XLIII CONGRESO DE LA SOCIEDAD ESPAÑOLA DE GENÉTICA - SEG2023

VALENCIA, DEL 21 AL 23 DE JUNIO DE 2023

LIBRO DE RESÚMENES

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01 COMITÉS

COMITÉ ORGANIZADOR:

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Rafael Lozano Ruiz
Catedrático de Genética de la Universidad de Almería

Gemma Marfany Nadal
Catedrática de Genética de la Universidad de Barcelona

Lluis Montoliu
Investigador Científico del CSIC (CNB)

José Alonso
William Neal Reynolds Distinguished Professor of Plant and Microbial Biology
de la North Carolina State University

02 RESUMEN DEL PROGRAMA

	21 DE JUNIO		22 DE JUNIO	23 DE JUNIO
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09.00 - 09.15			Sesión 3: Genética de microorganismos Moderador: Antonio di Pietro Ponencia invitada: Álvaro San Millán Comunicaciones orales Flash talks	Sesión 6: Genética humana Moderadora: Gemma Marfany Ponencia invitada: Tomàs Marquès Bonet Comunicaciones orales Flash talks
09.15 - 09.30				
09.30 - 09.45	Sesión Satélite I: Genética y alimentación Moderador: Daniel Ramón Ponentes invitados: Diego Orzáez, Ana Ramírez de Molina, José Manuel Guillamón, Empar Chenoll			
09.45 - 10.00				
10.00 - 10.15				
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10.45 - 11.00			Pausa café + carteles	Pausa café + carteles
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11.45 - 12.00	Sesión Satélite II: Escuela de Divulgadores de la Genética Moderador: Lluis Montoliu Ponentes invitados: Cristina Nieto, Guillermo Peris, Tomàs Marquès Bonet, Helena González Burón		Sesión 4: Mejora genética y biotecnología Moderador: Rafael Lozano Ponencia invitada: Belén Picó Comunicaciones orales Flash talks	Sesión 7: Docencia de la Genética Moderadora: Rosario Linacero Comunicación oral Mesa redonda: Marcos Egea-Cortines, Ana Fita, Rosario Linacero, J. Alberto Marchal
12.00 - 12.15				
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13.00 - 13.15				Conferencia de Clausura: José Alonso
13.15 - 13.30				
13.30 - 13.45	Comida		Comida + carteles	Premios, próximo congreso, clausura
13.45 - 14.00				
14.00 - 14.15				
14.15 - 14.30				Comida
14.30 - 14.45				
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15.00 - 15.15	Apertura del congreso			
15.15 - 15.30	Sesión 1: Dinámica de cromosomas Moderadora: Mónica González Sánchez Ponencia invitada: Aurora Ruiz Herrera Comunicaciones orales Flash talks		Sesión 5: Genética de Poblaciones y Evolución Moderador: Miguel Burgos Ponencia invitada: Fernando González Candelas Comunicaciones orales Flash talks	
15.30 - 15.45				
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17.00 - 17.15				
17.15 - 17.30	Sesión 2: Expresión génica y epigenética Moderadora: Mª Teresa Roldán Arjona Ponencia invitada: Ozren Bodganovic Comunicaciones orales Flash talks			
17.30 - 17.45				
17.45 - 18.00			Asamblea de la SEG	
18.00 - 18.15				
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19.00 - 19.15				
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19.45 - 20.00	Conferencia inaugural (Jardín Botánico): Montserrat Elías Arnanz			
20.00 - 20.15				
20.15 - 20.30				
20.30 - 20.45	Recepción de bienvenida (Jardín Botánico)			
20.45 - 21.00				
21.00			Cena del congreso (Hotel Balneario las Arenas)	

03 PROGRAMA

21 JUNIO

08:30-13:30 Registro y colocación de carteles

09:30-11:15 **Sesión Satélite I: Genética y alimentación**

Moderador: Daniel Ramón (ADM Biopolis)

Ponentes invitados:
Diego Orzáez (Instituto de Biología Molecular y Celular de Plantas, CSIC)
Ana Ramírez de Molina (Instituto IMDEA Alimentación)

José Manuel Guillamón (Instituto de Agroquímica y Tecnología de Alimentos, CSIC)

Empar Chenoll (ADM Biopolis)

11:15-11:45 Pausa café

11:45-13:30 **Sesión Satélite II: Escuela de Divulgadores de la Genética**

Moderador: Lluis Montoliu (Centro Nacional de Biotecnología, CSIC)

Ponentes invitados:
Cristina Nieto (Centro de Recursos Fitogenéticos, INIA-CSIC)
Guillermo Peris (Universidad Jaume I)

Tomàs Marquès Bonet (Instituto de Biología Evolutiva, CSIC-Universidad Pompeu Fabra)

Helena González Burón (Big Van Science)

13:30-15:00 Comida

15:00-15:15 Apertura del congreso

15:15-16:45 **Sesión 1: Dinámica de cromosomas**

Moderadora: Mónica González Sánchez (Universidad Complutense de Madrid)

Ponencia invitada:
Aurora Ruiz Herrera (Universidad Autónoma de Barcelona): *3D chromatin remodelling in the germ line.*

Comunicaciones orales:
Mónica Pradillo Orellana: *The dynamic nature of the nuclear envelope is related to chromosomal behaviour during plant meiosis.*
Ismael Cross Pacheco: *Divergence and genomic evolution of repetitive elements in the sole Solea senegalensis.*
Ángela Patricia Vergara García: *ALK gene rearrangements and expression in a cohort of patients diagnosed with lung adenocarcinoma candidates for targeted therapy with inhibitors.*
Mª Ángeles Fernández Mimbrera: *Analyses of epigenetic signalling upon G2 decatenation checkpoint response in human cells.*

16:45-17:15 Pausa café + carteles

17:15-19:00 **Sesión 2: Expresión génica y epigenética**

Moderadora: Mª Teresa Roldán Arjona (Universidad de Córdoba)

Ponencia invitada:
Ozren Bogdanovic (Centro Andaluz de Biología del Desarrollo, CSIC): *Evolutionary conservation of embryonic DNA methylome remodeling in distantly related teleost species.*

Comunicaciones orales:
José Luis Micol Molina: *Cytokinins in simple leaf margin morphogenesis.*
Lluis Montoliu José: *New CRISPR genome-edited mouse models to investigate albinism.*
Eva Bastida Martínez: *Unraveling the peroxide stress response in Myxococcus xanthus and its regulation by a novel mechanism.*
Celeste Moya Valera: *Unveiling Type II Diabetes Epigenetics: Pre-Onset Exomic DNA Methylation Analysis.*

Flash talks:
Eduardo Larriba Tornel: *Functional analysis of the genes of the LYSINE SPECIFIC HISTONE DEMETHYLASE 1 (LSD1) family of Arabidopsis thaliana.*
Rosa Micol-Ponce: *Unraveling the role of the CAX-INTERACTING PROTEIN 4 (CXIP4) gene in Arabidopsis RNA metabolism.*
Pedro Perdiguero Jiménez: *Application of single cell transcriptomics to explore the rainbow trout (Oncorhynchus mykiss) immune system.*
André Vidal Capón: *Epigenetic mechanisms in bivalve tumorigenesis: a comparison of healthy and neoplastic cockles, Cerastoderma edule.*
Beatriz Suárez Quintero: *RNAPII-facilitated repair of ribosomal DNA breaks in nucleolar caps guards against genomic instability.*
Daniel Ramírez Torres: *Methylation and transcriptomic analysis in flatfish Solea senegalensis gonadal tissue.*

19:45-20:30 **Conferencia inaugural** (Jardín Botánico):
Montserrat Elías Arnanz (Universidad de Murcia): *A soil bacterium´s response to light and how it has led to discoveries important for optogenetics and for human biology and health.*

20:30 Recepción de bienvenida (Jardín Botánico)

22 JUNIO

09:30-10:45 **Sesión 3: Genética de microorganismos**

Moderador: Antonio di Pietro (Universidad de Córdoba)

Ponencia invitada: Álvaro San Millán (Centro Nacional de Biotecnología, CSIC): *Differences in vertical and horizontal transmission dynamics shape plasmid distribution in clinical enterobacteria.*

Comunicaciones orales:
Agustín Blasco: *Metagenomic data are compositional. The problem and its solution.*
Marta Fontes Bastos: *New extracytoplasmic function (ECF) sigma/anti-sigma pairs involved in the complex Myxococcus xanthus response to copper.*
S. Padmanabhan: *Regulated expression of a CRISPR-Cas defense island and its role against attack by myxophages.*
Susana Ruiz Ruiz: *Changes in the microbiota across life in a healthy Mediterranean cohort.*

Flash talks:
VicenteArnau Llombart: *Calculating genomic signature distances between phages and their bacterial host for distinguishing lytic and lysogenic phages.*
Rosario Gil García: *Molecular characterization of Wolbachia wMel in native populations of Drosophila for its application in biocontrol of the tiger mosquito in Valencia.*
Alba Gómez Gil: *Maternal microbial vertical transfer, before or after birth?*
Enrique Roig Tormo: *Exploring the Common Human Gut Eukaryotic Microbiota around the World.*
Alfonso López Rojo: *Genomic analysis of lytic myxophages and identification of Myxococcus xanthus genes conferring resistance.*
Irene del Rey Navalón: *Role of methionine sulfoxide reductases in the Myxococcus xanthus light response.*

10:45-11:45 Pausa café + carteles

11:45-13:30 **Sesión 4: Mejora genética y biotecnología**

Moderador: Rafael Lozano (Universidad de Almería)

Ponencia invitada: Belén Picó (Universidad Politécnica de Valencia): *Advances in Cucurbits Genetics and Breeding.*

Comunicaciones orales:
Clara Pons: *Trait stability and its genetic basis in European Traditional Tomato.*
Andrea Arrones Olmo: *Identification of SmAPRR2 and SmGLK2 genes as responsible for uniform chlorophyll distribution and netting in the eggplant fruit peel.*
María Salud Justamante Clemente: *Genomic landscape of wound-induced adventitious root formation in tomato.*
Alejandro Centeno Cuadros: *Towards a genetic-based toolkit for outdoor sex identification in aquaculture: an application in Solea senegalensis.*

