium dendrobatidis*** Mark Yacoub, Jason Stajich Microbiology and Plant Pathology, University of California, Riverside

The Global Pandemic Lineage (GPL) of the amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*) has been described as a main driver of amphibian extinctions on nearly every continent. However the genomic features that set *Bd*-GPL apart from other *Bd* lineages is not well understood. Although the contiguous genomes of three *Bd*-GPL strains have been sequenced, high-quality genome assemblies and annotations have not been provided for strains from any other lineage. We used long-read DNA sequencing to generate high-quality assembled and annotated genomes of three *Bd*-BRAZIL isolates and related saprophytic

chytrid, *Polyrhizophydium stewartii,* to compare with the aforementioned previously sequenced *Bd*-GPL strains. We demonstrate the utility of these genomes for pangenomic analysis. We identify the core genome of *Bd,* consisting of 6738 conserved gene families, and 202 *Bd*-BRAZIL and 172 *Bd*-GPL specific gene families. We discovered copy number variation in pathogenicity genes between *Bd*-BRAZIL and *Bd*-GPL strains though we did not observe any pathogenicity gene families consistently expanded in *Bd*-GPL vs. *Bd*-BRAZIL strains. Among the pathogenicity genes, we identify variation in sequence and protein domain counts between copies in *Bd* strains and saprophytic chytrids. We measured the effect of aligning transcripts from a *Bd*-BRAZIL strain against the *Bd* reference genome, JEL423. We identified 424 single-copy genes with over or under-estimated transcription when aligned to the reference genome compared to the genome of the RNAseq donor strain. This analysis reveals the genomic variation between strains in *Bd*-BRAZIL and *Bd*-GPL, and offers insights into the application of these genomes as reference genomes for future studies.

786B **Pathogenicity is associated with population structure in a fungal pathogen of humans** Anne Hatmaker[1], Amelia E Barber[2], Milton T Drott[3,4], Thomas J.C. Sauters[1], Ana Alastruey-Izquierdo[5], Dea Hermoso-Garcia[6], Oliver Kurzai[7], Antonis Rokas[1] [1]Biological Sciences, Vanderbilt University, [2]Friedrich Schiller University, [3]University of Minnesota, [4]USDA-ARS, [5]Instituto de Salud Carlos III, [6]Institut Pasteur, [7]Leibniz Institute for Natural Product Research and Infection Biology

The saprotrophic fungus *Aspergillus flavus* is a clinically and agriculturally important species responsible for devastating human infections and contamination of seed crops. To examine population structure and the pan-genome of *A. flavus*, we collected genomes from 250 (95 clinical and 155 environmental) isolates from 9 countries, including 70 newly sequenced clinical isolates. The core genome of *A. flavus* consisted of over 10,000 protein families present in at least 95% of isolates. Of these, 3,375 were single-copy orthologs present in all strains. Using over 900,000 single nucle otide polymorphisms, we identified five *A. flavus* populations; the five populations mostly corresponded to distinct clades in the genome-wide *A. flavus* phylogeny of the 250 isolates. Accessory genes, including genes previously associated with virulence and genes within biosynthetic gene clusters, were distributed unequally across the five populations. Strikingly, although clinical isolates were present in all but one population, we found that over 75% of all clinical isolates were from a single population. These results suggest that, in contrast, to the cosmopolitan major pathogen *Aspergillus fumigatus*, *A. flavus* pathogenicity is associated with population structure, highlighting the value of whole genome sequencing of geographically diverse clinical and environmental isolates of clinically relevant fungi.

787B **Developing genomic methods to dissect thermophilicity in *Myceliopthora thermophila*** Olusola A. Ogunyewo[1], Pierre Gladieux[2], Hanna Johannesson[3], N. Louise Glass[4], Lori B. Huberman[5], Rachel B. Brem[4] [1]Plant and Microbial Biology, University of California Berkeley, [2]PHIM Plant Health Institute, PHIM Plant Health Institute,Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, [3]Environment and Plants Sciences, Dept of Ecology, Stockholm University, [4]Plant and Microbial Biology, University of California, Berkeley, [5]dPlant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science Cornell University

A key goal of the bioenergy industry is to develop low-cost green energy technology from plant-based material without interfering in food markets. Fungi have the potential for major applications in this space, as they natively degrade plant biowastes to simple sugars which can then be converted to fuels and chemicals. However, most current bioproduction strains of fungi cannot withstand high-temperature treatment of lignocellulosic plant material, a foundational step in industry fermentation processes. Wild thermophilic fungi represent an exciting potential solution to this roadblock, especially if their genes or secreted factors can be used to confer thermophily to cultures of engineered bioproduction strains. To date, the mechanisms of thermophilicity in such systems have remained largely unknown. To begin to fill this knowledge gap, we have taken two genomic approaches focused on *Myceliophthora thermophila*, a thermophilic fungus frequently isolated from soil and self-heated compost. First, we have implemented molecular evolution analyses of 31 Sordariomycete genomes, including *M. thermophila* and its thermophilic relatives alongside farther-diverged mesophile species. In tests for loci with accelerated protein evolution in the ancestor of the thermophilic lineages, we identified 12 top-scoring loci, each of which represent candidate determinants of thermophilicity suitable for follow-up and validation. Separately, we have developed an experimental mutant screening approach to identify genes required for thermophilicity in *M. thermophila*. We have established a pipeline for mutagenesis of *M. thermophila* conidia with *Agrobacterium tumefaciens,* which inserts a barcoded transposon (T-DNA) randomly into the nuclear genome of each transformed clone. In a pilot experiment, we generated and pooled ~4,500 mutant conidia, followed by DNA isolation and preliminary low-depth sequencing of T-DNA insertion positions. Results revealed 2443 detectable insertions with uniform distribution across the *M. thermophila* genome, of which 864 were in genes. These results lay the groundwork for transposon mutant generation and phenotyping-by-sequencing on a genome-wide scale. Together, our methods open the door to evolutionary and genetic dissection in *M. thermophila*, with results relevant for thermophily engineering in green technology applications.

788B **Genome-wide association studies for the genetic basis of variation in fungicide sensitivity and mycotoxin production in U.S. isolates of *Fusarium graminearum*** Upasana Dhakal[1], John F Leslie[1], Christopher Toomajian[2] [1]Kansas State University, [2]Plant Pathology, Kansas State Univ

Fusarium graminearum causes Fusarium head blight (FHB), a devastating disease of wheat and barley. The pathogen also contaminates infected grains with the mycotoxin deoxynivalenol (DON), making them unsuitable for food and feed. DON, besides having health consequences, is also a virulence factor. The application of fungicides, such as demethylation inhibitors (DMIs) which inhibit ergosterol synthesis, is an important component of integrated FHB management. The objective of this study is to identify the genetic basis of variation in fungicide sensitivity and mycotoxin production using genome-wide association (GWAS). Genes responsible for the biosynthesis of DON and its derivatives (*e.g.*, 15ADON) cluster in three *TRI* loci, but genes outside the *TRI* loci may affect the amount of DON produced by different isolates. Similarly, *Fusarium* populations can develop fungicide resistance through mutations in their demethylases, encoded by CYP51 genes, yet other genes such as transporters could also affect fungicide sensitivity quantitatively. Thus, the use of GWAS can help to identify additional QTL for these two traits outside of the main candidates. We measured mycotoxins produced by 152 isolates in controlled laboratory experiments. The amounts of DON and 15ADON produced from isolates grown on rice cultures were measured using GC-MS. Sensitivity of isolates to two DMI fungicides, propiconazole and tebuconazole, was measured in lab experiments with 96-well plate assays. GWAS was conducted using multi-locus models. Five SNPs were significantly associated with DON, and three with 15ADON production. One associated SNP is common to DON and 15ADON production and falls in a polyketide biosynthesis gene cluster. Other associated SNPs are found in, or are in linkage disequilibrium with, SNPs in: an efflux family gene or genes that code for a non-ribosomal peptide synthetase and an MFS transporter. Sensitivities to the two fungicides were highly correlated, with 21 SNPs significantly associated with propiconazole sensitivity, 13 associated with tebuconazole sensitivity, and an additional 9 associated with both fungicides. Candidate genes for fungicide sensitivity included transcription factors and transport-related genes. Many associations we identified are novel and provide new candidates for functional studies. Once verified, they can serve as markers to monitor field populations for fungicide sensitivity and DON production potential, which can inform management decisions.

789B **Developmental regulation of transposon activity drives adaptation in the clonally evolving fungal pathogen *Fusarium oxysporum*** Ana Rodríguez López[1], Cristina López Díaz[1], Dilay Hazal Ayhan[2], Li-Jun Ma[2], Antonio Di Pietro[1] [1]Genetics, University of Córdoba, [2]Biochemistry and Molecular Biology, University of Massachusetts Amherst

Transposable elements (TEs) represent an important source of genetic variation and are considered major drivers of genome evolution in eukaryotic organisms. The clonally propagating fungus *Fusarium oxysporum* (*Fo*) causes devastating vascular wilts in a wide range of crops and life-threatening opportunistic infections in humans. The ability to infect organisms from different kingdoms makes *Fo* an ideal model to study the genetic mechanisms underlying adaptation to different host environments. Previous work revealed a pivotal role of DNA transposons in rapid adaptation of the tomato pathogenic isolate Fol4287 during serial passages conducted under different environmental conditions. More than 80% of the TE insertions detected in experimentally evolved lines were caused by *Hormin*, a non-autonomous miniature DNA element derived from the hAT superfamily TE *Hornet*. Here, we studied the expression of *Hornet* transposase, responsible for the mobilization of *Hormin*, during different stages of fungal development. RT-qPCR performed during coordinated growth in liquid medium revealed a marked upregulation of *Hornet* transcript levels during the early stages of asexual sporulation, with a peak of expression in freshly produced microconidia. Our findings suggest that stage-specific regulation of TE activity promotes environmental adaptation in this clonally evolving fungal pathogen.

790B **Population genetic consequences of introduction and invasion in *Suillus luteus*, an ectomycorrhizal fungus co-introduced with exotic forestry** Yi-Hong Ke[1], Anna Bazzicalupo[2], Joske Ruytinx[3], Lotus Lofgren[1], Thomas Bruns[4], Sara Branco[5], Nhu Nguyen[6], Peter Kennedy[7], Alejandro Rojas[8], Hui-Ling Liao[9,10], Alan Kuo[11], Kerrie Barry[11], Igor Grigoriev[4,11], Ursula Peintner[12], Jonathan Plett[13], Leho Tedersoo[14], Jason Hoeksema[15], Brian Looney[1], Dai Hirose[16], Rytas Vilgalys[1] [1]Duke University, [2]Royal Botanic Gardens Kew, [3]Vrije Universiteit Brussel, [4]University of California Berkeley, [5]University of Colorado Denver, [6]University of Hawai'i at Mānoa, [7]University of Minnesota, [8]Michigan State University, [9]North Florida Research and Education Center, [10]University of Florida, [11]U.S. Dept of Energy Joint Genome Institute, [12]University of Innsbruck, [13]Western Sydney University, [14]University of Tartu, [15]University of Mississippi, [16]Nihon University

Introduced species often modify their mating systems and develop local adaptations to enhance fitness in novel environments. *Suillus luteus*, a prevalent ectomycorrhizal fungus in exotic pine forests, has been co-introduced with pines to multiple continents across the Southern Hemisphere, playing a pivotal role in pine invasions. Whole-genome analyses of *S. luteus* from native and introduced populations reveal a consistent increase in inbreeding coefficients within all introduced populations, which is likely to have resulting from smaller founder population sizes and limited habitat connectivity. Nonetheless, a predominant pattern of outcrossing persists in both native and introduced populations of S. luteus, consistent with the known mating system of this genus. Notably, selfing was rarely detected or absent within introduced populations, suggesting no change

in the mating system of *S. luteus*, which contrasts with other introduced/invasive fungal species. Analysis the distribution of mating types of HD MAT gene across introduced and native populations indicates that mating types are widely shared among populations, with the native European population exhibiting the highest mating type diversity. Despite smaller sampling sizes in introduced populations, we observed a higher frequency of duplicated mating types and genotypes, which we attribute to genetic bottlenecks during introduction process. We also investigated adaptive evolution associated with introductions by identifying genome regions with high divergence and selective sweeps shared across multiple introductions to different continents. Our findings reveal several regulatory functional genes showing signatures of selection, pointing to their crucial role of several genes in adaptation to diverse exotic environments.

791B **Exploring the role of Spoks (Spore Killers) in chromosome dynamics of *Fusarium oxysporum*** Gema Puebla Planas[1], Dilay H. Ayhan[2], Pilar Gutiérrez Escribano[1], Lucía Gómez Gil[1], Cristina López Díaz[1], Li-Jun Ma[3], Antonio Di Pietro[1], Manuel Sánchez López-Berges[1] [1]Genetics, Universidad de Córdoba, [2]Biochemistry and Molecular Biology, University of Massachusetts Amherst, [3]University of Massachusetts Amherst

The ascomycete *Fusarium oxysporum* causes vascular wilt disease in more than a hundred crop species and opportunistic infections in humans. Its genome is compartmentalized into conserved core chromosomes and lineage-specific accessory regions, which are involved in adaptation to different environments such as the plant host. Some of these regions are highly dynamic and undergo spontaneous loss or duplication, but the mechanisms controlling chromosome dynamics remain largely unknown. It was previously observed that the accessory genome of *F. oxysporum* contains multiple copies of Spoks (Spore Killers), a class of genetic elements that act as meiotic drivers by actively killing neighboring cells that lack the element. Interestingly, we found that a single copy of Spok was either present or absent on a conserved chromosome exhibiting differential stability in two different *F. oxysporum* isolates. To experimentally test the role of Spok in chromosome stability, we transferred either a wild-type or a loss-of-function copy of the Spok element from the strain with the stable chromosome into the strain with the unstable chromosome, together with a fluorescence and an antibiotic resistance marker. To test the effect of chromosome stability, passaging experiments are performed under conditions favoring loss of the unstable chromosome followed by quantitative monitoring of chromosome loss by flow cytometry. The results of this work will shed new light on the role of Spoks in the dynamics of accessory genomic regions.

792B **The impact of structural variations on reproductive barriers and speciation in the fungal morphospecies *Trichaptum abietinum*** Anders K Krabberød[1], Dabao S Lu[1], David Peris[1,2], Inger Skrede[1] [1]Dept of Biosciences, University of Oslo, [2]Dept of Biotechnology, Institute of Agrochemistry and Food Technology

Knowledge of the origin of reproductive barriers and speciation in fungi are important to understand species diversity and how species adapt to the changing climate. In the morphospecies *Trichaptum abietinum* reproductive barriers have been detected between both sympatric and allopatric populations around the world. Recent findings suggest that reproductive barriers have arisen rapidly, partly due to chromosomal rearrangements in this species complex. This study aims to investigate the role of structural variations within 16 genomes of *T. abietinum*. The genomes have been sequenced using PacBio HiFi reads, providing increased precision in the identification of longer structural variations. This combination of HiFi reads and pangenome construction enables the examination of much longer structural variations compared to previous methods. Focusing on diverse populations of *T. abietinum*, some of which display reproductive barriers, our investigation aims to understand how the identified structural variations may contribute to these barriers. By unraveling the interplay between genomic architecture and reproductive isolation, we seek insights into how structural variations influence the evolution of multiple species within the *T. abietinum* morphospecies. The study aims to contribute to the understanding of how structural variants may contribute to the adaptation and diversification of *T. abietinum* populations.

793B **A global pangenome of *Aspergillus fumigatus* reveals the origin of azole resistance** Johanna Rhodes[1,2], Harry Chown[2,3], Felicia Stanford[4], Rodrigo Leitao[2], Samuel Hemmings[2], Ali Abdolrasouli[2], Norman van Rhijn[3], Gillian Sigle-Hall[2], Zain Chaudhry[2], Iro Chatzidaki[2], Ben Simmons[2], Chuhan Qin[2], Darius Armstrong-James[2], Paul Verweij[1], Michael Bromley[3], Paul Dyer[4], Matthew Fisher[2] [1]Radboudumc, [2]Imperial College London, [3]University of Manchester, [4]University of Nottingham

Resistance to antifungal drugs in *Aspergillus fumigatus* infections is on the rise, posing a significant challenge in clinical settings. Despite this, our understanding of the temporal and spatial origins of drug-resistant genotypes remains limited. This study presents an investigation into the genomic dynamics of *A. fumigatus* through the creation of one of the largest fungal pangenomes, making it the largest for this species, to date.

Leveraging a diverse panel of over 1000 globally acquired isolates, we conducted a comprehensive assessment of the species' genomic plasticity. Employing clock-based phylogenetics, we traced the origins of azole drug resistance, shedding light on the anthropogenic drivers leading to resistance emergence. In addition to elucidating the temporal aspects, our analysis uncovered the contribution of accessory genes to novel resistance mechanisms and niche specificity through association tests. Furthermore, we test for evidence of horizontal gene transfer within the population, highlighting the ability of genetic exchange amongst *A. fumigatus*.

This research highlights the intricate interplay between antifungal usage and the development of drug resistance in *A. fumigatus.* Through analysis of a large-scale population pangenome we are able to obtain a nuanced understanding of the genetic repertoire that contributes to the species' resilience and adaptability in the presence of antifungals.