Flash talks:
Elena Benavente: *Relationship between gene sequence-based markers for glutamine synthetase loci and yield components in wheat.*
Marta Gavilán Camacho: *Role of Transcription Factors involved in the synthesis of wheat prolamins.*
María José Gonzalo Pascual: *Validation of heat tolerance QTLs in elite lines and pyramiding of QTLs related to abiotic stresses in tomato.*
Carlos Polanco de la Puente: *Using lentil wild relatives to identify genes related to Ascochyta blight resistance.*
Santiago García Martínez: *Start of a breeding program to introduce virus resistances in Flor de Baladre and Pimiento tomatoes (Solanum lycopersicum L.).*
Ilaria Macri: *Genetic analysis of Arabidopsis growth effect in bioreactors.*

13:30-15:00 Comida + carteles

15:00-16:45 **Sesión 5: Genética de Poblaciones y Evolución**

Moderador: Miguel Burgos (Universidad de Granada)

Ponencia invitada: Fernando González Candelas (Universidad de Valencia): *The population and evolutionary genomics of bacteria.*

Comunicaciones orales:
Beatriz Sabater Munoz: *Distinct transcriptional responses to acute and chronic oxidative stress in Saccharomyces cerevisiae.*
Vadim Pisarenco: *Understanding the genomic basis of adaptation: Lessons from the island radiation of the spider genus Dysdera.*
Cristian Cuevas Caballé: *Conservation genomics of the Balearic shearwater (Puffinus mauretanicus).*
Aureliano Bombarely: *Genetic Insights of Pawpaw (Asimina triloba [L.] Dunal), the North American Forgotten Fruit.*

Flash talks:
José Luis Horreo: *Genetic diversity, structure, and dynamics of the European polecat (Mustela putorius) in the Iberian Peninsula.*
Alejandro Hernández Delgado: *DNA-based species delimitation analyses reveal extensive lineage diversity in Haploginglymus, a groundwater amphipod genus endemic to the Iberian Peninsula.*
José Antonio Jurado Rivera: *Mitophylogenomics support the placement of the enigmatic crustacean order Thermosbaenacea within the peracarida.*
Pablo Presa Martínez: *Temporal incongruence between demographic and genetic metrics in fisheries assessment: the European hake case study.*
Rebeca Domínguez Santos: *Evolution and recovery of the gut microbiota during and after rifampicin treatment in Blattella germanica.*
David Saiz Martínez: *The role of extracellular vesicles in host-symbiont communication mediated by sRNA in Blattella germanica.*

16:45-17:45 Pausa café + carteles

17:45-18:45 Asamblea de la SEG

21:00 Cena del congreso (Hotel Balneario Las Arenas)

23 JUNIO

09:00-10:45 **Sesión 6: Genética humana**

Moderadora: Gemma Marfany (Universidad de Barcelona)

Ponencia invitada: Tomàs Marquès Bonet (Instituto de Biología Evolutiva, CSIC-Universidad Pompeu Fabra): *A global catalog of genome diversity across the primate radiation.*

Comunicaciones orales:
Vasileios Toulis: *Knocking-out USP48 in human iPSCs using CRISPR/Cas9 to generate 3D retinal organoids as a retinal disease model.*
Jesús J. Ferre Fernández: *Overexpression of Myocilin in Transgenic Adult Zebrafish Results in Retinal Alterations and Variable Ocular Anterior Segment Defects Associated with Extracellular Matrix Abnormalities.*
Gema Garrido Martínez: *Update of the genetic diagnosis of albinism in Spain.*
Ismael Ejarque Doménech: *Referral criteria to clinical genetics from Primary Care: Consensus document.*

Flash talks:
José Martín Nieto: *Locating the α-dystroglycan O-mannosylglycosylation pathway in mouse retinal cells.*
José Daniel Aroca Aguilar: *Generation and functional analysis of a zebrafish knockout line for the congenital glaucoma gene CYP1B1.*
Ángel Tévar Saiz: *Functional interaction between zebrafish adamtsl4 and cpamd8 matrix metalloproteinase-related genes and its implication in early-onset glaucoma.*
María Elena Quiroz Rodriguez: *Genetic interaction between liver enzyme levels and the risk of developing diabetes mellitus 2 (T2D) over time in a Spanish population.*
Mónica Centeno Pla: *Truncated MAGEL2 and its novel subcellular localisation in Schaaf-Yang syndrome.*
David López López: *Circulating cell free DNA (ccfDNA) as a biomarker of biological age in humans.*

10:45-11:45 Pausa café + carteles

11:45-12:45 **Sesión 7: Docencia de la Genética**

Moderadora: Rosario Linacero (Universidad Complutense de Madrid)

Comunicación oral:
Marcos Egea-Cortines: *The phylogenetic map of genetics teaching in Spanish Universities.*

Mesa redonda:
Marcos Egea-Cortines (Universidad Politécnica de Cartagena)
Ana Fita (Universitat Politècnica de València)
Rosario Linacero (Universidad Complutense de Madrid)
J. Alberto Marchal (Universidad de Jaén)

12:45-13:30 **Conferencia de clausura: José Alonso** (North Carolina State University)

13:30-14:00 **Premios, próximo congreso, clausura**

14:00-15:30 Comida

04 COMUNICACIONES

CONFERENCIA INAUGURAL

CI-01 A soil bacterium´s response to light and how it has led to discoveries important for optogenetics and for human biology and health

Montserrat Elías Arnanz[a]
[a]*Departamento de Genética y Microbiología, Área de Genética (Unidad Asociada al IQFR-CSIC), Universidad de Murcia, Murcia, Spain.*

Bacteria are masters at adapting their cellular physiology to multiple external stimuli, with the cell envelope at the frontline in sensing signals. Light, a crucial external cue for most organisms including bacteria, affects various cellular pathways. We study how light is sensed and transduced in the bacterium *Myxococcus xanthus* to achieve regulated carotenogenesis and combat photooxidative stress. This has unearthed new paradigms in light sensing, signal transduction and gene regulation, and led to the discovery of prototypical members of widely distributed protein families with novel functions (1,2). These include one of the earliest members of the large group of the extracytoplasmic function sigma factors, a pillar of bacterial signal transduction (1,2), as well as a family of RNA polymerase-binding global regulators essential in many bacteria including pathogens (3). A major discovery was the CarH family of bacterial photoreceptors, which use a specific form of vitamin B_{12} to directly sense light. Light and B_{12} modulate CarH oligomerization and DNA binding/repressor activity, and this can occur with remarkable plasticity (4-7). Discovery of CarH photoreceptors not only revealed a new biological facet of vitamin B_{12}, but also provided a valuable tool for optogenetics and synthetic biology (6,8). Our studies on the *M. xanthus* light response have also unmasked a long-sought lipid desaturase indispensable for human plasmalogen biosynthesis and established the broad evolutionary sweep of this enzyme, thus opening a crucial door to study plasmalogen biogenesis, functions, and roles in human health and disease (9). Additionally, a novel role for these lipids in signaling photooxidative stress has emerged (9). Some factors involved in the response to light also act in other cellular processes, such as in copper and general oxidative stress responses, or in CRISPR-Cas-mediated phage defense (10), laying the groundwork for our ongoing efforts to decipher their underlying biology and cellular mechanisms.

1. Elías-Arnanz et al. (2011). Curr Opin Microbiol 14, 128-135.
2. Padmanabhan et al. (2021). Microorganisms 9, 1067.
3. García-Moreno et al. (2010). Nucleic Acids Res 8, 4586-4598
4. Ortiz-Guerrero et al. (2011). Proc Natl Acad Sci (USA) 108, 7565-7570.
5. Jost et al. (2015). Nature 526, 536-541.
6. Padmanabhan et al. (2017). Annu Rev Biochem 86, 485-514.
7. Pérez-Castaño et al. (2022). Environ Microbiol 24, 1865-1886.
8. Padmanabhan et al. (2019). Curr Opin Struct Biol 57, 47-55.
9. Gallego-García et al. (2019). Science 366, 128-132.
10. Bernal-Bernal et al. (2018) Nucleic Acids Res 46, 6726-6745.

CONFERENCIA DE CLAUSURA

CC-01 The challenging journey from mutants to metabolic pathways and translational regulation

José M. Alonso
Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA.

Plants are sessile organisms and their physiological and developmental processes need to be finely tuned to the everchanging environmental conditions. Years of research show that plant hormones play a central role in the integration of environmental signals with the internal physiological and developmental programs in plants. To start unveiling the molecular mechanisms underlying these integration processes, we are employing a combination of genetic, genomic, and computational approaches. In this presentation, I will describe the journey that has taken us from the identification of hormone interaction mutants to uncovering how the plant hormone auxin is produced in plants and the role the regulation of this process plays in the control of plant development and response to environmental changes. I will also describe how classical genetic and genomic approaches have given us a new appreciation for the importance of gene-specific translation changes in the orchestration of gene expression regulation. Finally, I will show how by studying the mechanisms underlying translation regulation, we are not only expanding our basic biological understanding of how cells respond to different internal and external clues, but also identifying potential biotechnological applications of this new knowledge.

Acknowledgments & Funding. I wish to thank all the present and past members of the Alonso-Stepanova lab as well as the financial support from the National Science Foundation.

SESIÓN 1: DINÁMICA DE CROMOSOMAS

Ponencia invitada:

I1-01 3D chromatin remodelling in the germ line

Aurora Ruiz-Herrera [a,b]

[a]Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. [b]Genome Integrity and Instability Group, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

The spatial folding of chromosomes inside the nucleus has regulatory effects on gene expression, yet the intricacies of this process and the impact of genome reshuffling on the 3D genome remains unclear. This is of relevance since chromosomal fusions represent the most common chromosomal rearrangement in nature (from plants to mammals), and are linked to recurrent miscarriages, infertility, and aneuploid offspring in humans. In fact, it has long been suggested that the presence of chromosomal fusions in the germ line can alter segregation patterns. In this talk I will resume our recent results on 3D chromatin remodelling in the germ line. Moreover, I will discuss on the effect of chromosomal fusions on the higher-order chromatin organization and recombination landscapes in germ line using the house mouse as a model system. Our results indicate that chromosomal fusions can alter the nuclear architecture during meiosis, including an increased rate of heterologous interactions in primary spermatocytes, and alterations in both chromosome synapsis and axis length. These disturbances in topology were associated with changes in genomic landscapes of recombination, resulting in detectable genomic footprints. Overall, chromosomal fusions impact the dynamic genome topology of germ cells in two ways: (i) altering chromosomal nuclear occupancy and synapsis, and (ii) reshaping landscapes of recombination.