794B **Geno mic factors shape carbon and nitrogen metabolic niche breadth across an entire subphylum** Dana A Opulente[1], Abigail Leavitt LaBella[2], Antonis Rokas[3], Chris T Hittinger[4] [1]Villanova University, [2]University of North Carolina - Charlotte, [3]Vanderbilt University, [4]University of Wisconsin - Madison

Organisms exhibit extensive variation in ecological niche breadth, from very narrow (specialists) to very broad (generalists). The existing paradigms attributing to this variance explore the interplay of factors such as trade-offs between performance efficiency and breadth, external environmental influences, and inherent genomic traits. Our study leveraged a comprehensive dataset comprising genomic information sourced from 1,154 yeast strains across 1,051 species in the subphylum *Saccharomycotina*, metabolic capacity encompassing growth under 24 carbon and nitrogen conditions, and an innovative hierarchical environmental classification system. We found that variation in carbon utilization breadth across species primarily arise from intrinsic differences encoded in genes governing specific metabolic pathways. In contrast, our investigation yielded limited evidence for the influence of external factors and the trade-offs. This comprehensive analysis strongly advocates that intrinsic genomic factors serve as the principal drivers shaping the breadth of microbial ecological niches.

795B **Entanglement of transposable elements and virulence in rapid crop pathogen adaptation** Daniel Croll University of Neuchatel

Adaptation in plant pathogens proceeds at speeds that easily overwhelm the rate of resistant cultivar deployment and fungicide development. Hence, understanding the molecular basis of adaptation is critical to define more sustainable containment strategies. Key features of genomic variation in crop pathogens are transposable elements (TEs). Many pathogen species carry compartmentalized genomes with gene-rich and gene-poor regions where genes involved in virulence (i.e., effectors) are often located near TEs. TEs govern the regulation of effector genes in TE-rich compartments through epigenetic effects. This enables tight regulatory timing with the plant infection stages from early contact to the establishment of large lesion areas. De-repression of TE control during infection imposes though a challenge to maintain genome integrity as TEs may jump and insert into new locations in the genome. How pathogens may benefit or suffer from active TEs remains largely unexplored. I will address this question using large-scale genomic and transcriptomic datasets of *Zymoseptoria tritici,* a major fungal pathogen of wheat having spread to all continents over the past centuries. Using a reference-quality pangenome and large resequenced panels of strains across the world, I will recapitulate first the spread of the pathogenicity-associated *Styx* TE. The element likely originated in the *Zymoseptoria* genus and underwent multiple independent reactivation events. Importantly, we find that new copies of the element are not affected by genomic defenses revealing a recent loss of control against the element. Beyond the *Styx* element, the species experiences a broad pattern of TE reactivation concurrent with weakened genomic defences. The newly inserted TEs make a vast contribution to regulatory variation within populations and are overpresented at loci associated with variation in virulence. In conjunction, the pool of active TEs in the species underpins both a vast potential to adapt to the host and carries long-term risks to the integrity of the genome.

796B **Assessing Roles for Dynamic *Magnaporthe oryzae* Mini-Chromosomes in Host Adaptation** Tyler D Suelter[1], Guifang Lin[2], Ravi Bika[1], Lidia Calderon Daza[1], Giovana Cruppe[1], Sanzhen Liu[1], David Cook[1], Barbara Valent[1] [1]Plant Pathology, Kansas State University, [2]Basic Forestry and Plant Proteomics Research Center, Fujian Agriculture and Forestry University

Magnaporthe oryzae (synonym of *Pyricularia oryzae*) is a phytopathogenic, filamentous ascomycete that causes devastating blast disease epidemics in rice, wheat and other cereal and forage crops around the globe. Since its first report in Brazil in 1985, the *M. oryzae Triticum* pathotype (MoT) which causes wheat blast disease, has gained aggressiveness in causing disease on wheat, overcome deployed (2NS) resistance, and spread to wheat-growing regions on new continents. The genome of *M. oryzae* has seven core chromosomes and often contains one or more supernumerary chromosomes, known as mini-chromosomes. We hypothesize

that the presence of mini-chromosomes in the *M. oryzae* genome play an important role in host adaptation as they contain effector genes which code for proteins that modulate host cell metabolism and immune responses, and they appear to be passed horizontally between fungal strains.

We are focusing on mini-chromosome dynamics including detailed analyses of historical South American MoT isolates from 1985 to 1989, and recent MoT isolates from South America, South Asia and Africa. We have previously shown that known effector genes *PWL2* (prevents pathogenicity to weeping lovegrass) and *BAS1* (highly expressed cytoplasmic effector), which are located on different core chromosomes in the rice pathogen reference genome 70-15 (MG8), are located side-by-side on the mini-chromosome of the reference wheat pathogen B71 (Peng et al, PLoS Genetics, 2019).

The *PWL2* and *BAS1* genes exhibit in planta-specific expression, and so far, they only occur on mini-chromosomes in the MoT population. Additionally, *BAS1* and *PWL2* co-occur in 6% of wheat isolates collected between 1986 and 1989, and in 91% of wheat isolates collected in 2017 and 2018, indicating that the overall frequency of wheat field isolates containing these two genes has increased through time. Experiments are underway to determine if BAS1 and PWL2 might be playing a role in the enhanced aggressiveness of recent MoT strains towards wheat. We will also report progress in understanding the role of the parasexual sexual cycle in horizontal transfer of mini-chromosomes within the *M. oryzae* fungal population.

797B **Tracing parallel evolution in a clonal lineage of the rice blast fungus for over a century** Sergio M. Latorre[1], Eva Morisot[1], Joe Win[2], Sophien Kamoun[2], Hernán A. Burbano[1] [1]Dept of Genetics, Evolution & Environment, University College London, [2]The Sainsbury Laboratory

Pandemic clonal lineages of the rice blast fungus, *Magnaporthe* (Syn. *Pyricularia*) *oryzae*, are a threat to global rice production. Despite the absence of sexual reproduction and consequently lack of meiotic recombination, these clonal lineages successfully colonize diverse environments and adapt to the full range of rice subspecies and varieties. By utilizing a combination of contemporary isolates and herbaria-derived historical samples , we have ascertained the century-scale genetic continuity of a rice blast fungus pandemic lineage, despite the widespread pest control strategies employed in modern agriculture. Our goal is to comprehend the mechanisms and strategies involved in the generation of diversity by the rice blast fungus, as well as its adaptation to strong selective regimes. However, the limited impact of meiotic recombination poses challenges for conventional methods aimed at detecting positive selection, as they predominantly rely on patterns of linkage disequilibrium. The integration of phylogenetics and homoplasy identification can shed light on instances of parallel adaptation, wherein disparate genetic lineages and sublineages independently succeed in colonizing similar hosts and overcoming shared environmental constraints. By interweaving genetic, historical, and ecological perspectives, we offer insights into understanding *Magnaporthe oryzae*'s evolutionary tactics.

798B **Demographic history and effects of habitat loss on the genetic structure in a red listed forest fungus** Ine-Susanne Methlie[1], Jørn Henrik Sønstebø[2], Inger Skrede[1], Håvard Kauserud[1], Jenni Nordén[3], Sundy Maurice[1] [1]Dept of Biosciences, University of Oslo, [2]Dept of Natural Sciences and Environmental Health, University of South-Eastern Norway, [3]Norwegian Institute for Nature Research, Oslo, Norway

Amylocystis lapponica is a rare and red-listed wood decay fungus in northern Europe, here fruiting mainly on Norway spruce logs in old-growth boreal forests. In northern Europe, the population size of *A. lapponica* has declined during the last decades, primarily due to loss of old growth forests and an increase in habitat fragmentation. Here, we investigate the present population structure and genetic diversity, as well as the demographic history of *A. lapponica,* and discuss the results in light of the habitat fragmentation the species have been exposed to. A total of 83 specimens of *A. lapponica* were collected in 17 different forests across northern Europe, from which we did whole genome sequencing and population genomic analyses. The sampling area stretches across regions with different intensities of forestry management.

We hypothesize that: *(H1), A. lapponica* migrated westwards into northern Europe from an east-European refugium after the last Ice Age, together with its host tree species. *(H2)* The genetic diversity is lower and level of inbreeding is higher in the westernmost parts of its distribution range in northern Europe, towards the leading edge of the species range expansion. Finally, since the species is rare and only occurs in old-growth forests, we hypothesize that *(H3)* the species is dispersal limited and populations within Northern Europe are consequently isolated by distance.

The findings of our study will be important for future conservation work for this rare species, but also relevant for other dead-wood dependent fungi with similar ecologies and habitat requirements.

799B **Inferring molecular bases of the *Rhizopus microsporus* – *Mycetohabitans* symbiosis by genome-wide positive selection analysis** Margaret E Branine[1], Teresa E Pawlowska[2] [1]Graduate Field of Microbiology, Cornell University, [2]School of Integrative Plant Science, Cornell University

The early-diverging fungal phylum Mucoromycota displays a high degree of coevolution with bacteria as several lineages harbor highly coevolved and ancient bacterial endosymbionts. The mucoromycete *Rhizopus microsporus* and its endosymbiont *Mycetohabitans* spp. are emerging as a model system for studying the evolution and molecular bases of such symbioses due to its experimental tractability and genomic resources. In this symbiosis, *Mycetohabitans* provides its host with secondary-metabolite toxins, controls fungal asexual and sexual propagation, and alters the lipid metabolism of the host. We hypothesize that the strong influence of *Mycetohabitans* on the biology of *R. microsporus* has significant consequences for the evolutionary trajectory of host fungi. Importantly, *R. microsporus* isolates naturally free of endosymbionts (i.e., nonhosts) permit comparative analyses into if and how endosymbiotic bacteria influence the evolution of their fungal hosts. To begin addressing this question, we are implementing a genome-wide positive selection analysis in host and nonhost strains of *R. microsporus* by calculating the non-synonymous to synonymous substitution rate ratio (dN/dS) of all protein-coding genes using the CODEML program within the PAML package. We hypothesize that many of the genes under positive selection (dN/dS>1) in hosts, but not in nonhosts, are significant within symbiosis maintenance and establishment. In addition to characterizing the evolutionary consequences of harboring bacterial endosymbionts, we anticipate that the candidate symbiosis regulators we identify may represent novel therapeutic targets for *R. microsporus* as one of the most prominent causative agents of mucormycosis and post-harvest spoilage, as targeted disruption of these proteins should disturb the symbiosis at the fungal host's expense.

800B **Parallel evolution in gene expression during the spore germination of the mycoparasites, *Trichoderma asperelloides* and *Tolypocladium ophioglossoides*** Yen-Wen Wang[1], Zheng Wang[2], Oded Yarden[3], Jeffrey Townsend[2] [1]Biostatistics, Yale University, [2]Yale University, [3]The Hebrew University of Jerusalem

Mycoparasitism evolved independently multiple times in the kingdom of fungi. However, little is known about the genes that govern the virulence traits necessary for pathogenic mycoparasitism. The conidial germination provides the first means of these fungi to sense and interact with the hosts. Analyses on the gene expression during this process give us opportunity to reveal the genetics underlying mycoparasitism. This study focuses on *Trichoderma asperelloides* and *Tolypocladium ophioglossoides*, both Sordariomycetes mycoparasites, offering a powerful framework to understand the evolution of mycoparasitism. In this study, we ascertained genome-wide gene expression associated with spore germination in *Tr. asperelloides*, *To. ophioglossoides*, and four other Sordariomycetes encompassing non-pathogenic or pathogenic species affecting plants or insects. For all six species, cultivated on potato dextrose agar, gene expression was quantified at four key conidial germination stages: fresh conidia, onset of polar growth, doubling of the long axis, and first hyphal branching (infection stage). By reconstructing the ancestral expression pattern, 27 genes were identified to have undergone parallel evolution, including one that is involved in chitin degradation. Additional transcriptomic data from culture on mushroom-powder agar medium provided further insights into the functionality of these genes. To investigate the gene regulatory networks in the two mycoparasites, Bayesian networks were constructed for these genes and known pathogenic genes, allowing identi fication of regulatory rewiring that associates with the mycoparasitism. Analyses of lineage-specific expression changes from ancestors of these fungi with diverse life histories identified genes that likely contributed to parallel-evolution in their fungal pathogen-host association.

801C **Fungi grown by insects, Nature's prototype of a Biorefinery, can guide design of industrial enzyme blends and elucidate fungal adaptation to domestication** Lene Lange[1], Kristian Barrett[2], Morten Schiøtt[2], Anne S Meyer[2], Michael Poulsen[3] [1]LL-BioEconomy, [2]DTU BioEngineering, Technical University of Denmark, [3]Institute of Biology, University of Copenhagen

The co-evolution of fungus-growing insects is highly specialized, well-studied symbiotic, biological systems. Most pronounced is the digestive part of the symbiosis, leveraged by primarily fungal-derived plant biomass degrading enzymes. We here take next steps to learn from nature: To guide design of new biomass-converting enzyme blends; and to expand our evolutionarily insight into domestication of fungi. The leaf cutter ants and termites grow fungal colonies of *Leucoagaricus gongylophorus* and *Termitomyces*. The leaf cutter ants are feeding *Leucoagaricus* fresh green leaves only, and termites are feeding *Termitomyces* with dry lignocellulosic plant materials only. These two high-ecological-impact biological systems can be seen as Nature's prototype of the Green Biorefinery, e.g., valorizing green leaves or grass, converted into food, feed and soil improvers. And the Yellow Biorefinery, converting wheat straw into e.g., animal gut-health feed and C6 sugars for producing biobased chemicals, materials, or fuels. In this study we analyze the secretome composition, (blend of different types of enzymes) of *Termitomyces* and *Leucoagaricus,* using peptide-based prediction of enzyme function by CUPP; and Enzyme Profile Relatedness-derived "Function;Family" annotation. Such insight can be used for designing optimized enzyme blend for biomass conversion in

the green biorefinery, e.g., for conversion and valorizing pectin; and in the yellow biorefinery for optimized blend for valorization of lignin. Furthermore, molecular studies of these two biological systems provides basis for comparing the evolutionary adaptation of CAZyme secretome composition of free-living Agaricomycetous fungal species of the same (or closely related) genera: Comparing *Leucoagaricus gongylophorus* to *L. leucothites*; and comparing termite symbiont *Termitomyces* with species of closest sister-group relatives of *Termitomyces,* the saprotrophic *Tephrocybe* (e.g., *T. rancida*), *Arthromyces* (e.g., *A. matolae*), and *Blastosporella*, (e.g., *B. zonata*). The following hypothesis is tested: Insect-grown fungi, converting one specific type of biomass, have lower biomass-degrading enzyme function-specificity diversity. Further, the need for same enzyme function to be present in several types of proteins is lower, as environmental conditions (temperature, humidity, pH) are rather stable in the subterranean insect nests. Similarly, a lower level of auxiliary activity enzymes is found among fungal insect symbionts.

802C **Heat stress, genetic changes and thermal adaptation in *Cryptococcus deneoformans*** Paola A Ramos, Eva Mei Shouse, Asiya Gusa Duke University

Human body temperature (37°C) acts as a thermal defense against environmental fungal infections. However, as global temperatures continue to rise, fungi are adapting to higher temperatures, enhancing their pathogenic potential and increasing the likelihood of new fungal diseases. The emergence of novel pathogenic fungal species and the expanding distribution of existing pathogens signal the contribution of climate change to the growing threat of fungal diseases. *Cryptococcus deneoformans* has been associated with skin infections and, to a lesser extent, fatal cryptococcal meningitis in Europe. Systemic infections frequently lead to mortality due to antifungal drug resistance that evolves during infection. *C. deneoformans,* as a less virulent, less thermotolerant disease-causing species, is an ideal model for studying the effects of heat stress on thermal adaptation and pathogenicity in fungi. Our group has shown that heat stress at 37°C significantly increases drug resistance and transposable element (TE) mutation rates in *C. deneoformans* (Gusa et al, *PNAS* 2020 and 2023). Additionally, we found that a spontaneous thermotolerant mutant of *C. deneoformans* exhibits increased resistance to cell wall stressors (unpublished). We identified mutations in uncharacterized genes in this isolate that may contribute to the observed heat adaptation. To more broadly explore the genetic basis of heat adaptation, we generated ten thermally adapted lines in *C. deneoformans* by passage at 37°C for 400 generations. Preliminary assessment reveals that multiple passaged lines outcompete the WT strain at 37°C and above. Southern analysis shows evidence that TE mutations have occurred in several lines and these changes may contribute to the observed increase in thermotolerance. In this study, we seek to define the genetic changes that lead to acquired thermotolerance by performing whole genome assembly of passaged lines to identify the causative mutations of the thermotolerance phenotype. Additionally, we will investigate whether increased thermal tolerance results in a fitness benefit or fitness loss for virulence-related phenotypes and pathogenicity. By shedding light on the genetic basis and phenotypic outcomes of thermal adaptation, this study will contribute to a better understanding of fungal responses to heat stress in a changing climate.