Comunicaciones orales:

O1-01 The dynamic nature of the nuclear envelope is related to chromosomal behaviour during plant meiosis

Nadia Fernández-Jiménez[a], María Cuacos[b], Stefan Heckmann[b], Félix Gil-Dones[a], Nieves Cuñado[a], Isabel María Serrano-León[c], Pilar Prieto[c], Mónica Pradillo[a]

[a]Departamento de Genética, Fisiología y Microbiología, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, Spain, [b]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) OT Gatersleben, Seeland, Germany, [c]Plant Breeding Department, Institute for Sustainable Agriculture, Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC), Córdoba, Spain

Breakdown of the nuclear envelope (NE) during cell division is one of the most dramatic structural and functional changes in eukaryotic cells. The NE forms a selective boundary around the chromosomes and is composed of two membrane sheets, the inner and outer nuclear membranes (INM and ONM), which are connected by channels occupied by nuclear pore complexes (NPCs). The NPCs play key roles in diverse nuclear processes, including mRNA export and DNA damage response (1, 2), however, their function in other cellular contexts remains largely uncharacterized. During meiosis, the specialized cell division required for sexual reproduction, the NE provides a platform through which telomeric ends glide under a cytoskeletal driving force. In this cell division, NE-associated chromosomal movements are dependent on LINC (LInkers of Nucleoskeleton and Cytoskeleton) complexes (3), but until now NPCs have not been linked to chromosome dynamics in meiosis. In this work, we examined the position of various nucleoporins during male meiosis in *Arabidopsis thaliana* and hexaploid wheat (*Triticum aestivum* cv. Chinese Spring). Our results, applying fluorescence *in situ* hybridization (FISH), immunofluorescence and live-cell imaging experiments, reveal a non-random distribution pattern of these proteins during early prophase I, opening a window into the dynamics of NPC components that could be correlated to chromosome and nucleolus dynamics.

1. Strambio-De-Castillia *et al.* (2010). *Nature Reviews Molecular Cell Biology* 11, 490–501.
2. Buchwalter *et al.* (2019). *Nature Review Genetics* 20, 39–50.
3. Varas *et al.* (2015). *Plant Journal* 81, 329–46.

This work has been supported by Ministerio de Ciencia e Innovación (PID2020-118038GB-I00).

O1-02 Divergence and genomic evolution of repetitive elements in the sole *Solea senegalensis*

Ismael Cross[a], Silvia Portela-Bens[a], Manuel Alejandro Merlos[a], Rafael Navajas-Pérez[b], María Esther Rodríguez[a], Aarón Gálvez-Salido[b], Alberto Arias-Pérez[a], Alejandro Centeno-Cuadros[a], Francisca Robles[b], Carmelo Ruiz-Rejón[b], Laureana Rebordinos[a]

[a]Laboratorio de Genética, Facultad de Ciencias del Mar y Ambientales. Instituto de Ciencias Marinas (INMAR). Puerto Real, Cádiz. España. [b]Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada, Spain

The Senegalese sole, *Solea senegalensis*, is a flatfish of major interest in fisheries and aquaculture with a high market value, mainly in Europe. Due to its high interest, genomic studies on this species have been increasing in the last decade, leading finally to the publication of its genome[1,2]. However, from the published genome, there are no specific studies of the distribution, abundance or chromosomal evolution of repetitive elements. These elements play a fundamental role in the evolution of genomes and their adaptation to changing environmental conditions. Therefore, in this work, an analysis of transposable elements (TEs) and satellite DNA, as the main components of the repeat fraction of the genome, has been carried out in *S. senegalensis*. The results have shown that the repetitive elements coverage in its genome is 35.0%. In addition, the coverage of the main TE families has been analysed in 7 other flatfish species belonging to 4 families, and two other species outside this group, *Seriola aureovittata* and *Sparus aurata*, with the latter having the highest coverage (47.29%) of repetitive elements and *Cynoglossus semilaevis* (Pleuronectiforme, Cynoglossidae) the lowest. However, *S. senegalensis* has the highest number of annotated loci per Mb of genome (NL/Mb=3373) of all the species analysed. A more detailed analysis of the TEs in Senegalese sole revealed that the most abundant elements are LINEs (LINE-L2) followed by Class II transposons (hAT-Ac) and helitrons. The study of centromeric satellite families has also revealed a high concentration of helitron families around the centromeres of a large number of chromosomes. Divergence studies of TE families by chromosomes have been carried out, as well as phylogenomic studies of subfamilies mapped in the genome. A bimodal distribution in the divergence of TEs on the 21 chromosomes of the species has been observed, indicating differences in genome evolution of transposon families.

1. De la Herrán et al. (2023). Mol Ecol Resour. 00:1–19
2.- Guerrero-Cózar et al. (2021). Scientific Reports, 11, 13460

This study was supported by the Spanish Ministry of Economy, Industry and Competitiveness -FEDER: RTI2018-096847-B-C21 and Regional Government of Andalusia - FEDER: P20-00938.

O1-03 ALK gene rearrangements and expression in a cohort of patients diagnosed with lung adenocarcinoma candidates for targeted therapy with inhibitors

Luisa Sará[a], Ana Shaia Clavijo[a], Jesus David Niño-Torres[b], Luisa M Solarte[c], Ángela Vergara[a], Olga M Moreno[a], Jorge L Rodríguez[c], Adriana Rojas[a]

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The rearrangement of the anaplastic lymphoma kinase (ALK) gene, located on the short arm of chromosome 2 (2p23) (1), can lead to the activation of signaling pathways involved in cell survival and proliferation (2), therefore its identification has become a standard diagnostic test in patients with advanced NSCLC due to the response to targeted therapies with specific inhibitors (3). In this study, we evaluated the status of the ALK gene by fluorescence in situ hybridization (FISH); and the expression of the tyrosine kinase domain of the human ALK protein in samples from 18 patients with lung adenocarcinoma, 12 women and 6 men, between 29 and 85 years old, from the Pathology Department of the San Ignacio University Hospital. In FISH, a minimum of 100 paraffin-embedded cells per individual were analyzed and the ALK rearrangement positivity limit was established at 15%. There were 13 concordant results (9 negative and 4 positive) and 5 discordant between the techniques. The discordant results were characterized by a FISH pattern with deletion of the 5' end and positive cytoplasmic immunostaining. The frequency of rearrangement patterns makes it possible to differentiate real positive or negative cases from false positives or negatives cases. However, positive FISH results for gene rearrangements do not imply expression of the TK domain of the protein. The identification of patients with discordant patterns is important because even if they have an alteration, they are not direct candidates for treatment with specific inhibitors. It is pertinent to evaluate in future studies the moments before and after the treatments in order to establish if some patterns are related to favorable cellular response, resistance or increased clonality.

1. Villalobos P, Wistuba II. Lung Cancer Biomarkers. Hematol Oncol Clin North Am. 2017 Feb;31(1):13-29. doi: 10.1016/j.hoc.2016.08.006. PMID: 27912828; PMCID: PMC5137804.
2. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer. 2008 Jan;8(1):11-23. doi: 10.1038/nrc2291. PMID: 18097461.
3. Lin C, Shi X, Yang S, Zhao J, He Q, Jin Y, Yu X. Comparison of ALK detection by FISH, IHC and NGS to predict benefit from crizotinib in advanced non-small-cell lung cancer. Lung Cancer. 2019 May;131:62-68. doi: 10.1016/j.lungcan.2019.03.018. Epub 2019 Mar 20. PMID: 31027700.

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O1-04 Analyses of epigenetic signalling upon G2 decatenation checkpoint response in human cells

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Removal of chromatid catenations along chromosome arms and centromeres is mandatory for successful anaphase segregation. Recent data indicate that some specific epigenetic marks functionally interact with TOP2A to promote decatenation during mitotic division in human cells. Perturbing such interaction results in ultra fine bridges (UFBs), indicative of a major failure in resolving entangled sister chromatids. We are currently investigating if such interplay might be operating early at the onset of G2 phase, being a major determinant for the outcome of the G2 decatenation checkpoint response. To address this we are making use of human cells depleted of MCPH1 function, as they sustain a permanent G2 arrest upon catalytic inhibition of TOP2A which is accompanied by abnormal chromatin hypercondensation. Our analyses have shown first that when those cells are challenged with low doses of TOP2A catalytic inhibitor there is an increase in the UFBs rate in further mitosis. Moreover, we are interested in comparing 3D chromatin compaction and the level/spatial distribution of some epigenetic marks of interest (e.g. H3K27me3, H3K9ac, H3K9me3, H4K8ac) in cells with different checkpoint response to persistent chromatid catenations during G2. The preliminary data obtained form our study will be presented, which might be of interest to better understand epigenetic signaling contribution during TOP2A-related checkpoint response.

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Comunicaciones póster:

P1-01 The domesticated transposable element *MUG1* is essential for proper meiosis in *Arabidopsis thaliana*

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MUSTANG (*MUG*) genes are a family of domesticated transposable elements (TEs) conserved in all angiosperms. Although their exact function is not known, previous works have revealed that *MUG* genes are important during plant development, as well as for ensuring adequate fertility, although their possible function in meiosis has not been described (1). In the present work, we describe the phenotypic consequences in plant and flower development, pollen viability, and male meiosis produced by the upregulation of *MUG1* in an *Arabidopsis thaliana* T-DNA mutant. The results obtained show that in the mutant there are multiple abnormalities during meiosis, which ultimately lead to a decrease in fertility. We detected alterations in chromatin condensation, multinucleated cells, and chromatin bridges and fragments, among other defects. Furthermore, more than 50% of the meiocytes were polyploid. *MUG* sequences are similar to ancestral TEs called *Mutator-like* elements (*MULEs*), but unlike *MULEs*, *MUG* genes lack the TE termini required for mobilization and are collinear in multiple genomes, indicating that it is highly unlikely that they function in transposition (2, 3). In addition, *MUG* genes are highly conserved, they are expressed in diverse tissues and present active site residues, suggesting that they may also have a function involving DNA binding. Indeed, transcriptomic analyses of samples enriched in meiocytes have revealed that there are alterations in the expression of more than 2,500 genes. The differentially-expressed genes (DEGs) identified are involved in multiple pathways such as environmental information processing and adaptation, metabolism, and cell division, among others. These results suggest that *MUG1* is likely playing a role as a transcription factor. We will discuss which of the DEGs might be involved in the altered meiotic phenotype we have observed.

1. Joly-Lopez *et al.* (2012). *PLoS Genetics* 8, e1002931.
2. Yu *et al.* (2000). *Genetics* 156, 2019-2031.
3. Cowan *et al.* (2005). *Molecular Biology and Evolution* 22, 2084-2089.

P1-02 Chromosome dynamics during meiosis in wheat in the context of breeding

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Wheat is one of the most important crops in the world. In the context of breeding, genetic crosses are developed between wheat and related species which carry desirable agronomic traits to be transfer into the crop. But associations between wheat chromosomes and those from the donor species do not occur during meiosis, the process to generate gametes in organisms with sexual reproduction, what difficult the introgression of genetic variability into wheat. In this work we study wheat genome organisation and chromosome interactions to clarify the distribution pattern of homologous chromosomes within the cell nucleus. We study putative Interactions between homologous chromosomes in premeiotic stages that could facilitate chromosome associations and recombination at the beginning of meiosis using cytogenetics tools. An extra pair of barley homologous chromosomes introgressed in the wheat genetic background allowed us the study of the spatial distribution, arrangements and interactions occurring exclusively between this pair of homologous chromosomes during premeiosis by fluorescence *in situ* hybridization (FISH). Our results suggest that homologous chromosome interactions can be initiated before meiosis, which could contribute to facilitate the processes of specific chromosome recognition and association occurring during meiosis in wheat.

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SESIÓN 2: EXPRESIÓN GÉNICA Y EPIGENÉTICA

Ponencia invitada:

I2-01 Evolutionary conservation of embryonic DNA methylome remodeling in distantly related teleost species

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Methylation of cytosines in the CG context (mCG) is the most abundant DNA modification in vertebrates that plays crucial roles in cellular differentiation and identity (1). After fertilization, DNA methylation patterns inherited from parental gametes are remodelled into a state compatible with embryogenesis. In mammals, this is achieved through the global erasure and re-establishment of DNA methylation patterns (2). However, in non-mammalian vertebrates like zebrafish, no global erasure has been observed (3). To investigate the evolutionary conservation and divergence of DNA methylation remodelling in teleosts, we generated base resolution DNA methylome datasets of developing medaka and medaka-zebrafish hybrid embryos. In contrast to previous reports, we show that medaka display comparable DNA methylome dynamics to zebrafish with high gametic mCG levels (sperm: ~90%; egg: ~75%), and adoption of a paternal-like methylome during early embryogenesis, with no signs of prior DNA methylation erasure. We also demonstrate that non-canonical DNA methylation (mCH) reprogramming at TGCT tandem repeats (4) is a conserved feature of teleost embryogenesis. Lastly, we find remarkable evolutionary conservation of DNA methylation remodelling patterns in medaka-zebrafish hybrids, indicative of compatible DNA methylation maintenance machinery in far-related teleost species. Overall, these results suggest strong evolutionary conservation of DNA methylation remodelling pathways in teleosts, which is distinct from the global DNA methylome erasure and reestablishment observed in mammals.

1. Schubeler (2015). Nature 517, 321–326.
2. Hackett et al (2013). Philos Trans R Soc Lond B Biol Sci 368, 20110328.
3. Potok et al (2013). Cell 153, 759–772.
4. Ross et al (2020). Nucleic Acids Res 16, 12675-12688.

This work was supported by the ARC Discovery Project (DP190103852), Ramón y Cajal fellowship (RYC2020-028685-I), Proyecto de Generación de Conocimiento 2021 (PID2021-128358NA-I00), CEX2020-00108-M Unidad de Excelencia María de Maeztu, DOC Fellowship from the Austrian Academy of Sciences, FWF START program (Y 1031-B28) grant, ERC CoG 101044495/GaMe, HFSP Career Development Award (CDA00066/2015), HFSP Young Investigator Award (RGY0079/2020) and the FWF SFB RNA-Deco.

Comunicaciones orales:

O2-01 Cytokinins in simple leaf margin morphogenesis

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**These authors contributed equally to this work.*

The interplay between auxin and cytokinins governs different plant developmental events, as ovule formation, lateral root initiation and organ emergence from the shoot apical meristem (SAM). Moreover, cytokinins favor complexity in the development of *Solanum lycopersicum* and *Cardamine hirsuta* compound leaves. Nevertheless, no role has been proposed for cytokinins in patterning the margins of the simple leaves of Arabidopsis, a process that is assumed to be sufficiently explained by auxin activity. Here, we discuss evidence supporting the hypothesis that cytokinins play a role in simple leaf margin morphogenesis via crosstalk with auxin (1). Indeed, treatment with the synthetic cytokinin 6-BAP increases serration in Col-0 leaves (2), while mutant or transgenic Arabidopsis plants defective in cytokinin biosynthesis or signaling, or with increased cytokinin degradation have leaf margins less serrated than the wild type. For instance, overexpressing *CYTOKININ DEHYDROGENASE/OXIDASE* (*CKX*) genes, which encode enzymes that irreversibly inactivate cytokinins, like occurs in the *ANT:CKX3* transgenic line, which overexpresses *CKX3* under the control of the promoter of *AINTEGUMENTA* (*ANT*), a gene specifically active during leaf primordia development, produces a smoother leaf margin than in the wild type (3). IPT enzymes catalyze the first step of cytokinin biosynthesis, with IPT3, IPT5, and IPT7 being most specific to the vegetative phase. Similar to the *ANT:CKX3* transgenic line, the *ipt3 ipt5 ipt7* triple mutant presents leaves with smoother margins than the Col-0 wild type (4). Type-B ARRs are the final effector targets of the phosphorylation signaling cascade of cytokinins. Therefore, the *arr1 arr10 arr12* triple mutant, which carries loss-of-function alleles of three members of the major subfamily of type-B ARRs, also shows smoother leaf margins (5). Since cytokinins promote leaf compoundness in plants with compound leaves, studying their role in margin morphogenesis of dicotyledonous simple leaves will provide additional insight into leaf development.

(1) Navarro-Cartagena and Micol (2023). Trends Plant Sci 28, 54-73. (2) Steiner et al. (2012). Plant Cell 24, 96-108. (3) Holst et al. (2011). J Plant Physiol 168, 1328-1334. (4) Miyawaki et al. (2006). Proc Natl Acad Sci USA 103, 16598-16603. (5) Argyros et al. (2008). Plant Cell 20, 2102-2116.

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O2-02 New CRISPR genome-edited mouse models to investigate albinism

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Albinism is a rare genetic condition affecting the visual system which might also present with alterations in pigmentation patterns (1,2). It is genetically heterogenous, being associated with as many as 22 genes whose mutations cause the corresponding subtype of albinism. All these human genes have a counterpart in the mouse genome. Hence, we have been applying the newest CRISPR genome editing tools to generate patient-specific mouse mutants, which we call "avatar mice" (3). Most of our mouse models accumulated over the years refer to mutations in the mouse *Tyr* gene, homologous to the human *TYR* locus, whose mutations cause OCA1, the common oculocutaneous albinism type 1 (4). In the *Tyr* gene we have functionally analysed the role of several regulatory elements surrounding the locus whose presence is instrumental for the faithful regulation of the expression of this gene (5, 6).

To date we have generated CRISPR-based avatar mouse models of several different types of oculocutaneous albinism (OCA1, OCA2, OCA4, OCA6, OCA7), ocular albinism type 1 (OA1) and FHONDA (foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis) type, whose affected genes are *Tyr*, *Oca2*, *Slc45a2*, *Slc24a5*, *Lrmda*, *Gpr143* and *Slc38a8*, respectively. In this presentation we will be update our efforts and progress towards completing the phenotyping attempts (with a wide range of tests, including melanin quantification, histology analysis, optomotor test for visual acuity and the study of chiasmatic connections) to describe these various new mouse models of albinism and their use to better understand the genetic condition of albinism and as future subjects to develop gene therapy approaches to fix these mutations

(1) Bakker R et al. Prog Retin Eye Res. 2022 Nov;91:101091.
(2) Kuht HJ et al. Ophthalmology. 2022 Jun;129(6):708-718.
(3) Perez CJ et al. Exp Dermatol. 2022 Mar;31(3):330-340.
(4) Seruggia D et al. Pigment Cell Melanoma Res. 2021 Mar;34(2):212-221.
(5) Seruggia D et al. Sci Rep. 2020 Sep 23;10(1):15494.
(6) Seruggia D et al. Nucleic Acids Res. 2015 May 26;43(10):4855-67

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O2-03 Unraveling the peroxide stress response in *Myxococcus xanthus* and its regulation by a novel mechanism

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Photooxidative stress triggers a transcriptional response involving novel factors that culminates in carotenogenesis for photoprotection in *Myxococcus xanthus* (1). But how this obligately aerobic, Gram-negative soil bacterium copes with general oxidative stress remains largely unexplored. Peroxiredoxins, a ubiquitous family of thiol-based peroxidases, remove >90% of cellular peroxides in most organisms. AhpC, a highly conserved and widespread peroxiredoxin with broad substrate specificity, efficiently scavenges low levels of H_2O_2, while catalases detoxify high levels of extracellular H_2O_2 (2-3). *M. xanthus ahpC* gene deletion produced a pleiotropic phenotype, including growth and plating defects, reduced thresholds for signals activating carotenogenesis, and enhanced tolerance to H_2O_2 due to overexpression of the catalase gene *katB.* Expression of *ahpC* and *katB* in most bacteria is usually regulated by the peroxide sensors OxyR or PerR through a redox switch (4), but both are absent in *M. xanthus*. Rather, we found that *M. xanthus* and related myxobacteria conserve putative σ^{54}-dependent promoters upstream of both *ahpC* and *katB* genes and a gene adjacent to *ahpC* encoding an enhancer-binding protein (EBP), a member of a class of AAA^+ ATPase DNA-binding proteins typically involved in activating σ^{54}-dependent promoters. We show that under normal growth conditions EBP partially represses the σ^{70}-dependent expression of *ahpC,* whereas under peroxide stress EBP activates *ahpC* and *katB* expression by binding to pseudo-palindromic repeats upstream of their σ^{54}-dependent promoters. Interestingly, deletion of *ahpC* together with that of *katB* or of the EBP gene was synthetically lethal, suggesting that the EBP- mediated compensatory *katB* upregulation in *ahpC*-deficient cells is essential for viability. Moreover, we demonstrate that its N-terminal GAF domain inhibits EBP activity, and that this is relieved when the GAF domain, through its possible non-heme iron center, senses peroxide. This would then trigger EBP-driven activation of σ^{54}-dependent *ahpC* and *katB* expression to counter oxidative stress.