803C **Genotype phenotype associations reveal genome scale convergence across 993 budding yeasts** Kyle T David[1], Joshua G Schraiber[2], Marie-Claire Harrison[1], Dana A Opulente[3], Abigail L LaBella[4], John F Wolters[5], Xiaofan Zhou[6], Xing-Xing Shen[7], Marizeth Groenewald[8], Chris Todd Hittinger[5], Matt Pennell[2], Antonis Rokas[1] [1]Vanderbilt University, [2]University of Southern California, [3]Villanova University, [4]University of North Carolina at Charlotte, [5]University of Wisconsin-Madison, [6]South China Agricultural University, [7]Zhejiang University, [8]Westerdijk Fungal Biodiversity Institute

One of the fundamental goals of biology has been to map genotype to phenotype. Recently, new approaches leveraging phylogenetic comparative methods have made significant strides in this area. For example, mapping SNPs to climate adaptation in tomatoes, coding sequences to aging in primate, or nonexonic conserved elements to flightlessness in birds. While sequence evolution undoubtedly plays a significant role in phenotypic diversity, the consequences of genome-scale evolution such as duplication, loss, and transfer events, have remained unexplored. We sought to estimate the extent to which gene family evolution mirrors trait evolution by measuring patterns of gain and loss between 18,031 gene families and 56 metabolic traits across 993 species of yeast. Using a phylogenetic comparative method to reduce known issues of spurious correlations, we discovered evidence for extensive parallelism, pleiotropy, and convergence across the tree. On average, 692 gene families were significantly associated with each trait, demonstrating repeated coevolution with the same phenotype. Additionally, the same families often contributed to multiple phenotypes, with 192 families significantly correlating with over 25% of all metabolic traits, indicating large scale pleiotropy on the gene family level. In this study, we demonstrate the power of phylogenetic comparative methods in gene family evolution toward identifying genes of interest underlying a broad range of traits and functions. Furthermore, we recover extensive gene family convergence in metabolic trait evolution, identifying hundreds of gene families that have repeatedly evolved to produce both similar and distinct phenotypes across yeasts.

804C **Paternity test: Identifying the parental genomic contributions to the important biological control, *Trichoderma* strain T22** Tammy Stackhouse[1], Daren Brown[2], Tim Satterlee[2], Alfredo Martinez-Espinoza[1], Anthony Glenn[2], Scott Gold[2] [1]University of Georgia, [2]USDA-ARS

The fungal strain, *Trichoderma* T22, has been used commercially as a biocontrol agent and biofertilizer to protect a wide range of host plants from five key fungal genera of root pathogens. T22 was originally patented in 1990 as a protoplast fusion product of two auxotrophic mutants derived from the biological control strains, T12 and T95. These two strains were selected because one (T12) was an effective biocontrol agent against plant pathogenic soil-borne fungi, while the other (T95) had the strong capacity to colonize the plant rhizosphere. *Trichoderma* strain T22 has been used internationally to control root pathogens and promote root health, but no information is available regarding the genetic contributions of the fusion participant strains used to create it. Whole genome sequencing was performed using Nanopore sequencing for all three strains and Illumina for T12 and T95 and analyzed, along with the previously published JGI T22 Illumina data. MaSuRCA was used for hybrid genome assembly and Geneious Prime BLAST was used to map single nucleotide polymorphisms (SNPs) of T12 and T95 to the T22 genome. While we expected that the fusion product strain, T22, would have roughly evenly divided genomic contributions from the two fusion participant strains, we found there were roughly 40-fold more SNPs in T95 compared to T12. Further, many of the genomic regions that matched T95 were very short, consisting of a few hundred to a few thousand base pairs. We conclude that the T22 genome is mostly comprised of T12, with minor interspersed contributions from T95. We hypothesize that the regions from T95 provided strain T22 its enhanced rhizosphere competence and that T12 primarily contributed to its strong biocontrol capabilities. Moving forward, T12 and T95 contributions will be discerned in genes that have been previously published and associated with biocontrol and rhizosphere, respectively. We anticipate that this research can be used to enhance targeting of select genetic traits for future biocontrol strain improvement.

805C **Elucidating the population genomic structure of *Malassezia* species: Implications for sexual reproduction** Marcia David-Palma[1], Marco A Coelho[1], Leyna Díaz[2], F. Javier Cabañes[2], Joseph Heitman[1] [1]MGM, Duke University, [2]Dept of Animal Health and Anatomy, Universitat Autonoma de Barcelona

Malassezia species are lipid-dependent yeasts that inhabit the skin of warm-blooded hosts, playing both commensal and opportunistic pathogenic roles. Emerging evidence suggests a potential for sexual reproduction in these species, despite the absence of a confirmed sexual cycle. Building upon these results, here we explored the genomic diversity and population structure of a collection of *M. pachydermatis, M. sympodialis,* and *M. vespertilionis* isolates from different geographical locations and hosts.

For *M. pachydermatis*, phylogenetic analyses based on single nucleotide variants revealed three distinct populations, regardless of host, health status, or geography. Phylogenetic network analysis showed potential reticulate events (such as recombination or hybridization) within these populations, but limited between populations. Despite that, several *M. pachydermatis* heterozygous diploid strains were identified with genetic contributions from two distinct parental populations, with most strains displayed a pairing of two *PR* alleles and two *HD* alleles, each originating from distinct parental populations. As for *M. sympodialis*, no clear population structure was found, which is congruent with the network analysis suggesting recombination has occurred within the analyzed isolate set. In contrast, *M. vespertilionis* seems to exhibit population differentiation roughly associated with geographical origin, and possible reticulate evolution was noted among the California population, the sole population where two distinct *PR* alleles were identified.

Sequence analysis of the *HD* loci across all three species revealed multiple *HD* alleles at the DNA level, but with limited differences at the protein level, which may suggest a different mechanism of mating-type determination in these species. Although preliminary co-culture experiments did not detect morphological features of sexual reproduction between potentially compatible strains, the study opens avenues for further investigation into *HD* allele compatibility and its role in mating-type determination, aiming to elucidate the mechanisms of sexual reproduction in *Malassezia*.

This study enhances our understanding of the genomic diversity within the *Malassezia* species and the potential for sexual reproduction, contributing to the broader knowledge of its biology and the roles it plays in the mycobiome of humans and other animals.

806C **Exploring the Genetic Diversity of *Arthrobotrys oligospora* Wild Populations in Taiwan** Guillermo Vidal-Diez de Ulzurrun[1], Ching-Ting Yang[2], Jason E Stajich[3], Rachel Brem[4], Erik C. Andersen[5], Yen-Ping Hsueh[1] [1]Academia Sinica, [2]IMB, Academia Sinica, [3]UC Riverside, [4]UC Berkeley, [5]Johns Hopkins University

Arthrobotrys oligospora, a nematode-trapping fungus, exhibits a dual lifestyle as both a saprophyte and predator, showcasing remarkable versatility in adapting to diverse environmental conditions and nutrient availabilities. In its carnivorous mode, it traps, kills, and consumes nematodes, ensuring its success even in nutrient-deficient environments. However, the number of traps and their efficacy varies widely among *A. oligospora* isolates, even when exposed to similar environmental conditions and nematode cues. To gain deeper insights into the population dynamics of this nematode-trapping fungus, we collected and sequenced over 180 *A. oligospora* wild isolates from various locations across Taiwan. We assembled pangenomes for each isolate, revealing substantial variation in genome size and gene count—some differing by up to 3 Mb and lacking up to 1000 genes compared to the reference genome. Variations also extend to the number and distribution of transposable elements within our population. Identification of orthologs among isolates and related species allowed us to categorize genes unique to *A. oligospora* and pinpoint potential candidates involved in the trapping process. Our investigation into population structure and phylogeny of *A. oligospora*wild isolates revealed eight distinct subpopulations, hinting at possible interbreeding among them. We computed trapping phenotype for selected subpopulations, aiming to discern genes and g enomic features associated with trapping ability. Our genome-wide association study (GWAS) identified mutations in genes correlated with the ability to form traps across isolates, unveiling novel candidate genes potentially linked to the still elusive trapping process of nematode-trapping fungi. This research constitutes the most comprehensive study to date of a natural population of nematode-trapping fungi. We believe that our findings will contribute to a better understanding of the genetic mechanisms governing the trapping process and the population dynamics of nematode-trapping fungi.

807C **Population genomic analyses reveal deep population subdivision in *Rhizoctonia solani* AG-1 isolates associated with different crops in North America and the Caribbean** Juanita Gil[1,2], Kensy Rodriguez[3], Vanina Castroagudin[4], Camila Nicolli[5], Felipe Dalla Lana[3], Xin-Gen Zhou[6], Sara Thomas-Sharma[3], Terry Spurlock[5], Jim Correll[1], Pierre Gladieux[7], Alejandro Rojas[2] [1]University of Arkansas, [2]Michigan State University, [3]Louisiana State University AgCenter, [4]USDA-ARS, [5]University of Arkansas System Division of Agriculture, [6]Texas A&M AgriLife Research Center, [7]University of Montpellier, INRAE, CIRAD, Institut Agro, IRD

Rhizoctonia solani (Basidiomycota: Agaricomycetes) is a species complex classified into anastomosis groups (AGs) based on hyphal compatibility. AG-1 is an important group and further divided into subgroups based on differences in host range and molecular diversity. *Rhizoctonia solani* AG1-IA is the causal agent of sheath blight in rice and aerial blight in soybean, two devastating diseases in economically important crops. These crops are often used in rotation, resulting in an increase in inoculum in the fields over seasons. Currently, there is limited resistance to sheath blight in rice, and no soybean cultivars are known to be resistant to aerial blight. Monitoring genetic variability and addressing host-pathogen dynamics in *R. solani* AG1-IA is necessary to understand the origin and colonization history of the pathogen and can assist in the screening and development of new resistant cultivars. We sequenced 165 *R. solani* isolates, representing four subgroups of AG-1, collected over three decades (1993 to 2022) from different hosts and states in the United States, as well as other countries in North America and the Caribbean. The genetic diversity of the AG1-IA subgroup was determined using high-quality single nucleotide polymorphisms (SNPs). Also, we examined the phylogenetic relationships of *R. solani* AG-1 subgroups based on the ITS region and genomic relatedness based on whole-genome sequence similarity. Illumina reads were mapped to the genome of *R. solani* AG1-IA isolate HG81, achieving an overall alignment rate of up to 92% within the AG1-IA subgroup. Mapping percentages between subgroups were below 55%. The population genetic structure of the AG-1 group was inferred with clustering approaches based on ca. 920,000 biallelic SNPs. These analyses revealed that isolates from Arkansas showed low genetic variability and were distinct from those from Cuba, Texas, and Louisiana. Population structure had a clear geographical component, although signs of recent admixture were also observed. Estimates of nucleotide divergence between subgroups of AG-1 suggested that the split between the different subgroups within AG-1 was relatively ancient. Our work contributes to a better understanding of the genome-wide variability of *R. solani* AG1-IA and the history of lineage divergence within the anastomosis group AG-1.

808C **Recent co-evolution of two pandemic plant diseases in a multi-hybrid swarm** Mostafa Rahnama[1], Bradford Condon[2], João P Ascari[3], Julian R Dupuis[4], Emerson M. Del Ponte[3], Kerry F. Pedley[5], Sebastián Martinez[6], Barbara Valent[7], Mark L Farman[8] [1]Tennessee Tech University, [2]University of Kentucky, [3]Universidade Federal de Viçosa, [4]Entomology, University of Kentucky, [5]USDA, [6]Instituto Nacional de Investigación Agropecuaria, [7]Plant Pathology, Kansas State University, [8]Plant Pathology, University of Kentucky

Pyricularia oryzae (synonymous with *Magnaporthe oryzae*) is primarily recognized as the "rice blast fungus" due to its significant and global impact on rice crops. More recently, it has become a concern for potentially affecting global wheat production. Wheat blast, a new disease, was first detected in 1985 in Paraná, Brazil, and by 1990, it was widespread throughout all wheat-growing regions and neighboring countries. In recent years, wheat blast outbreaks have been reported in Asia and Africa, making it an emerging concern for global agriculture. Another disease caused by *P. oryzae*, gray leaf spot (GLS), was initially identified in 1971 in annual ryegrasses in Louisiana and Mississippi, USA. By the mid-1990s, GLS had caused widespread outbreaks of perennial ryegrass and the related species, tall fescue, in the central United States. In this study, we reconstruct the evolutionary history of two recent populations of *P. oryzae* that are responsible for these two novel diseases (wheat blast and GLS). We provide evidence that wheat blast/GLS evolved through two distinct mating episodes: the first occurred around 60 years ago when an *Eleusine*-adapted fungal individual mated with a *Urochloa*-adapted individual. In the subsequent decade, a single progeny of this cross was mated with a small number of individuals from three additional host-specialized populations. As a result of these matings, non-functional alleles of two key host-specificity factors, whose recombination in a multi-hybrid swarm probably facilitated the host jump, were introduced into the population. Additionally, we demonstrate that a very small number of mutations have occurred since the founding event and that the majority are private to individual isolates. Finally, our results showed adaptation to the wheat or *Lolium* hosts appears to have been instantaneous and driven solely by selection on repartitioned standing variation, with no apparent role being played by newly formed mutations.

809C **Dynamics of copy-number variation in response to fluconazole are dependent on drug concentration and temperature** Saaz Sakrikar, David Gresham New York University

Treatment of fungal infections relies on a limited series of drugs of which triazoles (like fluconazole) are the most commonly used class. However, heritable resistance to these drugs has been documented in common fungal pathogens. A commonly observed mechanism of resistance is the amplification of the ERG11 gene, whose product is the target of fluconazole.

Here, we investigate the dynamics of ERG11 copy-number variants (CNVs) in *Saccharomyces cerevisiae* in the adaptation to fluconazole. We use a fluorescent reporter system developed in the lab to track the CNVs at a single-cell level during experimental evolution in different fluconazole concentrations and growth temperatures.

We found that ERG11 CNVs arise repeatedly in the lower tested concentrations (16 and 32ng/µL), but not at higher concentrations or in the absence of fluconazole. Further, temperature plays a key role in the dynamics of ERG11 CNVs, with higher temperatures favouring quicker emergence and high prevalence of these CNVs. Sequencing revealed that the evolved strains were found to be whole chromosome aneuploidies, rather than local amplifications. This study clarifies the role of aneuploidies in rapid adaptation to a widely used drug, and will be used as a basis for further work in pathogenic fungal species, as well as longer-term evolution to understand the stability of CNVs as an adaptive mechanism.

810C **Genomic insights into the evolution of virulence in tan spot disease** Ryan Gourlie[1], Mohamed Hafez[2,3], Megan McDonald[4], Rodrigo Ortega Polo[2], Marcelo Carmona[5], Francisco Sautua[5], Carolina Moffat[6], Paula Moolhuijzen[6], Pao Theen See[6], Reem Aboukhaddour[1] [1]AAFC, Agriculture and Agri-Food Canada, [2]Agriculture and Agri-Food Canada, [3]Ag, [4]University of Birmingham, [5]Universidad de Buenos Aires, [6]Curtin University

Tan spot (Pyrenophora tritici-repentis) has emerged as a destructive foliar wheat disease fifty years ago in North America, Australia and worldwide. In this study, we present research advances from our lab exploring this pathogen's genome anatomy: its pangenome, genome architecture in relation to effector gene evolution, allelic diversity of its effector genes (ToxA and ToxB) across various species and global origins. We have utilized a large collection (427) of tan spot single spore isolates, representing diverse regions, including South and North America, Australia, the Fertile Crescent and encompassing regions, North Africa, Europe and Japan. We examined all identified pathotypes as well as isolates obtained from 1950s to present. A combinations of short (40) and long read sequences (24) were utilized to explore its pangenome and chromosomal structure analysis to detail the inheritance of its effector genes. Our finding reveals that tan spot has an open pangenome with one-speed compartment. We identified the involvement of various forms of starships elements in ToxA evolution and mobility within this species, and defined the presence

of novel ToxA haplotypes in Japan, South America, Canada and North Africa. Additionally, we investigated the architecture of multicopy ToxB and its surrounding region, and showed its unidirectional tandem repeats with a Helitron transposable element-mediated evolution of this multicopy virulence gene and its surrounding sequences. We identified the involvement of a Copia like element driving the loss of ToxB function, and defined the supernumerary nature of ToxB and its surrounding sequences in the tan spot species. Overall, this study provides the most comprehensive insight into the tan spot genome, highlighting its highly plastic nature characterized by gain or loss of effectors, gene duplication and the involvement of various types of transposable elements in shaping the evolution of virulence.