(1) Gallego-García A, et al. (2019) Science **366**: 128-132. 2. **(2)** Cao Z, Lindsay JG (2017) *Subcell Biochem* **83**: 127–147. **(3)** Nóbrega CS, Pauleta SR (2019) *Adv Microb Physiol* **74**: 415-464. **(4)** Sem A, Imlay J (2021) *Front Immunol* **12**: 667343.

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O2-04 Unveiling Type II Diabetes Epigenetics: Pre-Onset Exomic DNA Methylation Analysis

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Type 2 diabetes (T2D) is a chronic disease caused by a complex interaction of genetic and environmental factors. Epigenetic modifications such as DNA methylation (DNAm) may have contributed to the development of this disease since DNAm modulates genetic regulation. DNAm changes can occur throughout life, including embryonic development. Therefore, DNAm alterations may contribute to the development of T2D. By identifying the differentially methylated regions and cytosines (DMRs and DMCs), involved in T2D, we can gain insight into genes and mechanisms that are implicated in the disease. Furthermore, this data will allow us to identify individuals who may be at risk. We have analysed DNAm in the exome of 20 samples from the Egabro-Pizarra study. The cases had developed T2D up to 5 years after the blood samples were taken. We used open source software (HPG-Dhunter, Bicycle and Bismark) to identify DMRs and DMCs for further data analysis. We identified DMRs in 7 different genes (*IRS2*, *ADARB2*, *KLK7*, *OAZ1*, *FEM1A*, *UTP11*, *DNASE2*) and one pseudogene (*HSPD1P4* located in the promoter region of *RBMS2*) with different methylation patterns between those who develop T2D and those who do not. Some of these genes have previously been implicated in T2D. These DNA methylation changes in individuals prior to disease onset highlight the potential importance of epigenetic modifications in disease development. These data support the idea that T2D may be programmed by methylation changes in DNA. Future research in larger populations is needed to confirm these findings.

1. Fernández, Lisardo, *et al*. "HPG-DHunter: an ultrafast, friendly tool for DMR detection and visualization." *BMC bioinformatics* 21.1 (2020): 1-27.
2. Graña, Osvaldo, *et al.* "Bicycle: a bioinformatics pipeline to analyze bisulfite sequencing data." *Bioinformatics* 34.8 (2018): 1414-1415.
3. Krueger, F., & Andrews, S. R. (2011). Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. *Bioinformatics*, 27(11), 1571-1572.

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Comunicaciones Flash Talk y póster:

FP2-01 Functional analysis of the genes of the LYSINE SPECIFIC HISTONE DEMETHYLASE 1 (LSD1) family of *Arabidopsis thaliana*

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We previously found that mutations in a homolog of a gene encoding the highly conserved LYSINE SPECIFIC HISTONE DEMETHYLASE 1 (LSD1) enhanced wound-induced adventitious root (AR) formation in tomato seedlings. Four *LSD1-LIKE* (*LDL*) homologues have been identified in *Arabidopsis thaliana*, namely *LDL1*, *LDL2*, *LDL3* and *FLOWERING LOCUS D* (*FLD*) [1]. We are studying the effect of loss-of-function mutations in *LDL* genes on AR formation after injury and the results found suggest both their specialization and their partial redundancy in specifying the size of the AR founder cell population. Using a whole transcriptome RNA sequencing approach, we have identified specific genomic targets regulated by LDL and likely involved in *de novo* organ formation in response to injury. Finally, we have initiated a tandem affinity purification (TAP) approach coupled with mass spectrometry [2], which is allowing us to identify LDL-containing protein complexes that are involved in chromatin remodeling. Following this approach, we have identified 43 LDL1-interacting proteins, some of which are known to be involved in broad epigenetic regulation, such as SUVR5, which mediates H3K9me2 deposition and silencing, and whose previous link to LDL1 function has been reported but not yet clarified [3]. Our results suggest that LDL-mediated chromatin modifications repress cell reprogramming during organ regeneration in *A. thaliana* explants.

1. Martignago et al. (2019). Frontiers in Plant Science 10, 669.
2. García-León et al. (2018). Methods in Molecular Biology 1794, 297-309.
3. Caro et al. (2012). PLOS Genetics 8, e1002995.

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FP2-02 Unraveling the role of the *CAX-INTERACTING PROTEIN 4* (*CXIP4*) gene in Arabidopsis RNA metabolism

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MORPHOLOGY OF AGO1-52 SUPPRESSED 2 (MAS2) is involved in pre-mRNA splicing, mRNA export and ribosome biogenesis in Arabidopsis (1). In a search for MAS2 interactors based on the yeast two-hybrid assay, we identified CAX-INTERACTING PROTEIN 4 (CXIP4), a plant-specific protein that contains a zinc knuckle motif commonly found in proteins involved in RNA metabolism (2). The putative human ortholog of CXIP4 is SREK1-interacting protein 1 (SREK1IP1), which interacts with the serine-arginine (SR)-rich splicing regulatory protein SRrp86 (3). We obtained two insertional, recessive alleles of *CXIP4*: *cxip4-1* causes early post-embryonic lethality, and *cxip4-2* plants were viable and showed slow growth and late flowering, as well as pointed leaves, a common trait among mutants affected in genes encoding ribosomal proteins and ribosomal biogenesis factors (RBFs). RT-PCR analyses indicated that *cxip4-1* and *cxip4-2* are null and hypomorphic alleles of *CXIP4*, respectively. A transgene harboring a wild-type copy of the *CXIP4* gene fully rescued the lethality of *cxip4-1,* and the pleiotropic phenotype of *cxip4-2*. Wild-type plants overexpressing *CXIP4* did not exhibit any abnormal phenotypes. We found that CXIP4 is primarily localized in the nucleoplasm, with some presence in the nucleolus. The *cxip4-2* mutant showed nuclear accumulation of polyadenylated RNAs, suggesting that CXIP4 is required for proper nuclear mRNA maturation and/or export. RNA gel blot analyses also revealed overaccumulation of 18S rRNA precursors in *cxip4-1*, a trait seen in mutants carrying alleles of genes encoding other MAS2 interactors that act as RBFs. We obtained double mutants combining *cxip4-2* with alleles of genes encoding RBFs, which showed synergistic phenotypes. Our results indicate that CXIP4 plays a dual role, as MAS2 does, acting in rRNA and mRNA metabolism.

1. Sánchez-García *et al.* (2015). *Plant Cell* **27**, 1999-2015.
2. Aceituno-Valenzuela *et al.* (2020). *Cell. Mol. Life Sci.* **77**, 3991-4014.
3. Hense *et al.* (2004). *J. Mol. Neurosci*. **24**, 269-275.

Research in the laboratory of M.R.P. is supported by grants from the Ministerio de Ciencia e Innovación of Spain (MCI; BIO2017-89728-R and PID2020-117125RB-I00 [MCI/AEI/FEDER, UE]) and the Generalitat Valenciana (GV; PROMETEO/2019/117). U.A.-V. held a predoctoral contract from the GV (GRISOLIAP/2016/134), and R.M.-P. holds a María Zambrano postdoctoral contract from the Ministerio de Universidades of Spain.

FP2-03 Application of single cell transcriptomics to explore the rainbow trout (*Oncorhynchus mykiss)* immune system

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The recent development of single cell sequencing technologies (scRNA-seq) has revolutionized the state-of-art of cell biology, allowing the simultaneous measurement of thousands of genes in thousands of single cells at once. Although current protocols are mainly adapted to human and mouse, this technology is easily transferable to non-model species allowing the study of cells with a resolution that was unimaginable decades ago. Considering that fish represent the first species in evolution with a well formed adaptive immune system, the availability of this new high throughput tools is highly interesting allowing to perform deep comparative immunology studies focused on leukocyte populations from rainbow trout.

Using 10x genomics, we initially characterized the different B cell populations present in rainbow trout blood. We were able to differentiate cellular subgroups differentially expressing specific genes as well as long non-coding RNAs, that suggested specific functional characteristics of these subpopulations (1). A deep analysis of B cell receptor (BCR) from these B cells allowed us to describe that teleosts express multiple IgLs encoded by different CL genes simultaneously in the same cell. Using single cell transcriptomics and selectively amplifying the receptors of these multiple IgLs we demonstrated that in many of the individualized B cells of rainbow trout the different $V_LJ_LC_L$ genes had undergone somatic recombination, evidencing that, unlike mammals, teleosts present greater laxity of the isotype exclusion that forms the B cell receptor. These results contrast with the established one-cell-one-antibody paradigm suggesting that fish B cells can simultaneously produce antibodies with different specificities in the same cell (2). A new analysis is allowing us to explore the white cell populations in blood of rainbow trout identifying a set of cell markers shared with mammals in addition to another set of fish specific cell markers that could be used in future to further characterize these subpopulations. Additionally, we are exploring the induced molecular response that each population shows in response *in vitro* stimulation with infectious pancreatic necrosis virus (IPNV) (3). Finally, these technologies are being applied in TeLymSeq project in order to explore the different maturation and differentiation stages of rainbow trout adaptive immune cells.