811C **Genomic Insights into the Population Structure and Reproductive Strategies of the rice pathogen *Cercospora janseana*** Jacob Searight[1], Adam N. Famoso[2], Xin-Gen Zhou[3], Vinson Doyle[2], Jonathan Richards[2] [1]Plant Pathology & Crop Physiology, Louisiana State University Agricultural Center, [2]Louisiana State University Agricultural Center, [3]Texas A&M AgriLife Research and Extension Center

Cercospora janseana, the causal agent of narrow brown leaf spot (NBLS) in rice, poses a persistent threat to rice production in the southern United States. Resistance breeding efforts have been ongoing since the 1940s and the recent identification of the *CRSP2.1* resistance locus marked a significant advance. Host resistance tends to breakdown rapidly over seasons, presumably due to a rapidly changing pathogen population. However, *C. janseana* genetic diversity and population dynamics remain unexplored. Our study addresses this knowledge gap by employing whole-genome sequencing and population genomic analyses to characterize the genetic diversity, population structure, and reproduction of *C. janseana*. We collected diseased rice samples from Louisiana and Texas in 2019 and 2020. Whole-genome resequencing of 136 isolates derived from these collections and read alignment to the newly generated *C. janseana* reference genome identified 808,859 single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). Population structure analyses revealed two lineages: NA1, which comprised 85% of the total samples and NA2. The presence of both lineages in each state/year sampled and low pairwise F_{ST} among sampling populations indicated migration is occurring among regions. A notable exception was the higher F_{ST} value (0.63) between the lineages, suggesting substantial genetic differentiation. We next examined genomic signatures of recombination within these lineages to infer modes of reproduction. The NA1 lineage showed evidence of recent or ongoing sexual reproduction, as indicated by rapid linkage disequilibrium decay, low index of association, and a significant pairwise-homoplasy index test. Furthermore, frequency of mating type idiomorphs did not deviate from a 1:1 ratio expected under random mating. In contrast, the NA2 lineage exhibited strong signs of clonal reproduction. These insights into the genetic and reproductive dynamics of *C. janseana* are crucial for developing effective and sustainable disease management strategies, particularly in the context of deploying resistant cultivars.

812C **Investigating the history and consequences of secondary contact between divergent populations of *Trichaptum abietinum* in Europe** Dabao S Lu[1], Ine-Susanne Hopland Methlie[1], Michelle Vera Castellanos[1], Jørn Henrik Sønstebø[2], Anneli Andersen[1], Kathleen T Helleland[1], David Peris[3], Sundy Maurice[1], Håvard Kauserud[1], Inger Skrede[1] [1]Dept of Biosciences, University of Oslo, [2]2Dept of Nature, Health and Environment, University of South-Eastern Norway, [3]Institute of Agrochemistry Food Biotechnology (IATA) CSIC

The glacial cycles of the Quarternary have been a major factor in shaping the present-day genetic structure of species on the northern hemisphere. During the glacial periods, populations of species were commonly isolated in multiple glacial refugia where they could diverge in allopatry. In the ensuing interglacials these diverged populations expanded their ranges and came into secondary contact, and the resulting admixture of genomes in these hybrid zones can be seen as natural experiments for the study of speciation. There is limited information on postglacial migration of fungi, and it is unclear to what extent they have followed the same recolonization route as plants and animals. Here we trace the postglacial history of the widespread wood-decay fungus *Trichaptum abietinum* in Europe using population genomic analyses of 138 genomes. We detected a Mediterranean lineage in Southern Europe and a Boreal lineage in Eastern Europe and Fennoscandia, that we connect to glacial survival in southern and eastern refugia, respectively. These two lineages form an admixture zone in Central Europe, and local ancestry analyses of the admixed individuals reveal that the second half of their largest chromosome is entirely inherited from the Boreal lineage, indicating either strong selection or genomic incompatibilities. To understand this pattern further we have generated F1 lab hybrids between the Boreal and Atlantic lineages to compare to the natural hybrids, which our analyses suggest originated from secondary contact at least 600 generations ago. In addition, a fourth Atlantic lineage was detected in western Scandinavia and Europe, and we connect its origin to a western refugia. Intriguingly, the Atlantic and Boreal lineages appear to have limited admixture in Scandinavia despite being in proximity and able to mate in the lab. We therefore assess the fitness of lab generated hybrids of the Atlantic and Boreal lineages with growth experiments. In conclusion, the postglacial history of *T. abietinum* in Europe resemble what has been found in numerous plants and animals, with survival and divergence in multiple separate glacial refugia and the formation of hybrid zones in Central Europe.

813C **Experimental Evolution of *Benniella erionia* and Mollicutes-Related Endobacteria** Reid Longley[1], Aaron Robinson[2], Gregory Bonito[3], Patrick Chain[2] [1]Bioscience Division, Dept of Genomics and Bioanalytics, Los Alamos National Laboratory, [2]Los Alamos National Laboratory, [3]Michigan State University

It has become clear that interactions between diverse bacteria and fungi are common. However, many of these interactions can be short-lived making them difficult to study in laboratory settings. However, diverse Mucoromycota fungi are known to host gram-positive Mollicutes-related endobacteria (MRE) and gram-negative *Burkholderia*-related endobacteria (BRE) which make ideal model systems for studying bacterial-fungal interactions due to the long-term nature of the interactions. Our recent work demonstrated that MRE have highly reduced genomes and metabolic capacity, likely making MRE completely dependent on their fungal hosts. Additionally, MRE genomes are characterized by a high degree of rearrangement leading to low levels of synteny between closely related taxa. To further investigate the evolution of MRE and their fungal hosts, we have carried out a long-term evolution experiment on *Benniella erionia* GBAus27B and it's MRE. Briefly, fungal hosts containing endobacteria and those cured of endobacteria are transferred every three weeks to fresh 1% Malt Extract Agar (MEA) and 0.1% MEA to assess the impacts of nutrient availability on evolution of fungal hosts and MRE. Endobacterial load is tracked using QPCR and evolution of hosts and MRE is assessed using periodic (every six months) genome sequencing. Preliminary results indicate that *Benniella erionia* has maintained large populations of endobacteria (> 10^5 cells/mg of fungal tissue) for a period of 48 weeks. However, in low nutrient conditions, MRE populations have steadily declined over the experimental period indicating that in stressful low nutrient conditions, MRE populations may not be maintained to due to an expected nutritional burden on the fungal host. Genomic sequencing after six months of experimental evolution indicated that MRE genomes have undergone more insertions/deletions and rearrangements compared to other types of mutations while fungal host genomes remain largely unchanged. This work will give important insights into the role of rearrangement in genome evolution of endosymbionts which are expected to be valid in diverse endosymbiont systems across the Eukaryotic tree of life.

815A **Expanding the repertoire of fungal heterologous hosts for the expression of natural products** Adrian Gadar, Pablo Cruz-Morales DTU biosustain

Natural products (NP) are widely appreciated for their bioactivities and are a very important source of drugs. Nevertheless, due to their extraordinary chemical diversity, they can be used to make polymers, fuels, and pesticides, replacing petroleum-derived products.

The biosynthesis of natural products is driven by biosynthetic gene clusters (BGC) which are often triggered by specific environmental cues, hindering their production in the laboratory. Heterologous expression bypasses native regulation allowing controlled production of the NP. Fungi are highly attractive hosts for sustainable production of chemicals as they can use lignocellulosic and waste-derived feedstocks. However, the number of fungal hosts for natural products production is limited to a few species of *Aspergilli* and *Saccharomycotina* yeast.

In this project, we established four novel fungal heterologous hosts capable of growing on second generation feedstocks. We developed genetic engineering tools for all of them using either classical or CRISPR-Cas9-based methods. For proof-of-concept we challenged our hosts for (i) productivity and (ii) proficiency.

For productivity (i) we tested the expression of the model polyketide synthase 6-methylsalicylic acid (6-MSA). We quantified and compared the strain's performance which showed that our hosts can reach gram per liter titers.

For proficiency (ii)we tested the expression of six PKSs with different architectures including a previously undescribed highly reducing, methylated PKS-NRPS hybrid. Our results showed that our hosts can express complex NPs that cannot be expressed in *Saccharomyces cerevisiae.*

We believe that our new hosts will allow the systematic expression and engineering of polyketide synthases which hold promise for the sustainable production of hydrocarbon products.

816A **Learning chemistry from fungi to make sustainable chemicals** Pablo Cruz-Morales Center for Biosustainability, Technical University of Denmark

Transportation, manufacturing, and agriculture depend on petroleum and produce large amounts of greenhouse gas emissions which are accelerating climate change. We need sustainable alternatives to make food, pesticides, fuels, and materials. Fungal natural product metabolism covers a huge chemical space, we believe that a new generation of industrial chemistry can be developed upon this chemical and enzymatic repertoire. We are cataloging the fungal natural products chemical and genetic space. We are using this information to program well-known and novel fungal hosts to make new molecules using sustainable and inexpensive feedstocks.

Our platform is useful for heterologous production of chemicals using fungal Polyketide Synthases (PKSs) and Non-Ribosomal Peptide Synthetases (NRPSs). We have developed bioinformatics and synthetic biology toolkits to mine thousands of fungal genomes for NRPSs and PKSs, we have selected synthases for heterologous production and engineering using fungal hosts. To test our platform, we assembled a strain collection of >100 *Hypocreales* fungi. We sequenced their genomes and used tandem mass spectrometry to assess their chemical repertoire. We have found the BGCs for many new and known molecules and created a catalog of bioparts for heterologous expression. In this chance, I will present the platform and show case its application on the production of insecticides and fuels and polymers.

817A **Investigation of the biosynthetic gene cluster for the production of the blue-green pigment xylindein by *Chlorociboria* species** Yanfang Guo[1], Jorge Navarro-Muñoz[2], Caroline Rodenbach[1], Elske Dwars[1], Chendo Dieleman[1], Bart van den Hout[3], Bazante Sanders[3], Miaomiao Zhou[3], Russell J Cox[4], Arnold J M Driessen[5], Jérôme Collemare[1] [1]Westerdijk Fungal Biodiversity Institute, [2]Wageningen University and Research, [3]Avans University of Applied Sciences, [4]Institute for Organic Chemistry and BMWZ, Leibniz Universität Hannover, [5]University of Groningen

Xylindein is a vibrant blue-green pigment produced by the Leotiomycetes fungi *Chlorociboria aeruginascens* and *Chlorociboria aeruginosa*. The stunning color and promising optoelectronic performance makes xylindein valuable in textile coloration, decorative wood industry and as natural semiconductor material. Production of xylindein for industrial applications has remained very challenging. Elucidating the biosynthetic pathway of xylindein in *C. aeruginascens*, and using a heterologous expression strategy represent a promising alternative for producing pure xylindein. Previously, we mined the genome of *C. aeruginascens* and identified a unique candidate biosynthetic gene cluster (BGC), with a non-reducing polyketide synthase (nrPKS) gene which is closely related to the viriditoxin *vdtA* nrPKS gene. First attempts to express the genes responsible for xylindein intermediate in the heterologous host *Aspergillus oryzae* were unsuccessful for reasons that remain to be determined. Here, we sought for additional evidence to link the candidate BGC to xylindein by sequencing the genome of the other known producer *C. aeruginosa*, and by performing RNA sequencing during the time course of xylindein production. In addition, heterologous expression of the *vdtA* gene encoding a nrPKS was performed to obtain a first polyketide backbone that will allow further investigations of xylindein tailoring enzymes encoded in *Chlorociboria* genomes.

818A **Characterization of two landing sites for genomic integration in the Sordariales *Podospora anserina*** Camille Guilly[1], Herve Lalucque[2] [1]Laboratoire Interdisciplinaire des Energies de Demain, Université Paris Cité, [2]Laboratoire Interdisciplinaire des Energies de Demain, Universite Paris Cite

Secondary metabolites (SM), a.k.a specialised metabolites and natural products, are compounds that are defined as non-essential for the survival of the producing organism, but confer a selective advantage in its biotope. Due to their properties, these molecules are often of interest in the pharmaceutical, industrial and food sectors. Analysis of fungal genomes reveals a wealth of genes involved in the biosynthesis of secondary metabolites, although only a limited number of molecules have been identified. This may be explained by the fact that biosynthetic genes are silent under laboratory culture conditions. Various strategies have been developed successfully to induce gene expression, however, many molecules remain to be discovered and identified, requiring new tools.

In recent years, advancements in synthetic biology with Golden Gate cloning and the MoClo syntax facilitate the construction of complex DNA plasmids from previously validated parts (biobricks), opening up the possibility of modifying the structure and regulation of these SM genes. This requires the characterization of genomic insertion sites that allow efficient integration and proper expression of the transgene. We present here the characterization of two integration sites in the Sordariales *Podospora anserina*, a species that grows in an original and highly competitive biotope, namely herbivore drops. These two landing sites allow integration and expression of selection markers and reporter genes. Progress in the characterization of promoters to allow fine control of the expression of transgene of interest in *P. anserina* will be presented.

819A **Probing the limitations of synthetic biology platforms through *in situ* biosynthetic gene cluster reconstruction** Daniel Berry, Luke Stevenson, Rosannah Cameron, Emily Parker The Ferrier Research Institute, Victoria University of Wellington

Fungal genomes contain many biosynthetic gene clusters (BGCs), each of which encodes the proteins required to manufacture a specific natural product. Advances in synthetic biology have greatly enhanced our ability to transfer BGCs between fungi, enabling the biomanufacture of useful natural products from intractable donor organisms using more suitable hosts. Coordinated expression of transferred genes is required to achieve biosynthesis of the target natural product, which can be achieved by assembling a synthetic BGC that combines the coding sequences from a donor BGC with regulatory sequences from a host BGC that is expressed under desirable culture conditions. For example, we have successfully used the regulatory sequences from the paxilline BGC (*PAX*) of *Penicillium paxilli* to transfer biosynthesis for more than a dozen different indole diterpene natural products into that host. However, little is known about the regulation of the *PAX* cluster, which lacks an integrated transcription factor gene, nor do we understand how *PAX* regulatory sequences perform in these synthetic BGCs relative to their native setting.

Aiming to identify any factors limiting our synthetic biology platform, we performed *in situ* replacement of the *PAX* cluster with a synthetic BGC designed to replicate the native *PAX* cluster as faithfully as possible. While tandem integration of the synthetic *PAX* BGC restored paxilline production to WT levels, the single copy integrants that most accurately replicate the native state underperformed substantially. More surprisingly, we found that reconstruction of paxilline production was dependent on the presence of a positive selection gene cassette that included the promoter sequence from the *Aspergillus nidulans* gene *trpC*; reconstruction strains generated from a marker-free synthetic *PAX* BGC were unable to produce paxilline. We hypothesised that this deficiency might be due to low gene expression caused by the alteration of DNA methylation patterns and/or subtle sequence differences inherent to the synthetic *PAX* BGCs, with the *trpC* promoter able to partially ameliorate expression for nearby genes through recruitment of transcriptional regulators. We therefore synthesised and tested performance for a series of *PAX* BGCs that incrementally eliminated each of these variables, the results and implications of which will be presented here.

820A **Leverage of fungal growth data through modelling: applications to the study of antifungal drugs** David Canovas Genetics, University of Sevilla

Microbial growth analysis serves as a valuable tool for extracting information from various strains growing in a variety of conditions, which is an established knowledge among microbiologists. However, it is common for such analyses to solely focus on the qualitative assessment of the quantitative data obtained experimentally. In order to fill this gap, in this study I tested different mathematical growth models using real experimental datasets. Among the tested models, one function emerged as the most cost-effective in fitting the growth datasets. To validate this model, I employed growth datasets obtained by growing *Aspergillus nidulans* under varying nitrogen concentrations and different inoculum sizes. This model yields four parameters delineating the growth curve characteristics. Altering nitrogen concentration impacted both maximum growth and growth rate, while inoculum size showed an inverse correlation with the inflection time. Once validated, the model was utilized to analyse the growth characteristics of the fungal human pathogen *Aspergillus fumigatus* in response to antifungal drugs. Voriconazole at subinhibitory concentrations primarily reduced the growth rate, leaving other parameters unaffected. Moreover, the *ΔnctA* and *ΔnctB* mutants, which have been reported to have an increased resistance to triazoles, exhibited an increased fitness in growth rate, but not in any other growth paramenter. This effect aligns with the primary effects of subinhibitory concentrations of voriconazole in the wild-type strain observed in this study. The method developed herein facilitates automated characterization of hundreds of samples simultaneously, presenting promising applications in high-throughput antibiotic drug screenings.

821B **Towards the development of a safeguarding CRISPR RNA-guided gene drive to mitigate the impacts of the non-native fungal pathogen *Sphaerulina musiva* on managed ecosystems** Joshua Sparks[1], Kelsey Sondreli[2], Cole Sawyer[1], Tomas Rush[1], Dana Carper[1], Wellington Muchero[1], Daniel A Jacobson[1], Carrie Eckert[1], Paul E Abraham[1], Jared LeBoldus[2], Joanna Tannous[1] [1]Oak Ridge National Laboratory, [2]Oregon State University

Invasion of non-native fungal species is acknowledged as one of the major external drivers altering the structure, biodiversity, and function of ecosystems. Understanding the mechanisms of establishment of these invaders and developing mitigation approaches to manage them is a critical aspect of sustaining native biodiversity and normal ecosystem functions. The fungal pathogen *Sphaerulina musiva* is a well-characterized example of an invasive species spread unintentionally by human activities. Originally native to Eastern North America, *S. musiva* was only recently introduced and established into the Pacific Northwest of North America resulting in deleterious effects on susceptible *Populus* species/genotypes, a foundational bioenergy crop, and a keystone tree species in forested ecosystems.

In our efforts to establish a safeguarding CRISPR RNA-guided gene drive to mitigate *S. musiva*'s diseases on *Populus* plantations, we recently developed a CRISPR-Cas9 system to genetically manipulate this non-model fungal species. This tool has been used to identify and validate genetic determinants of establishment and pathogenicity in *S. musiva* including effector genes and secondary metabolite biosynthetic genes. Those target genes will guide engineering risk mitigation strategies such as gene drives for more sustainable and productive ecosystems.