(1) Perdiguero, P.; Morel, E.; Tafalla, C. 2021. Diversity of Rainbow Trout Blood B Cells Revealed by Single Cell RNA Sequencing. Biology, 10, 511.
(2) Perdiguero, P.; Morel, E.; Díaz-Rosales, P.; Tafalla, C. 2021. Individual B cells express multiple immunoglobulin light chains in teleosts. iScience. 24(6), 102615
(3) Perdiguero, P.; Abos, B; Docando, F.; Díaz-Rosales, P.; Tafalla, C. 202X. A Single-Cell Atlas of rainbow trout white blood cells and their molecular regulation during in vitro interaction with the infectious pancreatic necrosis virus (IPNV). In preparation.

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FP2-04 Epigenetic mechanisms in bivalve tumorigenesis: a comparison of healthy and neoplastic cockles, *Cerastoderma edule*

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Cancer is a complex genetic-based disease characterized by the acquisition of several hallmarks, including sustained proliferative signalling, resistance to growth suppressors, evasion of cell death, and immunodepression (1). Modifications on the epigenetic regulation mechanisms, such as alterations in chromatin access via histone modification and cytosine methylation, play a role in cancer development (2). While most neoplastic processes arise from congenital genetic deregulation of somatic cells, some transmissible cancers in which neoplastic cells are horizontally transmitted between individuals have been described in some mammals (3, 4) and various bivalve species, the later referred to as disseminated neoplasia (5). To understand the molecular mechanisms underlying the development of these processes, we investigated whole-genome methylation patterns in healthy and tumoral cockles *Cerastoderma edule* (Linnaeus, 1758) using nanopore sequencing.

In both healthy and neoplastic animals CpG methylation was globally low but condensed on gene bodies and positively correlated with gene expression, consistently with previous studies on oysters (6) and in contrast to vertebrates, where CpG methylation is generally associated with gene repression. Notably, neoplastic samples showed global hypomethylation, with some genes preferably up-methylated and, likely, up-transcribed. Furthermore, we detected a significant increase in hydroxymethylcytosine in neoplastic animals, although its levels were overall very low in both conditions, and its transcriptional effect remains to be understood. Our study provides novel insights into the epigenetic regulation of cancer development in bivalves. These findings highlight the differences in the function of methylation in bivalves compared to vertebrates. Further investigations are required to understand the functional consequences of these alterations and their potential as biomarkers for tumorigenesis in bivalve species.

1- https://doi.org/10.1158/2159-8290.cd-21-1059
2- https://doi.org/10.1200/jco.2004.07.151
3- https://doi.org/10.1111/j.1558-5646.2009.00724.x
4- https://doi.org/10.3390/tropicalmed5020050
5- https://doi.org/10.1016/j.cell.2015.02.042
6- https://doi.org/10.1007/s10126-013-9523-2

FP2-05 RNAPII-facilitated repair of ribosomal DNA breaks in nucleolar caps guards against genomic instability

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The nucleolus, a non-membrane organelle whose canonical function is ribosome biogenesis, is a cellular sensor for multiple external and internal molecular stress. Oversized nucleoli and enhanced ribosome biogenesis are hallmarks of cancer, reflecting tumor cells' aberrantly high demand for proteosynthesis during tumorigenesis (1). The high-copy ribosomal DNA (rDNA) genes are highly transcribed by RNAPI and inherently unstable, often subject to DNA double-strand breaks (DSBs) caused by transcription-replication conflicts (2). Recently, it has been shown that upon rDNA DSBs, ATM-TCOF axis activation promotes transcriptional inhibition and rDNA DSBs relocation to the nucleolar periphery for repair by homologous recombination (HR) in newly formed nucleolar caps. However, molecular mechanisms underlying these processes are incompletely understood (3,4,5). Last years, RNA polymerase II (RNAPII) and RNA polymerase III (RNAPIII) were reported to have an essential role for cell choice between HR and non- homologous end joining (NHEJ), with nascent RNA being a key step in promoting HR (6). This work assesses the theory that rDNA DSBs are actively moved out to the nucleolar periphery to interact with RNAPII and promote the HR pathway. Taking advantage of an U2OS-based cell model that stably expresses the endonuclease Cas9 and a GFP-tagged version of the DDR protein NBS1, we can induce DSBs specifically in the nucleolus by transfection with guide RNA targeting rDNA. Using this cell model, we have observed that rDNA DSB translocation and nucleolar cap formation require presence and activity of RNAPII. Nascent RNA deficiency upon RNAPII inhibition in damaged rDNA cells provokes rDNA instability and cell death. Finally, the enhanced cytotoxic effect of combined RNA transcription inhibition and rDNA damage is consistent with addiction of cancer to nucleolar function, indicating an emerging targetable vulnerability of cancer cells that may inspire innovative treatment strategies in oncology.

1. Zisi et al. (2022). Cancers (Basel) 14.
2. Lin et al. (2012). Curr Genomics 13, 65-73.
3. Van Sluis et al. (2015). Genes Dev 29, 1151- 1163.
4. Kruhlak el at. (2007). Nature 447, 730- 734.
5. Larsen et al. (2014). Nat Cell Biol 16, 792- 803.
6. Gomez- Cabello et al. (2022). Nat Commun 13, 5303.

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FP2-06 Methylation and transcriptomic analysis in flatfish *Solea senegalensis* gonadal tissue

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Solea senegalensis is a species of high commercial value that presents reproductive dysfunction in males born and bred in captivity (F1) which hampers its aquaculture production. Understanding the biological mechanisms related to reproduction, sexual determination, and the effects of external stimuli on gonadal development is essential for their aquaculture exploitation [1]. DNA methylation is an epigenetic mechanism that involves the enzymatic enhancement of methyl (CH3-) groups to the DNA molecule through the conversion of cytosine (predominantly in CG or CpG dinucleotides) to 5′-methylcytosine by DNA methyltransferases (DNMTs). It influences a large part of the processes through the regulation of gene expression, acting in promoter regions and playing a key role in the response of an organism to internal and external stimuli [2]. The present study aims to study possible methylation differences in gonadal tissue of mature and immature male and female individuals, both wild and captive-bred (F1); For this, a total of 24 samples of *S. senegalensis* gonads of different origin, sex and degree of maturity were used for a transcriptomic and methylation analysis. Bisulfite sequencing deaminates those unmethylated cytosines in uracils, leaving the methylated cytosines intact, allowing analysis of methylation patterns. For sequencing, subsequent analysis and integration with expression data (RNA-seq), libraries were prepared from genomic DNA with the Diagenode Premium RRBS kit [3]. The integration of the study of methylation levels with RNA sequencing data makes it possible to study their connection with gene expression, contributing to the knowledge of the effects of methylation on the expression of certain genes and those functions regulated by this mechanism [2]. Knowing the mechanisms underlying reproduction, sexual determination, and the effect of external stimuli on gonadal development through neuronal communication is essential to promote aquaculture of this species [1].

References

1. de la Herrán R, Hermida M, Rubiolo JA, et al. A chromosome-level genome assembly enables the identification of the follicule stimulating hormone receptor as the master sex-determining gene in the flatfish *Solea senegalensis*. Mol Ecol Resour. 2023;(December 2022):1-19. doi:10.1111/1755-0998.13750
2. Mukiibi R, Peñaloza C, Gutierrez A, Yáñez JM, Houston RD, Robledo D. The impact of Piscirickettsia salmonis infection on genome-wide DNA methylation profile in Atlantic Salmon. Genomics. 2022;114(6). doi:10.1016/j.ygeno.2022.110503.
3. Veillard AC, Datlinger P, Laczik M, Squazzo S, Bock C. Diagenode® Premium RRBS technology: cost-effective DNA methylation mapping with superior coverage. Nat Methods. 2016;13(2):i-ii. doi:10.1038/NMETH.F.391

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Comunicaciones póster:

P2-01 Analysis of differential expression of genes associated with sexual differentiation mechanisms in *Solea senegalensis* (Kaup 1958)

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The Senegalese sole, *Solea senegalensis*, is a flatfish of the order Pleuronectiformes, a group that includes species of commercial interest. Within this order, several genes have been associated with sex differentiation mechanisms. For the Senegalese sole, which has an XX/XY sex determination system, recent works (1, 2) identify *fshr* as the gene responsible for sex differentiation. In similar species, such as *Cynoglossus semilaevis* or *Scophthalmus maximus* the responsible genes are *gsdf* (3) or *sox2* (4), respectively. In our study, to understand the mechanisms of sex differentiation in Solea senegalensis, we analyzed the complete transcriptome of 24 individuals under three different conditions: source (wild or F1), stage (mature and immature) and sex (males and females). Twenty-eight possible comparisons were made between individuals with different conditions. Changes in differential gene expression levels were observed when individuals were grouped by sex. In the Principal Component Analysis (PCA), the female samples showed a strong grouping indicating high similarity, while the males showed two groups differentiated by their origin, wild or F1. The highest number of differentially expressed genes was found in individuals of different sexes, regardless of their origin. Genes associated with sex differentiation mechanisms such as *fshr*, *dmrt1*, *amh*, *sox3* and *sox9a* were overexpressed in males while *hsdf17b1*, which catalyses sex steroid reactions, is downregulated in females.

1. Guerrero-Cózar et al., (2021). Scientific Report 11, 1-16.
2. Herrán Moreno et al., (2023). Molecular Ecology Resources 23, 886–904.
3. Zhu et al. (2018). Scientific Reports 9, 1-13.
4. Martínez et al. (2021). Genomics, 113, 1705-1718.

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P2-02 Artificial microRNAs designed to circumvent the lethality of null alleles of genes encoding ribosome biogenesis factors in Arabidopsis

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80S ribosome biogenesis is a complex process: it requires the coordinated action of three RNA polymerases for the synthesis of ribosomal RNA (rRNA) precursors, ribosomal proteins (RPs), and more than 200 ribosome biogenesis factors (RBFs). There is a wealth of information on yeast and human RBFs, but only a few have been studied in plants. The assembly and maturation of the 40S ribosomal subunit is carried out by a large ribonucleoprotein complex, termed the SSU processome or 90S particle, which was first discovered in yeast, where most of its components are essential (1). The SSU processome is assumed to be highly conserved, but its existence in plants has not yet been confirmed. DNA Polymerase V (Pol5), U3 Small Nucleolar RNA-Associated Protein 18 (Utp18), Utp22 and Ribosomal RNA Processing 36 (Rrp36) are essential components of yeast SSU processome. Using T-DNA insertional alleles, we found that the *POL5*, *UTP18* and *UTP22* Arabidopsis genes are essential for plant development, and no alleles of *RRP36* were available at stock centers. To circumvent the lethality caused by the null alleles of these genes, we constructed transgenes that produce artificial miRNAs (amiRNAs) designed to partially silence their expression. We obtained viable transgenic T_1 plants that are likely to produce amiRNAs, since they exhibited pointed leaves and slow growth, as usual for viable homozygotes for hypomorphic alleles of genes encoding RBFs or RPs. Our preliminary gel blot analysis of RNA from these transgenic lines indicate that POL5, UTP18, UTP22 and RRP36 are required for 18S rRNA maturation, which suggests that these proteins are components of the Arabidopsis SSU processome.