822B **High-throughput CAZyme production in *Aspergillus oryzae*** Martí Morera, Martzel Antsotegi, Lucas Levassor, Bernard P Henrissat, Uffe H Mortensen Bioengineering, DTU

To support the transition to a green and sustainable economy, novel industrial enzymes are necessary. Enzymes of particular interest in this context are Carbohydrate-Active EnZymes (CAZymes) where novel CAZymes targeting specific substrates are in great demand to address specific challenges in industrial applications. Fortunately, eukaryotic genome sequences show that there is a vast repertoire of uncharacterized CAZyme genes, which may deliver the desired activities. As our understanding of enzyme structure-function relationships advances, the engineering and discovery of new CAZymes hold immense potential for driving progress in various sectors of the bioeconomy, but for many of the novel genes it is still difficult to predict their function and to elucidate their substrate specificity, therefore it is necessary to characterize their gene products *in vitro*.

A successful strategy has been to heterologously produce the new enzymes in bacterial hosts followed by purification and characterization. However, this strategy has been less successful for the analysis of eukaryotic genes where enzyme yields have been low or absent. We hypothesize that a strategy for elucidating eukaryotic CAZymes based on fungal cell factories will result in a higher success rate, since fungi are better suited for eukaryotic enzymes, due to a dedicated secretory pathway that offers folding control and post translational modifications.

So far, this strategy has been hampered by the lack of specific tools for high-throughput strain engineering; and the goal of this project is to establish an automated setup that allows heterologous expression and characterization of new CAZyme genes. This system allows for mid-throughput when performed by hand, and high-throughput by using different liquid handlers (i.e. Tecan and Opentrons) and robotic equipment (i.e. QPix). To date, we have validated the method with a subset of 22 targets. We aim at automating all the fungal genetic engineering steps, which will allow us to express, produce and purify any desired target of enzymes in a high-throughput manner, with a vision to cover unexplored territory in the eukaryotic CAZyme map for novel enzyme discovery.

823B **Signal Peptide Engineering in Filamentous Fungi driven by lab automation and AI** Lucas Levassor[1,2], Martí Morera Gómez[1], Rasmus J.N. Frandsen[1], Uffe H. Mortensen[3] [1]Bioengineering, Technical University of Denmark, DTU, [2]Biosustain, Technical University of Denmark, DTU, [3]Technical University of Denmark, DTU

Filamentous fungi are key producers of industrial enzymes, widely used in the food and pharmaceutical industries. Leveraging their role as decomposers, these fungi exhibit enhanced enzyme secretion capabilities. To increase their promising protein secretion abilities several methods have been employed such as increasing gene expression but recently research has focused on the secretion pathway. The primary protein secretion pathway in these fungi involves signal peptides directing polypeptides to the ER membrane for subsequent cleavage. In eukaryotic systems, protein secretion has been increased by swapping homologous and heterologous signal peptides, while prokaryotic systems have seen advancements through the modification of peptide residues. These efforts mainly modify existing signal peptides rather than creating new ones.

De-novo design of proteins and peptides has long been a goal for biologists. However, while deep-learning and machine-learning approaches have yielded functional de-novo signal peptides, these advancements have yet to reach higher performance than native signal peptides. These models can decipher complex patterns in large datasets but the major hurdle lies in generating large-quality datasets for model training. This challenge could be met by employing automated biological labs with liquid-handling robots to facilitate data generation. Additionally, recent developments in open-source Python libraries have shown significant promise in streamlining microbial strain construction.

This study introduces an iterative learning cycle for engineering signal peptides in A. oryzae, anchored in the Design-Build-Test-Learn (DBTL) framework, to i) generate novel signal peptides using deep-learning and machine-learning across DBTL cycles; ii) apply literate programming for systematic, high-throughput strain design and integration of genetic elements; iii) test cross-species universal signal peptides for Aspergillus species. This innovative approach holds promise in advancing our understanding of signal peptides and enhancing the process of enzyme secretion in filamentous fungi.

824B **A Single Step Multi-Copy Integration System Based on Rolling-Circle Replication** Martzel Antsotegi[1], Martí Morera Gomez[1], Zofia Jarczynska[1], Katherina García[1], Vasil D'Ambrosio[2], Jean-Marie Mouillon[2], Uffe H Mortensen[1] [1]DTU Bioengineering, Technical University of Denmark (DTU), [2]Novozymes R&D, Novozymes

Fungi are often used as cell factories for heterologous production of enzymes and metabolites. One strategy to obtain high yielding strains is to enhance the expression level of the gene(s) responsible for production of the product, which can be achieved by inserting multiple copies of the gene(s) of interest. Typically, this is achieved by transforming NHEJ proficient strains with large amounts of a DNA vector, which randomly integrates in multiple copies at different loci, or more often, into a single locus with copies arranged as direct- and inverted repeats (DRs and IRs). The drawback of this method is that the resulting strains are often unstable and difficult to characterize. We speculated that unstable multi-copy arrays may mostly be due to the presence of IRs as they are known to cause genomic instability; and that arrays which are solely formed by copies arranged as DRs are more stable. To test this idea, we have developed a method that allows an array of DRs to be integrated into a single genomic locus. In our method, the DRs are obtained by Rolling-Circle replication of a circular vector containing a sequence of interest, e.g. a gene, and a sequence matching the desired target site in the genome. Rolling-Circle replication is initiated by a 3' DNA-end liberated from the target site in the genome by a DNA double-strand-break induced by Cas9. After Rolling-Circle replication, the resulting DRs are integrated into the target site as the DNA double-strand-break is sealed by Homology Recombination (HR). Moreover, exploiting the *in-vivo* assembly toolbox developed in our lab[1], we demonstrated that plasmids assembled from PCR fragments *in-vivo* after transformation can be used for Rolling-Circle replication allowing integration of DRs without any undesired *E. coli* sequences that would normally be part of a plasmid.

To demonstrate the potential of the method, we took advantage of our DIVERSIFY gene expression system[2] that provides landing platforms for integration of expression cassettes by Cas9 induced HR. As proof of concept, we have used the method to insert an RFP expression cassette into A*spergillus nidulans*, *Aspergillus oryzae*, and *Aspergillus niger*. In all species, the transformants exhibited a spectrum of copy numbers, but as expected, all copies were integrated as DRs in all arrays examined. Importantly, even for strains with 12 copies of RFP, production levels appear stable in a colony with a diameter of 10 cm.

References:

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825B **Characterizing the effects of simulated space environmental conditions on the biological and mechanical properties of fungal composite biomaterials** Rolando Perez[1,2], Katheryn Kornegay[2], Hannah Krivic[3], Victoria Porto[2], Sujith Pakala[4], Monika Brandic Lipinska[5], James Head[4], Christopher Maurer[6], Martyn Dade-Robertson[5], Maikel Rheinstadter[3], Debbie Senesky[2], Lynn Rothschild[7] [1]NASA Ames Research Center, Blue Marble Space Institute of Science, [2]Stanford University, [3]McMaster University, [4]Brown University, [5]Newcastle University, [6]Redhouse Studio, [7]NASA Ames Research Center

With increasing interest in long-term space exploration there is a need for technologies that enable *in situ* resource utilization (ISRU) to reduce the dependance of future missions on resupply services from Earth. For example, current proposals for lunar civil infrastructure construction, such as habitats for humans, use steel structures delivered from Earth. While it is acceptable to use steel infrastructure for lunar missions, and initial missions beyond the Moon, a sustained presence beyond the Moon would incur significant upmass costs in the long term. With resupply services to the Moon feasible, the lunar surface is increasingly being viewed as a testbed for technologies that enable longer-term and -distance space exploration. ISRU using lunar regolith for material production has been proposed but it relies on resource-intensive processes, such as lunar concrete production via laser melting. In contrast, biologically-based processes exhibit potential benefits, *e.g.*, regeneration. An alternative approach, working with fungi to bind biomass supplied from Earth or produced *in situ*, or lunar regolith mined *in situ*, could enable significant benefits in terms of cost, performance, function, and aesthetics. To this end, we have characterized the effects of simulated space environmental conditions, such as reduced pressure and UV exposure, on the biological and mechanical properties of fungal composite biomaterials, such as growth performance and compressive strength. Our initial results suggest that fungal composite biomaterials may be a viable alternative to current methods for producing valuable materials, *e.g.*, construction materials for habitats. If humanity is to explore beyond low-Earth orbit we will need advanced ISRU, material production capacities and bioregenerative life support systems to sustain life. Fungal composite biomaterials may be a viable solution to this need.

826C **Advancing Yarrowia lipolytica for heterologous production of production of fungal polyketides** Mihaela Bejenari, Sebastian Petzold, Jens Laurids Sørensen Dept of Chemistry and Bioscience, Aalborg University

The fungal world is full of colorful pigments, which often are based on quinones. These molecules are mainly polyketide-derived secondary metabolites. Besides their colorful appearance, quinones can several biological functions, including antimicrobial effects and repression of the immune response in insects. In our group, we have recently developed a redox flow-battery based on the subgroup of quinones for storing electricity from renewable sources [1, 2].

However, polyketide production in fungi is associated with several challenges: reduced production efficiency and increased costs, which hinder polyketide use for RFBs. To tackle these aspects, we switched from fungi to the yeast *Yarrowia lipolytica*, an easily cultivable heterologous host. As an oleaginous yeast, Y. lipolytica displays a high flux of acetyl- and malonyl-CoA precursors, which are also the building blocks of fungal polyketides. Despite its promising prospect, Y. lipolytica has so far only been used for heterologous production of simple type III polyketides from plants. We, therefore, examined the potential for more complex polyketide production in Y. lipolytica by targeting fungal polyketides derived from type I PKSs. In our study, we produced the quinone bostrycoidin by heterologously expressing three genes from *Fusarium vanettenii*. This resulted in a yield of 29 mg/L in shake flask conditions. We are currently optimizing this yield through fermentation in bioreactors, while also exploring production of other target quinones, which can subsequently be used for sustainable energy storage.

1. Wilhelmsen CO, Kristensen SB, Nolte O *et al* (2023): *Batteries & Supercaps*, 6: e202200365.

2. Wilhelmsen CO, Muff J, Sørensen JL (2023): *Energy Storage*, e450.

827C **Biomineralization-Enabled Self-Growing Building Blocks for Habitat Outfitting on Mars** Nisha Rokaya[1], Richard Wilson[1], Congrui Jin[2] [1]Plant Pathology, University of Nebraska-Lincoln, [2]Texas A&M University

The outfitting of inflatable habitat on Mars currently relies on launching necessary equipment from a second spacecraft. Rather than shipping prefabricated materials from earth, a biological toolkit that would allow to self-growing of the habitat outfitting on Mars by utilizing the initial in-situ resources on Mars, including sunlight, water, CO_2, N_2, and trace minerals enable long-term human space exploration and colonization. The focus of this research is the development of a synthetic lichen system using a phototroph-heterotroph symbiosis capable of thriving in the Martian atmosphere. The system would produce abundant biominerals and biopolymers, effectively binding Martian regolith into consolidated building blocks. To construct this synthetic community, two key species are employed: diazotrophic cyanobacteria, responsible for extracting CO_2 and N_2 from the air, and filamentous fungi, which excrete Ca^{2+} and stimulate the formation of substantial $CaCO_3$ precipitates. Experimental results confirm that these co-culture systems can grow very well on air and light in an inorganic liquid with a Martian regolith simulant (MRS) medium, without the need for additional carbon or nitrogen sources. Among ten tested filamentous fungi in co-culture with the diazotrophic cyanobacterium *Anabaena* sp., three fungi (*Trichoderma reesei, Aspergillus niger*, and *T. viride*) exhibited significantly enhanced growth of both cyanobacteria and fungi compared to the other seven fungi, highlighting the crucial role of mutual interactions with specific fungi. Since Mars' atmosphere consists of only traces of oxygen, we further tested axenic and co-culture growth of diazotrophic cyanobacteria and fungi under anaerobic conditions. Results reveal the oxygen produced by a diazotrophic cyanobacterium in 20% CO_2 and 80% N_2 supports the growth of fungi. In addition, the Mars atmosphere has a pressure of 7.5 mbar, 2.8% N_2, which is incompatible with the metabolism of most microbes. Therefore, to implement this technology, a photobioreactor will be utilized to provide the microbes with tightly regulated atmospheric conditions, as well as other necessary parameters inside the habitat, such as illumination, heating to optimal temperatures, and protection against harmful radiation.

828C **Towards Genetic Engineering in Anaerobic Fungi** Sarah Seagrave[1], Tejas Navaratna[2], Elaine Kirschke[3], Michelle O'Malley[3,4] [1]BioE, UCSB, [2]UCSB, [3]Chemical Engineering, UCSB, [4]Biological Engineering, UCSB

Anaerobic fungi, isolated from the guts of herbivores, contain unprecedented levels of carbohydrate active enzymes, which break down plant biomass at ambient conditions into highly valuable molecules. Despite the undeniable potential these microorganisms contain for green chemistry, the ability to genetically modify them has posed a challenge due to the uncharted novelty of their biology. This study involves efforts to create a comprehensive functional framework for genetic engineering within the anaerobic fungal clade *neocallimasticomycgota*, from refining and discovering elements for synthetic biology (i.e. promoters, safe landing sites, etc.) to enhancing the delivery of foreign nucleic matter into the nuclei. The findings from this study aim to broaden the horizons of synthetic biology within fungal systems, unlocking novel avenues for scientific exploration and innovative applications.

829C **Synthetic expression system enhances recombinant protein production in *Aspergillus oryzae*** Casper R. B. van der Luijt[1,2,3,4], Vayu Maini Rekdal[3,5,6], Yan Chen[3,4], Christopher J. Petzold[3,4], Jay D. Keasling[2,3,4,5,7,8], Leonie J. Jahn[2], Morten O. A. Sommer[2] [1]Dept of Food Science, University of Copenhagen, [2]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, [3]Joint BioEnergy Institute, [4]Biological Systems & Engineering Division, Lawrence Berkeley National Laboratory, [5]Dept of Bioengineering, University of California, Berkeley, [6]Miller Institute for Basic Research in Science, University of California, Berkeley, [7]Dept of Chemical and Biomolecular Engineering, University of California, Berkeley, [8]Center for Synthetic Biochemistry, Institute for Synthetic Biology, Shenzhen Institutes of Advanced Technologies

For centuries, the koji mold *Aspergillus oryzae* has played a crucial role in East Asian food fermentations, producing culinary staples such as soy sauce, miso, and sake. More recently, its proficiency in secreting enzymes has positioned it as a cornerstone in industrial biotechnology. This makes *A. oryzae* uniquely suitable for the heterologous production of food proteins for use in novel meat and dairy alternatives. However, there is a critical lack of well-characterized strong constitutive promoters for *A. oryzae*.

To address this, we established a synthetic expression system for *A. oryzae*. This modular system involves a synthetic transcription factor in one locus and a gene of interest under control of a core promoter and upstream activating sequence (UAS) in the other. These elements interact with the synthetic transcription factor, allowing precise control over gene expression.

Using the synthetic expression system, we screened a curated library of thirteen core promoters, derived from *A. oryzae* genes with high expression levels. This revealed a wide dynamic range, enabling the fine-tuning of gene expression levels. The most potent core promoter, in conjunction with six repeats of the UAS, enabled a remarkable sixfold increase in mycelial fluorescent protein levels compared to the strong native alpha-amylase promoter (PamyB) under PamyB-inducing conditions, and was equally effective on minimal glucose media. To our knowledge, this makes it the strongest promoter for *A. oryzae* published to date.

Furthermore, by combining UASs with two core promoters oriented in opposing directions, we engineered synthetic bidirectional promoters. These substantially reduce the time it takes to optimize production strains by enabling multiplex gene integrations in a single transformation.

Collectively, these results will contribute to the establishment of *A. oryzae* as a reliable platform for more efficient and sustainable recombinant protein production and help pave the way for novel precision fermentation processes.

830C **Development of bacterially-mediated transformation methods for anaerobic gut fungi** Hugh M Purdy[1], Sarah Seagrave[2], Elaine Kirschke[1], Tejas Navaratna[1], Noa Margalith[3], Michelle O'Malley[1,2] [1]Chemical Engineering, University of California, Santa Barbara, [2]Bioengineering, University of California, Santa Barbara, [3]University of California, Santa Barbara

Microorganisms hold great promise for applications in biotechnology, including the renewable production of fuels and chemicals, the biosynthesis of therapeutics, and even the creation of novel materials. However, it is well known that only a relatively small percentage of microbial species are isolated and characterized sufficiently to be amenable for engineering purposes, thus limiting our repertoire of available biological tools. This issue is particularly true for hard-to-culture organisms. One such group of organisms, the anaerobic gut fungi (phylum Neocallimastigomycota), hold significant, untapped biotechnological potential. These fungi, which are found predominantly in the digestive tracts of herbivores, possess an expansive array of uncharacterized carbohydrate-active enzymes, indicating a high-degree of potential for applications involving the processing and conversion of lignocellulosic material. Furthermore, as these fungi natively exist in a competitive microbial environment, they are believed to possess diverse secondary metabolites with potential for therapeutic applications.

However, studies of anaerobic gut fungi are severely limited by an almost complete lack of genetic tools. The genetic intractability of these organisms also hinders efforts at strain engineering for biotechnological applications. To overcome these restrictions, we are in the process of developing tools and techniques for the genetic manipulation of these fungi. A critical first step in this regard is to develop methods for introducing exogenous DNA into the fungal cells for stable or transient expression. We are investigating multiple mechanisms to carry out this genetic transformation, though this work will focus on our efforts to develop a bacterially-mediated transformation protocol. *Agrobacterium*-mediated transformation is well established in many aerobic fungi, though successful use of this system in anaerobic conditions has not been reported and is one focus of our investigation. Additionally, we are studying the potential of bacteria-to-fungi transformation using bacterial plasmid-conjugation systems, as has recently been reported in both yeast and several species of algae. The development of these systems into functional transformation tools in anaerobic gut fungi will significantly expand the scope of studies that can be performed on these organisms and will usher in the development of engineered fungal strains to address pressing biotechnological applications.

831C **A Biofoundry for Synthetic Biology and Genetic Tool Development of Anaerobic Gut Fungi** Elaine Kirschke[1], Michelle O'Malley[2] [1]Institute for Collaborative Biotechnologies, University of California Santa Barbara, [2]Chemical Engineering, University of California Santa Barbara

Anaerobic microbial communities possess many characteristics that make them attractive system for industrial application especially for the degradation of recalcitrant polymers and the conversion of renewable resources such as agricultural plant biproducts to biofuels and other value add chemicals. In particular, the anaerobic gut fungi (AGF) found in the digestive tracks of large herbivores, such as *Neocallimastix sp.*, poses impressive ability to break down lignocellulose plant fibers. Correlating with this, their genomes possess a significant expansion of genes associated with lignocellulose degradation. Moreover, recent findings from the O'Malley lab suggest that AGF harbor molecular machinery with the ability to facilitate bond cleavage in one of the most recalcitrant plant polymers, lignin, utilizing a novel uncharacterized anaerobic mechanism. However, our ability to characterize, understand, and exploit these fungal systems and their molecular machinery is severely limited. Challenges include technical consideration in handling obligate anaerobes along with a lack of genetic tools and a limited biological understanding of these understudied organisms. To further scale and accelerate our efforts, and those of others working with anaerobic microbial systems, we are establishing a user facility at UCSB that will house an automated robotic system equipped for high throughput workflows. This fully automatable system consisting of 13 different integrated instruments will enable synthetic biology and genetic tool development of anaerobic microbes and microbial communities. Leveraging the high throughput workflows enabled by this system we aim to develop efficient transformation procedures for AGF and the necessary tools for genome editing and synthetic biology applications. Together, this work should further our understanding of these fungal system and allow their industrial potential to be more fully explored.

832A **Myco-Ed: Mycological Curriculum for Education and Discovery** Stephen J Mondo[1], Amy Honan[2], Sara Branco[3], Andrew Wilson[4], Kerrie Barry[5], Lotus Lofgren[6], Chinyere Knight[7], Sara Gremillion[8], Ami Wangeline[9], Jane Stewart[10], Alisha Quandt[11], Jayson Talag[12], Ellen Dow[13], Gerald Cobián[14], Victoria M Bunting[12], Christopher Bivins[15], Geoff Zahn[16], Tanya Cheeke[17] [1]Fungal and Algal Genomics, DOE Joint Genome Institute, [2]Oregon State University, [3]University of Colorado Denver, [4]Denver Botanic Gardens, [5]DOE Joint Genome Institute, [6]Duke University, [7]Tuskegee University, [8]Georgia Southern University, [9]Laramie County Community College, [10]Colorado State University, [11]University of Colorado Boulder, [12]Arizona Genomics Institute, [13]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, [14]California State University, Chico, [15]University of California Merced, [16]Utah Valley University, [17]Washington State University

In a collaborative effort, mycologists from diverse institutions are developing Myco-Ed, a Course-based Undergraduate Research Experience (CURE) to train undergraduate students in fungal biology and comparative genomics. The learning outcomes for students include fungal culturing, experimental design, DNA amplification, and how to conduct comparative genomic analyses. Students in undergraduate mycology courses isolate fungi, assay them under specific conditions, document phenotypic responses, and harvest fungal tissue for genome sequencing. Harvested tissue from species without a corresponding genome are first passed to the Arizona Genomics Institute for DNA extraction and then to the Joint Genome Institute (JGI) for genome sequencing. Using the data generated by the JGI, students conduct bioinformatic analyses to learn comparative genomic techniques. Through analysis of novel biosynthetic gene clusters, fungal mating types and more, students provide important contributions to our collective knowledge of fungal biology. Myco-Ed is designed to be modular, allowing instructors to incorporate any aspect of the curriculum that fits their teaching goals. The MycoEd CURE has been successfully piloted in three classrooms and two more pilots are underway in teaching laboratories this Spring. As a result of the pilot, we have already targeted 20 fungal species for genome sequencing, and released several genomes via the JGI MycoCosm platform. We aim to scale this project to cover a wide range of institutions, introducing a diverse student body to mycology and genome science. Beyond providing training for the next generation, we hope to inspire students to pursue careers within the field of mycology by allowing them to meaningfully contribute to the advancement of our field.

833A **Controlling Fusarium head blight and mycotoxin contamination by exploring an endophytic fungal RNAi delivery system** Guixia Hao[1], Susan McCormick[1], Nicholas Rhoades[1], Guohua Yin[2], Martha Vaughan[1] [1]USDA/ARS, [2]USDA/ARS, ORISE

RNA interference (RNAi) technology has been widely used to control plant diseases and pests. Two most common methods for RNAi application include host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). However, HIGS application has been limited by availability of efficient plant transformation systems and public acceptance of genetically modified organisms. SIGS is convenient but it is limited by the cost of dsRNA synthesis and its instability. Therefore, it is critical to develop alternative RNAi production and delivery systems. Endophytes can inhabit the hosts without causing damage. Delivery of RNAi using endophytes is cost-effective and sustainable. Fusarium head blight (FHB) caused by *Fusarium* species is a major threat to food

safety and security by reducing crop yields and contaminating grains. *Sarocladium zeae* has been demonstrated to provide biocontrol function towards corn and wheat disease. To reduce FHB and mycotoxin contamination, we investigated if we could use an endophytic fungal strain *S. zeae* 34560 (Sz34560) to produce and deliver RNAi. For proof of the concept, we generated a Sz34560 RNAi strain expressing a hairpin RNA construct targeting GFP and examined the double stranded RNA (*GFP*-dsRNA) and small interference RNA (*GFP*-siRNA) production. We confirmed *GFP*-dsRNA production in the Sz34560 GFP-RNAi strain. Our analyses showed that Sz34560 GFP-RNAi strain significantly reduced GFP signals of a *F. graminearum* strain expressing GFP, suggesting the *S. zeae*-mediated RNAi delivery system is effective. Therefore, we generated a *F. graminearum* strain expressing an RNAi construct targeting the trichothecene biosynthetic gene *TRI5,* which is essential for trichothecene production. We showed that *F. graminearum* TRI5-RNAi strain significantly reduced *TRI5* expression and trichothecene production in toxin-inducing media. Then we introduced the *TRI5*-RNAi construct into Sz34560 and generated Sz34560 TRI5-RNAi strains. We detected *TRI5*-dsRNA in the Sz34560 TRI5-RNAi strains and showed reduced trichothecene level in *F. graminearum* and Sz34560 TRI5-RNAi co-cultures. Furthermore, FHB virulence assays showed disease and mycotoxin reduction in wheat seeds treated with Sz34560 *TRI5*-RNAi strains. Further investigations are underway to optimize treatments to improve the effectiveness of reducing FHB and toxin contamination and confirm dsRNA/siRNA presence in Sz34560 *TRI5*-RNAi treated plants.

834A **The *hdt4* transcription factor gene controls development and secondary metabolism in the fungus *Aspergillus flavus*** Farzana Ehetasum Hossain[1], Apporva Dabholkar[1], Sandesh S Pandit[1], Jessica M. Lohmar[2], Matthew Lebar[2], Jeffrey W. Cary[2], Ana M. Calvo[3] [1]Northern Illinois University, [2]Food and Feed Safety Research Unit, USDA/ARS, Southern Regional Research Center, [3]Dept of Biological Sciences, Northern Illinois University

Aspergillus flavus is an opportunistic fungal plant pathogen that colonizes several economically important crops, including corn, cotton, peanut, and tree nuts. When colonizing plant tissue, *A. flavus* produces a variety of potent mycotoxins, including aflatoxin B1, the most carcinogenic natural compounds known. *A. flavus* infection of crops and concomitant aflatoxin contamination causes an important health and economic impact worldwide. Current control methods are insufficient to control the detrimental effects of *A. flavus*. Molecular genetic studies could identify novel targets to develop new strategies against this fungus. Here, we determined the function of *hdt4*, a transcription factor gene whose expression is dependent on the regulatory gene *hbx1*, a homeobox domain transcription factor known to govern the expression of hundreds of genes in *A. flavus.* Our study revealed that *hdt4* regulates conidiation; deletion of *hdt4* results in a decrease in conidial production. In addition, *hdt4* is required for normal production of sclerotia in *A. flavus*, affecting number of sclerotia produced and maturation stage. Furthermore, our analysis revealed that *hdt4* regulates mycotoxin production. Removal of the *hdt4* gene results in a significant decrease in aflatoxin B1 production compared to the wild-type control. These findings indicate the potential of *hdt4* as possible target for a control strategy against *A. flavus,* to reduce health risks and economic losses associated with aflatoxin contamination.

835A **Fungal Genetics Stock Center: A Status Report** Jaideep Mallick Dept of Plant Pathology, Kansas State University

The Fungal Genetics Stock Center has expanded from 400 strains in 1960 to well over 90,000 strains from 40 species and almost a thousand plasmids. Our project with the K-State library to make all of the FGSC strain deposit sheets available through an on-line archive is nearly complete and will enable researchers to have access to strain details previously available only to FGSC staff. The most recent species addition is *Candida auris*, an emerging health risk. Orders are for about 500 strains per year and the revenue generated funds the salary for a half-time technician and operating supplies.

With Kevin McCluskey's departure, FGSC now has a new curator, Dr. Jaideep Mallick, who took up the position in the 4th quarter of 2022. Support for the curator is partially from K-State, a grant from Open Philanthropy, and a DoD grant that began in October 2022 and has a five-year term. The DoD project focuses on *Agrobacterium tumefaciens* and plasmids that can be used with it to transform fungi with genes that can be used to degrade wastes found in post-military environmental settings. The goal is to optimize transformation protocols that use *A. tumefaciens* for fungal transformations and to make strains, plasmids and protocols readily available to the fungal research community. Please continue to send your materials (and deposit sheets!) to us so that we can expand the collection further and keep it relevant!

836A ***Fusarium oxysporum* - the next model system to study melanoma** Shay Covo, Dibya Mukherjee Hebrew University
Melanoma is one of the deadliest and more common malignancies. Melanoma occurs due to exposure of skin cells to UV and consequently mutation in tumor suppressing genes and oncogenes. We know a lot of the UV-induced mutagenesis process as well as risk factors for melanoma from irradiating cells (bacteria, yeast human cell-lines) in culture. Yet, the data derived from this experiments is an average of the whole population. But what is there is an heterogenicity within the population? what if there are some risk factors for elevated mutagenicity? We propose to use Fusarium to address this questions. We found out that UV-

irradiated Fusarium population recovers in a heterogenic manner; some spores recover only 30 h after the first one started their recovery. We were able to collect based on the size UV arrested population. The plating efficiency of the population is lower than the total irradiated population and their mutagenicity is higher. There are also some rare UV mutations that are more common among colonies rescued from the arrested population. Interestingly, the level of DNA damage is actually low in the arrested population; it seems that most of the lesions were removed yet still the spore do not develop to a filament. By studying further the pattern of mutation, gene expression and protein phosphorylation we intend to understand why this population is arrested?

837A **Systematic characterization of GPI-anchored mannoproteins in *Cryptococcus neoformans*** yeqi li[1], Tuyetnhu Pham[2], Kenton Hipsher[1], Yumeng Fan[1], Youbao Zhao[1], Xiaofeng Xie[1], Xiaorong Lin[1,2] [1]Microbiology, University of Georgia, [2]Plant Biology, University of Georgia

Systemic cryptococcosis is fatal without treatment. Globally, this disease claims more than 180,000 lives each year, even with antifungal therapies. Unfortunately, there is no vaccine available against cryptococcosis. Thus, identifying protective antigens that could serve as vaccine candidates is of great importance. We previously discovered hyphal *ZNF2*^{oe} strains elicit protective host immune responses both in the live and heat-inactivated forms. We also found *ZNF2*^{oe} cells show increased antigens in the capsule. Consistently, immunoprotection offered by *ZNF2*^{oe} cells requires capsule. Capsule is a defining feature of *Cryptococcus* species and is composed of polysaccharides and mannoproteins. Mannoproteins are known to be the primary components recognized by the anti-cryptococcal cell-mediated immune response in mice, but few cryptococcal mannoproteins have been characterized. To systematically characterize the function of glycosylphosphatidylinositol/GPI-anchored mannoproteins (GPI-MPs), we identified 49 genes potentially encoding GPI-MPs in the genome of the *C. neoformans* reference strain H99. The dynamic changes of their expression during infection or in response to stress conditions suggest that GPI-MPs play important roles in cryptococcal adaptation to various environments. To functionally characterize their role in cryptococcal biology, we constructed both the deletion and overexpression mutant libraries of these 49 genes and screened the mutants for altered susceptibility to various stresses and phagocytosis by macrophages. One GPI-MP protein, Cig1, represents one of the antigens recognized by the host immunized with *ZNF2*^{oe} cells. Cig1 resides in plasma membrane and the protein level is highly induced by iron limitation. Currently, we are evaluating the potential use of Cig1 as an anti-cryptococcosis vaccine in murine models. Collectively, our findings highlight the importance of mannoproteins in cryptococcal biology and identify the surface protein Cig1 as one of the prominent antigens.

838A **A multifaceted approach to improving outcomes of cerebral aspergillosis** Martin Kelty[1], Aracely Miron-Ocampo[2], Sarah Beattie[1] [1]Pediatrics, University of Iowa, [2]Pathology, University of Iowa

Invasive mold infections are becoming more common with the introduction of novel immunomodulatory therapies and the emergence of new co-morbidities including COVID-19. The most common pathogenic mold is *Aspergillus fumigatus*, which is typically inhaled and causes invasive pulmonary aspergillosis (IPA). In a subset of patients with IPA, *A. fumigatus* disseminates to extrapulmonary organs including the brain, causing cerebral aspergillosis (CA). Once in the brain, *Aspergillus* is difficult to treat as most clinical antifungals do not readily penetrate the blood brain barrier (BBB). Thus, CA is one of the most fatal forms of invasive aspergillosis with mortality rates reaching 80-100%, even with treatment. Despite the high mortality rates of CA, the pathogenesis of *A. fumigatus* in the brain remains largely unexplored.

Our goal is to improve the treatment options and outcomes of this devastating disease using a multifaceted approach. First, we are developing mouse and cell culture models to identify fungal pathways that are essential for dissemination to the brain. We have demonstrated that C5-complement deficient mice develop robust cerebral infection with features of human disease and hallmarks of invasive aspergillosis. Using this model, we have shown that the pH responsive transcription factor, PacC, is required for dissemination to extrapulmonary organs including the brain. Work is ongoing to screen for additional transcription factors and kinases that are required for dissemination to or growth within the brain to elucidate mechanisms of pathogenesis in this niche.

Second, to directly address the need for central nervous system (CNS)-penetrant mold-active antifungals, we developed a luciferase-based, high throughput screening assay to screen directly against *A. fumigatus*. With this platform, we are screening a structurally diverse library of ~150,000 synthetic drug-like compounds, of which ~2/3 are predicted to penetrate the BBB. After screening through about half of this library, we have already been successful in identifying candidate scaffolds with desirable antifungal properties. These compounds are currently under investigation to identify the best candidates for lead development. Together, we hope our approaches will result in improved management of CA through better understanding of the pathogenesis within the brain and with the development of novel antifungals specifically targeted to treat CNS mold infections.