1. Phipps *et al.* (2011). *Wiley Interdiscip. Rev. RNA* **2**, 1-21.

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P2-03 Cross-kingdom conservation of Arabidopsis RIBOSOMAL PROTEIN S24 (RPS24) function in 18S rRNA maturation

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All the 81 ribosomal proteins (RPs) that form the Arabidopsis 80S ribosome are encoded by several paralogous genes. For example, the nearly identical RPS24A and RPS24B proteins are encoded by *RPS24A* and *RPS24B*, respectively. These genes exhibit combined haploinsufficiency, as at least two wild-type copies of either *RPS24A* or *RPS24B* are required for plant viability and at least three are required for normal plant development. Loss-of-function of either gene caused a pointed-leaf phenotype, which is typical of null or hypomorphic recessive alleles of genes encoding ribosome biogenesis factors (RBFs) or RPs. We also found that RPS24A and RPS24B act as RBFs during early stages of 18S ribosomal RNA (rRNA) maturation, as loss of RPS24A or RPS24B function reduced the 18S/25S rRNA ratio. An RPS24B-GFP fusion protein predominantly localized to the nucleolus. MRNA TRANSPORTER 4 (MTR4) and SMALL ORGAN 4 (SMO4) are Arabidopsis RBFs (1,2); the *rps24b-2* mutation strengthened the phenotypes of the *mtr4-2* and *smo4-3* mutants, which are defective in 5.8S rRNA maturation. This synergistic interaction might be an effect of increased 45S rDNA transcription, which we observed in the *rps24* mutants. Therefore, the Arabidopsis RPS24 proteins act as RBFs during 18S rRNA maturation, like their human and yeast putative orthologs (3,4). Only two plant RPs were previously shown to act not only as structural components of the ribosome but also as RBFs. We provide evidence that RPS24 proteins also regulate 45S rDNA transcription, which has not been described for their yeast or human orthologs.

1. Lange *et al.* (2008). *Plant J.* **68**, 51-63.
2. Micol-Ponce *et al.* (2020). *Plant Physiol.* **184**, 2022-2039.
3. Choesmel *et al.* (2008). *Hum. Mol. Genet.* **17**, 1253-1263.
4. Ferreira-Cerca *et al.* (2005). *Mol. Cell* **20**, 263-275.

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P2-04 Genetic characterization of RIBOSOMAL RNA PROCESSING 8 (*RRP8*), a putative ribosome biogenesis factor in Arabidopsis

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Ribosomal RNA Processing 8 (RRP8) is a known ribosome biogenesis factor in *Saccharomyces cerevisiae* and humans, which has not been studied in plants. Yeast Rrp8 acts as a methyltransferase in 25S pre-rRNA processing (1). Human RRP8 is a component of eNOSC (energy-dependent nucleolar silencing complex), which represses rRNA transcription depending on the energy status of the cell (2). To ascertain the role of Arabidopsis *RRP8* in ribosome biogenesis, we characterized the morphological and molecular phenotypes of two insertional lines, whose T-DNA insertions disrupt this gene in its first exon (*rrp8-1*) and third intron (*rrp8-2*). The leaves of these mutants are pointed and serrated, as shown by other mutants defective in ribosome biogenesis. A complementation assay confirmed allelism of *rrp8-1* and *rrp8-2*. RT-qPCR analysis of 45S rDNA transcript levels showed no differences with the Col-0 wild type. The 25S/18S mature rRNA ratio was also similar in these mutants and Col-0. An $RRP8_{pro}$:*RRP8* transgene did not rescue the leaf phenotype of *rrp8-1* and *rrp8-2*, and did not modify the morphological phenotype of Col-0 plants. To identify genetic interactors of *RRP8*, we are obtaining double mutant combinations of *rrp8-1* and *rrp8-2* with mutant alleles of genes known to encode RBFs. By analyzing the progeny of these crosses, we sought to uncover genetic interactions that may shed light on the complex network of molecular pathways underlying ribosome biogenesis. For example, we combined *rrp8-1* with *mtr4-2*, an allele of *MRNA TRANSPORT 4* (*MTR4*), and the morphological phenotype of the double mutant indicated that *MTR4* is epistatic over *RRP8*, which supports the hypothesis that RRP8 is an RBF also in Arabidopsis. Planned further studies include to ascertain the subcellular localization and the possible methyltransferase activity of RRP8, as well as its link to the energy status of the cell.

1. Peifer *et al.* (2013). *Nucleic Acids Res.* **41**, 1151-1163.
2. Murayama *et al.* (2008). *Cell* **133**, 627-639.

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P2-05 Challenging the genetic model of chemotype inheritance in *Cannabis sativa*

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Cannabis sativa, though long stigmatized, is now the source of a multibillion-dollar pharmaceutical industry due the compounds it produces, the cannabinoids. Specifically, THC (Δ-9-tetrahydrocannabinol) and CBD (cannabidiol) have several applications related to cancer treatment, anxiety, insomnia, and chronic pain among others. Hence, the most relevant trait of the plant is the quantity and relative abundance of THC and CBD it can produce, in other words its chemotype. Currently there is a simple genetic model that aims to explain the inheritance of the chemotype. This is a codominant model with two loci, one for the THC synthase (THCAS) and one for the CBD synthase (CBDAS). The loci comprise several paralogs tightly linked that get inherited, probably as a single haplotype. Only one of the paralogs of each synthase is expressed and thus the model is simplified to only two genes. The model is biallelic and assumes that there are functional and non-functional alleles for each synthase. Therefore, a plant with two, one and zero functional alleles of a particular synthase will produce a high, low, and null quantity of that cannabinoid respectively. However, this model fails to explain the differences in gene expression we have observed in our study. We have assembled the transcriptome of 19 different cannabis varieties, and we have compared both the gene expression level as well as the sequences of the synthases. The THCAS expression level is unable to discriminate the THC from the CBD chemotypes, unlike the CBDAS. Therefore, we propose that there must be some polymorphisms in *CIS* or *TRANS* regulatory elements, including promoters and transcription factors. These are independent of the polymorphisms in the coding region. Currently, we are validating the gene regulatory network of cannabis trichomes that we have inferred using different algorithms with Yeast One Hybrid assays. We will also retrieve and compare the promoter regions of CBDAS and THCAS from all 22 assembled genomes to identify potential sources of variation.

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P2-06 The unequal functional redundancy between the Arabidopsis ICU11 and CP2 putative PRC2 accessory proteins is not dependent on genetic background

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The Arabidopsis *INCURVATA11* (*ICU11*) and *CUPULIFORMIS2* (*CP2*) paralog genes encode components of the epigenetic machinery, which belong to the 2-oxoglutarate and Fe (II)-dependent dioxygenase superfamily (1). ICU11 is assumed to be a Polycomb Repressive Complex 2 (PRC2) accessory protein (2). We already studied the morphological and molecular phenotypes of *icu11* and *cp2* single mutants, and of *icu11 cp2* double mutants, with different and hybrid genetic backgrounds, respectively; their comparative analyses suggested unequal functional redundancy between *ICU11* and *CP2*. We isolated novel mutant alleles of *ICU11* in the Col-0 (*icu11-5* and *icu11-6*) and S96 (*icu11-4* and *icu11-7*) genetic backgrounds using CRISPR/Cas9 mutagenesis. We combined *icu11-5* and *icu11-6* with mutant alleles of *CP2* in the Col-0 background. The double mutants obtained exhibited the expected postembryonic lethal phenotype, similar to that of strong *embryonic flower* (*emf*) single mutants (3). These results confirm the previously proposed unequal functional redundancy between *ICU11* and *CP2*, as well as that it is not allele or genetic background specific. We also combined *icu11-5* and *cp2-3* null mutations with loss-of-function alleles of genes encoding known components of the epigenetic machinery. The double mutants involving *icu11-5* had synergistic phenotypes, while those involving *cp2-3* had additive phenotypes. Loss of function of *CP2* alone was not enough to modify the phenotypes caused by mutant alleles of other genes involved in epigenetic processes, except that of *ICU11* itself. We also found that increasing the sucrose content in the culture media partially rescued the early lethality of *icu11 cp2* double mutants, enabling us to study their morphological phenotype throughout their entire life cycle. We determined in this way that the ICU11-CP2 module is required for proper expression of flower organ identity genes.

(1) Mateo-Bonmatí *et al.* (2018). *Plant Cell* **30**, 1596-1616. (2) Bloomer *et al.* (2020). *Proc Natl Acad Sci USA* **117,** 16660-16666. (3) Goodrich *et al.* (1997). *Nature* **386,** 44-51.

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P2-07 Elucidating the function of the tomato *MARS1* gene encoding a LYSINE-SPECIFIC HISTONE DEMETHYLASE 1 (LSD1) protein during adventitious root formation

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Unlike what happens in animals, plants have a high regenerative capacity and under natural conditions they can form new organs and even complete individuals from a few cells present in adult tissues, either in response to injury or to the alteration of their environment. One of the most interesting mutants so far identified in our laboratory with alteration in wound-induced adventitious root (AR) formation is the so-called *more adventitious roots1-1* (*mars1-1*) mutant, which is affected in a tomato gene encoding a conserved lysine-specific histone demethylase which, in metazoans, regulates a diversity of processes such as cell proliferation, stem cell pluripotency and embryogenesis [1]. Two additional CRISPR/Cas9 null alleles, *mars1-2* and *mars1-3* also displayed the enhanced wound-induced AR phenotype. In addition, *mars1* fruits displayed a rough surface due to ectopic proliferation of subepidermal cells which form callus-like structures at the cuticle. We found high levels of H3K4me3 and H3K9me2 in *mars1* seedlings, suggesting that *SlLSD1* was required for demethylation of these two histone marks. Besides, we found increased levels of repressive H3K27me3 and of active H3K9/K27ac. These results indicate crosstalk between SlLSD1 function and other chromatin factors such as polycomb repressive complex 2 (PRC2) that requires further investigation. Finally, a time-course directional RNA-seq transcriptome analysis of protein coding genes and long non-coding RNAs (lncRNAs) in the *mars1* mutants suggest that LSD1 might be involved in the establishment and maintenance of silencing in specific genomic regions required for tissue-specific reprogramming.