839A **Production of poly(β-L-malic acid) by the yeast-like fungus *Aureobasidium pullulans*** Difan Xiao[1], Lars M Blank[2], Till Tiso[2] [1]Dept of Biology, Institute of Applied Microbiology, RWTH Aachen University, [2]Institute of Applied Microbiology

Aureobasidium pullulans is a polyextremotolerant fungus inhabiting many ecological niches. It can secrete a broad spectrum of biotechnologically relevant secondary metabolites like poly(β-L-malic acid) (PMA). PMA is a linear anionic biopolyester and its derivatives have some prospective applications as a novel drug delivery system, surgical sutures, nanoconjugates, and biodegradable plastics. In this study, we aimed to identify the dominant metabolic pathway employed for PMA precursor (malate) synthesis, the enzyme responsible for the polymerization of malate, and to increase the efficiency of PMA production by metabolic engineering. We first identified three possible pathways for malate synthesis *in silico*. The removal of the *cyMDH* gene encoding cytosolic malate dehydrogenase, a key enzyme in the reductive tricarboxylic acid (rTCA) cycle, allowed the resultant knockout strain to decrease the PMA yield (g/gCDW) by 61.4%. The disruptant where the *MSE* gene coding for malate synthase in the glyoxylate shunt was abolished did not affect PMA production. Deletion of the core synthetic gene in a nonribosomal peptide synthetase-like (NRPS-like) gene cluster completely abolished the polymerization of PMA while the cell biomass was higher than that from the wild-type strain. The deletant strain in which the *PKS2* (polyketide synthase) gene involved in the formation of liamocin was abolished could increase the PMA yield (g/g_{CDW}) by 2.2-fold. However, blocking of other unwanted byproducts formation such as melanin and pullulan did not significantly affect the production of PMA. The disruption of Crz1 (calcineurin-responsive zinc finger 1) could improve PMA synthesis but caused cell lysis. Moreover, the Crz1 deletion mutant showed an elevated sensitivity to high osmolarity, oxidative stress, alkaline environment, and cell membrane disruptive agents. However, the disruption of Crz1 reinforced the resistance to the high temperature of 37°C and the low temperature of 4°C. Taken together, it was concluded that intracellular malate was mainly derived from the rTCA cycle. The formed malate molecules were condensed into PMA mediated by a core synthetic gene in the NRPS-like gene cluster. The deletion of the *PKS2* gene can completely stop liamocin synthesis and contribute to higher PMA production. In addition, the knockout of transcription factor Crz1 also upregulated PMA production and conferred the cell's tolerance to high and cold temperatures.

840B **The myco-ecology of the *Stylophora pistillata* holobiont: a case study with two associated fungi - *Cladosporium halotolerans* and *Stachybotrys chlorohalonata*** Lior Granit[1,2], Nofar Lifshitz[1,2], Britt Ronen[2], Koren Karp[3], Shmuel Carmeli[3], Maoz Fine[1,2], Oded Yarden[1,2] [1]Hebrew Univ of Jerusalem, [2]The Interuniversity Institute for Marine Sciences, [3]Tel-Aviv University

Coral reefs are pivotal ecosystems sensitive to climate change. *Stylophora pistillata*, a Red Sea Scleractinian coral, accommodates repeatedly-isolated fungal genera, suggesting the presence of a coral-acclimated mycobiome that may have potential implications on coral well-being. *S. pistillata* nubbins were collected between 2018 and 2023 in the Gulf of Aqaba. 173 different fungal strains from 25 genera were isolated, 61% of which were found to be either *Aspergillus*, *Alternaria*, or *Cladosporium* spp. The abundance of other genera varied and included the isolation of at least two new species. To assess the fungi's impact on coral well-being, *S. pistilata* colonies were inoculated with conidia from prevalent or rare fungal species (*Cladosporium halotolerans* or *Stachybotrys chlorohalonata*, respectively). Inoculation with *C. halotolerans* yielded no visible effects within 24 hours, despite conidial adherence to the coral tissue surface. Transfer of the coral from ambient (25°C) to elevated (33°C) sea water temperature conferred a short-term detrimental effect, as evident by the reduced maximum photosynthetic yield of the algal symbionts, based on chlorophyll fluorescence. This effect was less pronounced in coral inoculated with *C. halotolerans*. In contrast, Inoculation with *S. chlorohalonata* led to visible coral bleaching after 24 hours. Conidial germination of *C. halotolerans* in culture was not affected by the presence of 0.66M NaCl (typical of the northern Red Sea) with ~60% of the tested conidia reaching the first branching stage within about 24 hours. However, hyphal growth was significantly enhanced by the presence of salt, as the colony area in the presence of 0.66M NaCl was 36% larger after 14 days of growth in comparison to medium lacking the NaCl amendment, suggesting the coral-derived isolate is well adapted for proliferation under saline conditions. The bleaching-inducing strain of *S. chlorohalonata* was found to secrete metabolites that significantly inhibited the lag phase of several tested gram-positive (yet not gram-negative) bacteria. We propose that at least some of the anti-bacterial activity observed was due to the production of saturated fatty acids by this strain. Taken together, we conclude that isolating the cultural components of the coral mycobiome can progress our ability to identify and dissect the potential contribution of fungal community members to coral well-being.

841B **The *Stylophora pistillata*-associated fungus *Cladosporium halotolerans* affects the expression of stress-related genes in the coral host following exposure to elevated sea water temperature** Rotem Levi[1,2], Nofar Lifshitz[1,2], Britt Ronen[2], Maoz Fine[1,2], Oded Yarden[2,3] [1]Hebrew Univ of Jerusalem, [2]The Interuniversity Institute for Marine Sciences, [3]The Hebrew University of Jerusalem

Corals are comprised of complex symbiotic relationships. These organisms face escalating threats from global climate change-induced sea temperature elevation, leading to widespread bleaching events and reef decline. *Stylophora pistillata* is a common stony coral in the Red Sea. The sensitivity of this coral to increased water temperature varies among the different coral hologenomes (the coral host and its microbiome). Fungi form an integral part of the coral's microbiome. However, their specific effects during periods of elevated seawater temperature, on the holobiont, are still unknown. Between 2018 and 2023 we isolated 173 fungal strains belonging to 25 genera. The most abundant (61%) genera found were *Aspergillus*, *Alternaria* and *Cladosporium*. To study the potential role of some of the isolated fungi in the response of the *S. pistiallata* holobiont to elevated seawater temperature, fragments of the coral were inoculated with conidia from one of three endogenous fungi: (i) *Cladosporium*

halotolerans, a highly-prevalent fungus associated with this host; (ii) *Aspergillus sydowii*, a species associated with disease in another coral species or (iii) *Stachybotrys chlorohalonata*, known as a producer of abundant secondary metabolites. 48 hours after inoculation, the fragments were rinsed and exposed to thermal stress in flowthrough seawater aquaria for 24 hours. The corals inoculated with *C. halotolerans* and *A. sydowii* did not show an observable phenotypic effect, while *S. chlorohalonata* led to bleaching, regardless of water temperature. To assess the effect on coral physiology, we analyzed the expression level of three genes that have previously been identified to exhibit elevated levels of expression in corals under stress: heat shock protein 70 (HSP70), linked to heat stress and B-cell lymphoma 2 (StyBcl-2-like, EU715319) and Caspase 3 (StyCasp3, EU715318) associated with apoptosis. When inoculated with C. halotolerans, the expression of all three stress-related markers was significantly lower (25-50%) in the sampled coral during thermal stress, when compared to the uninoculated control. These effects suggest that *C. halotolerans* may be involved in reducing the effective, or perceived, cellular stress levels in the coral after exposure to elevated temperature. This study highlights the diversity of the culturable mycobiome of *S. pistillata* and that some of its members have the potential to confer either detrimental or beneficial effects on the host.

842B **The proteomic response of Aspergillus fumigatus to Amphotericin B (AmB) reveals the involvement of the RTA-like protein RtaA in AmB resistance** Sophie M. Tröger-Görler[1], Ammar Abou-Kandil[2], Annica Pschibul[1], Thomas Krüger[1], Maira Rosin[1,3], Franziska Schmidt[1,3], Parastoo Akbarimoghaddam[4], Arjun Sakar[4], Zoltán Cseresnyés[4], Yana Shadkchan[2], Thorsten Heinekamp[1], Markus Gräler[5,6,7], Marc T. Figge[3,4], Axel A. Brakhage[1,3], Nir Osherov[2], Olaf Kniemeyer[1] [1]Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), [2]Dept of Clinical Microbiology and Immunology, Sackler School of Medicine, [3]Institute of Microbiology, Friedrich Schiller University (FSU), [4]Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), [5]Dept of Anesthesiology and Intensive Care Medicine, Jena University Hospital, [6]Center for Molecular Biomedicine (CMB), Jena University Hospital, [7]Center for Sepsis Control and Care (CSCC), Jena University Hospital

The opportunistic human pathogen *Aspergillus fumigatus* poses a significant threat by causing mycoses, which can be fatal especially in immunocompromised individuals. Due to the increase of azole resistance in *A. fumigatus*, treatment options are often limited to amphotericin B (AmB), a member of the polyene family of antifungals that has well known side effects. A rising number of resistant isolates against AmB as well as limited knowledge about resistance and compensatory mechanisms give rise to concerns.

To elucidate the effects of AMB on the fungal proteome, we conducted liquid chromatography-tandem mass spectrometry analyses to identify changes in the proteomic profiles of *A. fumigatus* treated with sublethal concentrations of AmB and its liposomal formulation. Selected proteins with significant increase in abundance upon AmB exposure were then characterized.

By comparison of the proteomic response of AmB-treated samples and untreated controls, we found significant increases in the abundance of proteins belonging to secondary metabolite biosynthesis gene clusters, proteins anchored to the membrane, involved in catabolic processes or aromatic acid degradation. One of the proteins with the highest increase in abundance was RtaA, a fungal Rta1-like family protein. While deletion of *rtaA* led to increased sensitivity against AmB, overexpression resulted in a two-fold increase of resistance. Interestingly, only treatment with AmB and nystatin resulted in a rise of *rtaA* transcript levels, which hints towards a specific protection mechanism against polyenes. Deletion of *rtaA* did not significantly change the ergosterol content and intracellular lipid droplets of *A. fumigatus.* While not being crucial for the virulence of *A. fumigatus* itself, RtaA is most likely involved in the resistance against AmB by maintaining lipid homeostasis and membrane stability. These findings reveal a novel polyene resistance mechanism.

843B **Fungal Flc/Pkd2 proteins, that are required for cell wall integrity and calcium homeostasis, belong to a distinct ancient eukaryotic transmembrane protein superfamily** Rachael M. Murray, Jelena Baranovic, Joanne Thompson, Edward W. J.
Wallace School of Biological Sciences, University of Edinburgh

Fungi express an essential family of transmembrane proteins known as Flc/Pkd2 proteins. They contribute to cell wall integrity and calcium homeostasis, and are necessary for virulence in diverse fungal pathogens. While data supports their important cellular roles in diverse fungi, little is known about their molecular structure or function. Early work annotated Flc/Pkd2 proteins as ion channel proteins belonging to the transient receptor potential (TRP) family, but this classification has not been substantiated with our phylogenetic or structural evidence.

We performed a thorough phylogenetic and structure-prediction analysis of Flc/Pkd2 protein homologs. We found that Flc/Pkd2 proteins are unrelated to TRP channel proteins and have a distinct domain architecture, membrane topology, and evolutionary

history. Instead, Flc/Pkd2 proteins belong to a transmembrane protein superfamily found in diverse eukaryotic phyla, and homologs are required for virulence in diverse eukaryotic pathogens. Members of this family share a conserved transmembrane domain with nine membrane-spanning helices. We did not detect structural homology between Flc-like proteins and any other family of verified calcium channels. Membrane topology predictions show that Flc-like proteins have variable extracellular N-terminal domains that have been extensively modified by domain gains and losses across the family. Fungal homologs have an extracellular ML-like domain that resembles MD-2-related lipid-binding proteins that bind to sterols. Furthermore, fungal homologs fall into two structurally distinct subtypes, and our phylogenetic analysis places all homologs with reported cell wall integrity phenotypes in the same subtype.

Importantly, we show that members of this new superfamily were lost in the ancestor of vertebrates. Future work will determine if and how the fungal Flc/Pkd2 proteins sense and transduce environmental signals including calcium, and how they affect cell wall structure.

844B **Bioremediation of heavy metals from wood preservatives by ectomycorrhizal fungi** Ray Van Court[1], Gerald Presley[2] [1]Wood Science and Engineering, Oregon State University, [2]Wood Science, Oregon State University

Leachate from heavy metal-based wood preservatives including copper chromated arsenic (CCA) and ammoniacal copper zinc arsenate (ACZA) poses an environmental threat, especially in areas of high concentration such as landfills. A potential option to ameliorate the effects of these toxic metals is bioremediation using ectomycorrhizal fungi. To this end, a screening of a range of fungal isolates was conducted to assess fungal tolerance of arsenic, copper, and zinc. Culture metal uptake was assessed using atomic absorption spectroscopy. Based on these results, *Laccaria laccata* ((Scop.) Cooke, Grevillea) was selected for RNA-seq analysis of differential gene expression when grown on arsenic, copper, and zinc treated plates. Putative genes associated with metal tolerance were identified. Following identification, gene expression in response to metal stressed conditions will be assessed using dPCR in metal stressed conditions in plate culture and mesocosm systems.

845B **Identification of *A. fumigatus* virulence factors by *in vivo* RNA-seq analysis** Hong Liu[1], Vincent M Bruno[2], Quynh T Phan[3], Scott G Filler[3,4] [1]Infectious Diseases, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, [2]University of Maryland, [3]Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, [4]David Geffen School of Medicine at UCLA

Background. Growth in the mammalian lung exposes *A. fumigatus* to a unique environment with multiple stressors. However, the transcriptional response of this organism during IA is incompletely understood. Our goal was to use a fungal RNA enrichment approach to identify *A. fumigatus* genes that encode GPI-anchored proteins that are highly expressed *in vivo* and determine their role in the host-pathogen interaction and virulence.

Methods. The transcriptional response of *A. fumigatus* Af293 in two mouse models of IA was analyzed using a fungal RNA enrichment technique followed by RNA-seq. Mice were immunosuppressed with cortisone acetate only (non-neutropenic) or cortisone acetate and cyclophosphamide (neutropenic) and then infected with *A. fumigatus*. After 2, 4, and 6 days of infection, the mice were sacrificed and RNA was isolated from the lungs for RNA-seq. Gene deletion mutants were constructed using CRISPR-Cas9 and tested for virulence in mice.

Results. The *A. fumigatus* transcriptomes in both neutropenic and non-neutropenic mice were very similar, with an R^2 value of 0.79. Gene Ontology enrichment analysis of these transcriptomes indicated that 138 genes involved in oxidative stress, pH response, lipid metabolic process, and ion homeostasis were highly expressed in neutropenic mice; 339 genes involved in nitrogen metabolic process were highly expressed in non-neutropenic mice. Of the 86 genes in the *A. fumigatus* genome that are annotated as encoding GPI-anchored proteins, 18 were found to be highly expressed during IA. From these 18 genes, 7 were selected for mutant construction and virulence testing. Two of the 7 mutants, Δ*gapA* and Δ*gapB,* had significantly attenuated virulence in non-neutropenic mice. Because the Δ*gapA* mutant had the greatest virulence defect, it was selected for further study. *In vitro*, the Δ*gapA* mutant had impaired invasion of A549 alveolar epithelial cells and HSAEC1-KT small airway epithelial cells. It also had increased susceptibility to BMDM killing, but wild type susceptibility to H_2O_2 and menadione.

Conclusions. Growth in the mammalian lung induces a unique transcriptional response in *A. fumigatus*. Although the fungal transcriptional responses in both neutropenic and non-neutropenic immunosuppressed mice are generally similar, growth in neutropenic mice induces a more diverse stress response relative to growth in non-neutropenic mice. A subset of genes predicted to encode GPI-anchored proteins are highly expressed *in vivo* and are likely to function in virulence. Of these genes, *gapA* encodes

a virulence factor that is required for maximal *A. fumigatus* invasion of pulmonary epithelial cells and resistance to macrophage killing. Thus, determining the *in vivo* transcriptome of *A. fumigatus* yields novel insights into pathogenicity.

846B **The histone deacetylase HosA regulates host cell interactions, resistance to intracellular oxidative stress, and virulence in *A. fumigatus*** Hong Liu[1], Pamela Lee[2], Alice Vo[2], Quynh T Phan[2], Vincent M Bruno[3], Mark Stamnes[4], Scott G Filler[2,5] [1]Infectious Diseases, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, [2]Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, [3]University of Maryland, [4]University of Iowa, [5]David Geffen School of Medicine at UCLA

Background. Invasive aspergillosis is a leading cause of morbidity and mortality in immunosuppressed patients. Epigenetic modifications in *A. fumigatus* can be induced by environmental changes and stresses such as those induced by interaction with host cells. However, very little is known about the role of epigenetics in the pathogenicity of *A. fumigatus*. *hosA* (Afu2g03810) encodes a class 1 histone deacetylase. Our aim was to investigate the role of HosA in the host cell interactions and virulence of *A. fumigatus*.

Methods. Δ*hosA* deletion and Δ*hosA*+*hosA* complemented strains were constructed in *A. fumigatus* Af293. Growth and conidiation were investigated in Sabouraud dextrose agar plates. Susceptibility to Congo red, calcofluor white, H_2O_2, me nadione, and protamine was determined in organisms grown on Aspergillus Minimum Medium (AMM) plates. The capacity of these strains to adhere to, invade, and damage A549 alveolar epithelial cells and HSAEC1-KT (HSAE) small airway epithelial cells was analyzed. The virulence of the various strains in triamcinolone immunosuppressed mice was evaluated by mouse survival and pulmonary fungal burden. The host inflammatory response was measured by Luminex multiplex cytokine array. For RNA-seq analysis, *A. fumigatus* strains were cultured in AMM with low zinc and low iron.

Results. The Δ*hosA* mutant had normal growth, morphology and conidiation when grown on Sabouraud dextrose agar media, but had a mild growth defect on AMM. The mutant had wild-type susceptibility to Congo red, calcofluor white, H_2O_2, and protamine. However, the Δ*hosA* mutant was sensitive to menadione, suggesting that HosA may induce resistance to intracellular oxidative stress. Although germlings of the Δ*hosA* mutant had normal adherence to A549 and HSAEC1-KT cells, they had 85% less invasion of A549 cells and 49% less invasion of HSAE cells. They also caused 55% and 25% less cell damage to A549 cells and HSAE cells, respectively. Mice infected with the Δ*hosA* mutant had significant reduced mortality compared to mice infected with the wild type or Δ*hosA+hosA* complemented strains. Surprisingly, the pulmonary fungal burden of mice infected with the Δ*hosA* mutant was similar to that of mice infected with the wild-type strain. Infection with the Δ*hosA* mutant induced lower levels of CXCL1, CXCL2, CCL2, IL-1α, and IL-6 in the lungs relative to the wild-type strain. RNA-seq data suggested that HosA may govern toxin production or antigen expression on the cell surface, which stimulates excessive inflammation and leads to increased host tissue damage.

Conclusions. The HosA histone deacetylase governs *A. fumigatus* pathogenicity. It is required for resistance to intracellular oxidative stress and for maximal invasion of and damage to pulmonary epithelial cells *in vitro*. HosA also contributes to virulence by inducing a pathogenic inflammatory response during invasive aspergillosis *in vivo*.

847B **Enhanced mycelial growth rate and fruit body yield *via* mycovirus elimination in the edible mushroom *Lentinula edodes*** Hayeon Song[1], Dae-Hyuk Kim[2,3], Jung-Mi Kim[1,4] [1]Institute of Life Science and Natural Resources, Wonkwang University, [2]Dept of Molecular Biology, Jeonbuk National University, [3]Institute for Molecular Biology and Genetics, Jeonbuk National University, [4]Dept of Life and Environmental Sciences, Wonkwang University

This study investigated the influence of mycoviral infections on the growth and fruiting body formation in *Lentinula edodes* by comparing isogenic virus-cured and virus-infected fungal strains. Virus-cured colonies were obtained through mycelial fragmentation and serial dilution plating, confirming the absence of all six previously reported viruses found in Korean wild-type strains of *L. edodes* using gel electrophoresis and reverse transcription-polymerase chain reaction (RT-PCR). Quantitative real-time PCR (qRT-PCR) and deep sequencing were employed to assess the occurrence of multiple viral infections in virus-cured and virus-infected fungal strains. Over a two-year period, all fungal cultures that were cured remained free of viruses. While no discernible changes in colony morphology were observed, the isogenic virus-cured *L. edodes* strain exhibited a higher growth rate and increased phenol oxidase activity compared to the virus-infected strain. Moreover, the cured strain demonstrated enhanced fruiting body production. These findings suggest that LeV infection negatively impacts the growth and fruiting body formation of *L. edodes* by reducing the activity of specific extracellular enzymes, notably phenol oxidase. The elimination of mycoviruses significantly appears to enhance spawn production and fruiting body yield in *L. edodes*.

848C
VOC profiles from a chestnut blight fungus *Cryphonectria parasitica* in response to hypovirus CHV1 Yo-Han Ko, Jeesun Chun, Kum-Kang So, Ngoc My Tieu Le, Dae-Hyuk Kim Jeonbuk National University

The c hestnut blight fungus, *Cryphonectria parasitica*, its hypovirus comprise useful model system to study fungus-virus interactions. Infection by hypovirus, Cryphonectria hypovirus 1 (CHV1) results in various phenotypic changes in the fungal host including hypovirulence and other associated symptoms such as altered metabolism, retarded development, and reduced sporulation. Although many studies regrading what are the factors affected by hypovirus infection and how these changes occurred, studies on VOC (Volatile Organic Compound) have not been conducted. In this study, we characterized VOC profiles of *C. parasitica* and analyzed the changes in VOCs by CHV1 infection over a 20 days growth period. A total of 65 predominant VOCs with high similarity to the known database were identified. Among these, 51 VOCs were identified from the virus-free EP155/2, 54 VOCs were from the virus-infected UEP1, and 40 VOCs were found from both strains. Among 51 EP155/2-released VOCs, phenylethyl alcohol is the most prevalent VOC but the most predominant VOC from UEP1 changed as culture proceeded, i.e., β-phellandrene (26.4% at 5-day culture), phenylethyl alcohol (32.7% at 10-day culture), and phenylethyl alcohol (51.2% at 20-day culture). Terpenes are most common members of VOCs from *C. parasitica*. Sesquiterpenes were more common in EP155/2 while monoterpenes were major in UEP1. VOC profiles changed greatly depending on the culture period. More importantly, CHV1 infection affected not only the component profiles of VOCs but also the amount of specific VOC released. Transcription analysis of genes responsible for the synthesis of corresponding VOCs revealed that the expression of genes was affected by the CHV1 infection suggesting the presence of hypoviral regulation of fungal metabolic gene expression. Interestingly, olfactory behavioral assay indicated that there was difference in attractiveness between virus-free and -infected strains, i.e., UEP1 showed greater attractiveness to insect than EP155/2, which suggests the difference in efficacy of insect-borne dissemination of this fungus.

849C **Effect of double-stranded RNAs on antifungal activity of *Trichoderma harzianum*** Jeesun Chun, Yo-Han Ko, Kum-Kang So, Ngoc My Tieu Le, Dae-Hyuk Kim Jeonbuk National University

Mycoviruses are widespread in most fungal groups. Here we report a novel mycovirus named Trichoderma harzianum alternavirus 1 (ThAV1). The genome of ThAV1 contains four dsRNA bands with lengths of 3.5, 2.6, 2.3 and 1.8 kbp, respectively. Among these dsRNAs, dsRNA1 at 3.5 kbp encodes RNA-dependent RNA polymerase (RdRp). dsRNA2, dsRNA3, and dsRNA4 at 2.6, 2.3, and 1.8 kbp, respectively, encode hypothetical proteins. BLAST search and evolutionary analysis of the amino acid sequences of RdRp and hypothetical proteins indicated that the dsRNAs represent segments of the genome of a novel alternavirus, as a novel member of the proposed family "*Alternaviridae*". Through repetitive single-spore isolation, we were able to cure mycovirus, which resulted in a virus-free strain. Virus-cured strain did not show any significant difference in growth rate, colony morphology, and conidia production from the virus-infected strain. However, the β-1,3-glucanase activity was increased in the ThAV1-infected strain. This study is the first report of an alternavirus of the proposed family "*Alternaviridae*" from *T. harzianum* and revealed the mycovirus-induced fungal enzyme activity.

850C **Fungi and humidity dynamics** Jan Dijksterhuis[1], Frank Segers[1], Han Wosten[2] [1]Westerdijk Fungal Biodiversity Institute, [2]Molecular Microbiology

A relatively small number of fungal species can grow at water activities at or below 0.8. These xerophilic fungi can cause problems in food (containing sugars or salt) or feed (dried) and during indoor conditions (were relative humidity is kept below 80%). Limits of fungal growth are often described under static conditions. For example, *Aspergillus niger* has a limit of growth, just below water activity 0.8, as measured on glycerol containing agar surfaces. The fungal genus *Cladosporium* belong the most abundant fungi in outdoor and indoor air. In our study it became clear that those species of *Cladosporium* that are isolated from indoor air and surfaces belong to the *C. sphaerospermum* species complex that exhibit growth limits at 0.82-0.85, lower as the other species groups within the genus. However, the limits of growth of *C. halotolerans*, one of these species, were not lower as is the case with *A. niger* and *Penicillium rubens*, two other indoor fungi that are less dominant in the indoor environment (the latter common after leakage or related problems). Transfer experiments were hyphae on membranes were subjected to one week long, defined lower relative humidities, showed that hyphae of this fungal species were able to survive dynamics in humidity much better. In all cases spores, being dormant, were able to survive a relative humidity of 33%, but germinated spores and growing hyphae stopped developing at 75% and did not recover in the case of *A. niger*, and only restrictedly so in the case of *P. rubens*. This was also confirmed with live-dead staining of hyphae. This indicates that some fungi a better able to survive humidity dynamics under growth, a topic relevant to find strategies to prevent indoor growth. Microcolonies of C*. halotolerans* showed characteristic morphological features that could help with this type of survival.

851C **Immune Mechanism of Intramuscular Vaccination against Cryptococcosis** Yu Zhang[1], Yina Wang[1], Keyi Wang[2], Amariliz Rivera[2], Chaoyang Xue[2] [1]Microbiology, Biochemistry and Molecular Genetics, Rutgers University, [2]Rutgers University
Cryptococcus neoformans is an encapsulated fungal pathogen that infects the lung and then disseminates to the central nervous system to cause deadly cryptococcal meningitis in mostly immunocompromised population, and is account for ~15% HIV/AIDS related deaths annually. The treatment options for cryptococcal infection are limited to small number of antifungal drugs that often have adverse effects. Moreover, there is no fungal vaccine available for clinic use. Thus, there is a critical need to develop a vaccine targeting cryptococcosis. Our previous studies identified a vaccine candidate (HK-fbp1) that is based on heat-inactivated *C. neoformans* mutant cells lacking the F-box protein Fbp1. Fbp1 belongs to the SCF (Skp1, Cullins, F-box proteins) E3 ligase, which is critical for controlling protein degradation and cryptococcus virulence. The HK-fbp1 vaccination is effective on both healthy and CD4 T cell-deficient mice and can achieve full protection against cryptococcosis when administrated intranasally. This administration route elicits a strong and long-term mucosal immunity that protects the respiratory tract - the first defending line against cryptococcal infection. Recently, we modified our HK-fbp1 vaccine that also allows us to successfully immunize animals through intramuscular and subcutaneous injection routes that are commonly used for other vaccines in clinic. To understand the mechanism, we analyzed the host immunity following vaccination and found high recruitment of neutrophils, monocytes, monocyte-derived dendritic cells, activation of CD4/CD8 T cells to the lung and robust IL-17A production by CD4 T cells in the respiratory tract on 7 days post-challenge. Overall, our preliminary data indicate a strong response of activation CD4 T cell and IL-17A mediated protective immunity against pulmonary cryptococcal infection and dissemination. The detailed immune mechanism remains a subject of intensive ongoing investigation. Our long-term goal is to develop an invasive vaccine that is capable of inducing potent and long-lasting antifungal immunity to combat cryptococcosis in immunocompromised patients.

852C **Evaluating the Performance of AlphaFold for Fungal Small Secreted Cysteine Rich Protein Structure Determination: A case for *Trichoderma* Hydrophobins** Zeynep Ozkeserli, Gunseli Bayram Akcapinar Dept of Medical Biotechnology, Acibadem University

Hydrophobins belong to the small secreted cysteine rich protein family of filamentous fungi. Hydrophobins' amphiphilic nature at the protein surface allows them to self-assemble into rodlet structures or amphipathic layers, allowing the fungi to grow and reproduce in various environments. As a result of their self-assembly properties, they play pivotal roles in biological processes governing the fungal lifestyle, showcasing significant potential for industrial and biological applications. A thorough understanding of their structural configuration is critical to understanding their functional mechanisms. Despite the diversity of fungal hydrophobins, structural studies on them are very limited.

Within the framework of this study, we focus on evaluating AlphaFold's performance in predicting the three-dimensional structures of fungal small secreted cysteine-rich proteins, specifically highlighting its application to *Trichoderma* hydrophobins. Alphafold database (https://alphafold.ebi.ac.uk/) currently hosts more than 50 *Trichoderma* hydrophobin structures belonging to UniProt reference <wt-ignore uuid="9db57b10-9f8e-466c-8750-6cc74f72f3bb" source="wt-feature-dismissed">proteomes. </wt-ignore><wt-ignore uuid="c0d038d2-80a9-4842-937a-10d 64b272ac3" source="wt-feature-result">Taking advantage of AlphaFold2's deep learning architecture, we assess its accuracy and reliability in predicting the structural features of these proteins.</wt-ignore> For this <wt-ignore uuid="aeaabd94-5184-49d4-8de3-cff8b1cf2090" source="wt-feature-result">purpose, the confidence</wt-ignore> metric outputs of AlphaFold2 and the Predicted Aligned Error metrics were collected. Solvent-accessible surface area calculations and hydrophobicity scores based on GRAVY calculations were determined and compared based on the AlphaFold models. Additionally, comparative analyses are conducted on selective representative hydrophobins to elucidate AlphaFold's efficacy in capturing the diverse characteristics of *Trichoderma* hydrophobins compared to alternative structure prediction methodologies such as ESMFold.

Our findings underscore the advancements and limitations of AlphaFold2 in accurately elucidating the structural landscape of these diverse fungal proteins, offering insights into their structure-function paradigm and <wt-ignore uuid="d8f2e417-77d0-4d5b-84e6-c2b36cfb920b" source="wt-feature-result">a basis</wt-ignore> for further refinement in the context of fungal small secreted cysteine-rich proteins.

853C **Vaccination with *ZNF2* overexpression strain provides cross-protection between serotypes** Nhu Pham, Yeqi Li, Xiaorong Lin University of Georgia
Systemic cryptococcosis is responsible for over 180,000 deaths annually, and the efficacy of current antifungal therapies are far from satisfactory. This disease kills 19% of AIDS patients and the fungus can infect both immunocompetent and immunocompromised individuals. Yet, there are no vaccines available to prevent cryptococcosis. The severity of the disease, the limitations of current antifungal therapies, and the lack of cryptococcal vaccines earned this fungus a top spot as a critical fungal

pathogen by the World Health Organization. Previously, we discovered that Znf2, a transcription factor that regulates *Cryptococcus* yeast-to-hyphal transition, could be a key player in vaccine development. Both live and heat-killed *ZNF2* overexpression strains provided host protection against the otherwise lethal challenge by the highly virulent clinical *C. neoformans* isolate H99. Heat-killed cells overexpressing *ZNF2* provide long-term protection in hosts that were challenged more than once. Importantly, immunization with heat-killed *ZNF2* overexpression strains or the short-lived cryptococcal cells overexpressing *ZNF2* offer significant protection to immunocompromised hosts whose immune system were suppressed even before vaccination. Due to the efficacy of this whole-cell vaccine against *C. neoformans*, we are currently testing its efficacy against the related *C. gattii* species, which predominately infects immunocompetent individuals. Cross-protection against both *C. neoformans* and *C. gattii* would be useful in finding a broad-spectrum vaccine against cryptococcosis.

854C **Gene expression analysis of *CpDmt2*-null mutant of *Cryphonectria parasitica* associated with hypoviral clearance** Jeesun Chun, Yo-Han Ko, Kum-Kang So, Ngoc My Tieu Le, Dae-Hyuk Kim Jeonbuk National University

We previously demonstrated that two representative DNA methyltransferases (DNMTases), *CpDmt1* and *CpDmt2* in chestnut blight fungus *Cryphonectria paraisitica*, showed sporadic viral clearance in hypoviral-infected colonies of both *CpDmt1*- (TdDMT1) and *CpDmt2*-null mutant (TdDMT2) as cultivation proceeded. In this study, gene expression analysis was performed based on Next Generation Sequencing (NGS) approach to describe the correlation of genetic changes and viral clearance. Comprehensive transcriptome analysis was conducted to identify differentially expressed genes (DEG) for hypovirus clearance using RNA-sequencing. In total, 1,150, 1,218, 3,028, and 2,362 unique transcripts were identified as DEGs by pairwise comparison of TdDMT2(V^+) vs TdDMT2, TdDMT2(V^+) vs TdDMT2-cured, TdDMT2(V^+) vs hypovirus-infected wild-type strain (UEP1), and UEP1 vs wild-type strain (EP155/2), respectively. Of these genes, a total of 174 DEGs were identified as common DEGs of the former three groups but excluding DEGs which were differentially expressed between EP155/2 and UEP1. The functional enrichment analysis demonstrated that most of DEGs were assigned to specific KEGG pathway such as SUMOylation of chromatin organization proteins and transcriptional regulation by small RNAs. Among those belonging top 10% in fold change, transporter and NAD binding domain were common representing 4 and 3, respectively. These help us understand molecular mechanism of hypoviral clearance.

855C **Vertical transfer of the core microbiome in ectomycorrhizal fungi - an example of the true truffle (Tuber aestivum)** Nejc Suban[1], Nataša Šibanc[1], Aleksander Mahnič[2], Cene Gostinčar[3], Tine Grebenc[4] [1]genetics and forest physiology, Slovenian Forestry Institute, [2]National Laboratory for Health, Environment and Food, [3]University of Ljubljana, [4]Slovenian Forestry Institute

Truffles are the fruiting bodies (ascocarps) of fungi belonging to the genus Tuber that are fruiting in the soil and are best known for their aromas. Truffles are regularly associated with bacteria and yeast that are hypothesized to contribute both to truffle aromas and to a better survival in next generation. Based on an extensive studies of truffles aromas in Europe and recent Tuber aestivum whole genomes population re-sequencing, we aimed to analyze and correlate the outcome of aroma analysis with the bacterial and fungal communities on surface and within ascocarps of the same truffle ascocarps that are expected to be associated in the truffles core microbiome. Both, the bacterial and the fungal associated communities were further assessed with site and ecological characteristics of each truffle genotype. Results of the preliminary analysis and statistical assessment of truffle genomes diversity and associated bacterial and fungal communities will be presented.

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