1. Ambrosio at al. (2017). Biochimica et Biophysica Acta 1860, 905–910.

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P2-08 Evolutionary conservation of the Arabidopsis PHOSPHORYLATED ADAPTOR FOR RNA EXPORT (PHAX) function

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Small nuclear and nucleolar RNAs (snRNAs and snoRNAs) are non-coding RNA molecules that act as critical components of the ribonucleoprotein complexes required for pre-mRNA splicing and ribosomal RNA (rRNA) maturation, respectively. The assembly of these complexes involves intricate and specific pathways, including nucleocytoplasmic transport of snRNAs and intranuclear transport of snoRNAs. In metazoans, the Phosphorylated Adaptor for RNA Export (PHAX) protein binds single-stranded RNA in a sequence-unspecific manner, and is required for the nuclear export of some spliceosomal U snRNA precursors, as well as the intranuclear transport of the U3 snoRNA precursor from the nucleoplasm to Cajal bodies; U3 snoRNA is essential for 18S rRNA maturation (1). AT3G20430 encodes the putative Arabidopsis ortholog of metazoan PHAXs, and is co-expressed with *RIBOSOMAL RNA PROCESSING 7* (*RRP7*; 2) and *SMALL ORGAN 4* (*SMO4*; 3), which encode factors involved in 5.8S and 18S rRNA maturation, respectively. We obtained two insertional alleles disrupting the Arabidopsis *PHAX* gene in its second and third intron, which we dubbed *phax-1* and *phax-2*, respectively. Only 23.88% of *phax* seedlings produced leaves, and showed a pleiotropic phenotype, including dwarfism, early flowering and sterility. The remaining *phax* seedlings produced only chlorotic cotyledons (65.67%) or normal cotyledons and two leaves (10.45%). Leaves of the viable *phax* plants were pointed, as expected from mutants affected in genes involved in rRNA maturation. RNA gel blot analyses of 45S pre-rRNA processing revealed overaccumulation of several pre-rRNAs in the *phax* plants. We are also obtaining double mutant combinations of *phax-1* and *phax-2* with loss-of-function alleles of other genes involved in pre-mRNA splicing and rRNA maturation. Furthermore, we are generating transgenes to analyze the effects of *PHAX* overexpression, as well as to assess the subcellular localization of the PHAX protein, and to complement the phenotype of the *phax* mutants. Our preliminary results strongly suggest cross-kingdom evolutionary conservation of PHAX function.

1. Mourão *et al.* (2010). *RNA* **16**, 1205-1216.
2. Micol-Ponce *et al.* (2018). *Plant Cell* **30**, 2855-2872.
3. Micol-Ponce *et al.* (2020). *Plant Physiol.* **184**, 2022-2039.

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P2-09 Main genomic characteristic and gene expression patterns of *hox* genes in Senegalese sole (*Solea senegelensis,* Kaup 1858*)*

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The hox genes encode transcription factors and are involved in morphogenesis and cell differentiation, which for the vertebrates are linked to the development of anterior-posterior structure and limbs, during the embryonic development (1). The body plan of the flatfishes are more complex than other teleost, because they suffer drastic changes during the larval development, mainly caused by the metamorphosis (2), such as the Senegalese sole (*Solea senegalensis*, Kaup 1858), a flatfish with high aquaculture interest, which larval culture and rearing, has several problems by presenting of skeletal malformations, being an important bottleneck (3, 4), but now the knowledge about the influence of genetic factor is limited (5), therefore, the comprehension of the role of *hox* gene in the body plan would be able a key to resolved. Hence, in this work the application of genomic and bioinformatics technologies has allowed to understand the main characteristic of gene *hox* in this species, for instance, specific localization into their cytogenetic map, molecular organization, similarities in the organization and phylogenetic relationship, with other teleost fishes, especially with other flatfishes, accumulation of repetitive elements into the each cluster (6), and gene expression patterns through embryonic stage, larval phases and metamorphosis stages. We found interesting results highlighting the cluster *hoxba.* The global result of this research close up to understand physiology function in relation to the morphology of the Senegalese sole.

1 Brown et al. (2020). Georgia Journal of Science. 78, 1–12.
2 Gibson et al. (2015). Flatfishes: Biology and Exploitation. 74–166.
3 Padrós et al. (2011). Journal of Fish Biology. 79, 3–32.
4 Fernández et al. (2018). Aquaculture. 496, 153–165.
5 Muñoz-Cueto et al. (2019). The Biology of Sole. 216–252.
6 Mendizábal-Castillero et al. (2022). Animals 12, 3586.

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P2-10 Mapping-by-sequencing platform at IB-UMH

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The manipulation of next generation sequencing (NGS) datasets has become a need in many biological laboratories for many purposes. One such purpose is mapping-by-sequencing: the combined use of NGS with classical genetic mapping by linkage analysis to establish gene to phenotype relationships. At the Instituto de Bioingeniería of the Universidad Miguel Hernández (IB-UMH), we implemented a platform for mapping-by-sequencing, a technique that combines NGS with classical genetic mapping by linkage analysis. We also developed a bioinformatic tool for mapping-by-sequencing: Easymap (1, 2), a versatile and easy-to-use package that performs automated mapping of point mutations and small indels in bulked segregant populations. Easymap also maps large DNA insertions. Easymap v.2 includes additional workflows to perform QTL-seq and variant density mapping analyses (3). Each of these mapping workflows can accommodate different experimental designs, including outcrossing, backcrossing and recurrent selfing, the use of F2, M2, and M3 mapping populations, chemically-induced mutation and natural variant mapping, input files containing either single-end or paired-end reads, of genomic or complementary DNA sequences, and alternative control sample files in FASTQ or VCF formats. Easymap v.2 can be used as a variant analyzer in the absence of a mapping algorithm, and includes a multithreading option. We are using this tool to help researchers in Arabidopsis and other plant and animal model systems to identify causal mutations for phenotypes of interest. Easymap v.2 runs within UNIX environments and can be installed in Windows 10 within the Ubuntu apps available in Microsoft Store, in virtual machines running a Unix-based OS, and remotely in a computational cluster or the Amazon Elastic Compute Cloud service. Easymap v.2 is available at http://genetics.edu.umh.es/resources/easymap/ and an interactive preview of the user interface can be accessed at http://atlas.umh.es/easymapv2.

(1) Lup *et al*. (2021). *Front Plant Sci* **12**, 655286. (2) Lup *et al*. (2022). *Methods Mol Biol* **2484**, 343-361. (3) Lup *et al*. (2023). *Front Plant Sci* **14**, 1042913.

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P2-11 Morphometry of bilateral symmetry and margin complexity in Arabidopsis leaves

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The margin of Arabidopsis simple leaves is patterned by the positions of auxin maxima in leaf primordia margins, which are specified by the PIN-FORMED1 (PIN1) auxin efflux carrier, the CUP-SHAPED COTYLEDON2 (CUC2) transcription factor, and auxin itself. *cuc2-3* and other loss-of-function alleles of *CUC2* cause the absence of auxin maxima and protrusions, resulting in smooth leaf margins. On the contrary, *cuc2-1D* and other gain-of-function alleles lead to expanded CUC2 expression domains, resulting in extra lobes and sinuses (1). We recently proposed that cytokinins play a role in margin patterning of simple leaves, in crosstalk with auxin (2). Indeed, Arabidopsis mutants defective in cytokinin biosynthesis or signalling have smoother leaf margins than wild-type leaves. For example, the *ipt3 ipt5 ipt7* triple mutant, which carries null alleles of three of the genes encoding the isopentenyl transferases (IPT) that catalyze the first step of cytokinin biosynthesis, exhibits smooth leaf margins. Loss-of-function alleles of the Arabidopsis *VASCULATURE COMPLEXITY AND CONNECTIVITY* (*VCC*) gene cause bilateral symmetry breaking in rosette leaves, due to the random position, size and number of leaf margin protrusions (3). We are gathering evidence that suggest that VCC influences both auxin and cytokinin activity during leaf margin morphogenesis. Additionally, we identified components of the fatty acid elongase complex as potential interactors of the VCC protein, which synthesizes very-long-chain fatty acids (VLCFAs) that have been proposed to inhibit cytokinin biosynthesis (4). We also developed an automated method based on ImageJ software to calculate the normalized difference margin complexity (NDMC) index (5) and the leaf symmetry index (LSI) (3). This allows to quantify the visible differences in leaf margin complexity (number and sizes of protrusions) and the extent of bilateral leaf asymmetry among the mutants under study.

(1) Bilsborough *et al.* (2011). *Proc Natl Acad Sci USA* **108**, 3424-3429. (2) Navarro-Cartagena and Micol. (2023). *Trends Plant Sci* **28**, 54-73. (3) Wilson-Sánchez *et al.* (2018). *New Phytol* **217**, 1307-1321. (4) Nobusawa *et al.* (2013). *PLOS Biol* **11**, e1001531. (5) Leigh *et al.* (2017). *Plant Cell Environ* **40**, 237-248.

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P2-12 *EODL* synergistically interacts with *VCC* in Arabidopsis leaf margin morphogenesis

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The Arabidopsis *VASCULATURE COMPLEXITY AND CONNECTIVITY* (*VCC*) gene acts in vascular pattern formation in cotyledons (1, 2) and bilateral symmetry in leaves (3). To study the role of *VCC* in leaf morphogenesis, we crossed *vcc* mutants to mutants affected in auxin transport and cytokinin biosynthesis in leaf primordia development, as well as to wild-type accessions that show different levels of expanded leaf serration. Our findings indicate the presence of *ENHANCER OF DEAL* (*EODL*), a modifier gene that significantly increases the expressivity and penetrance of the leaf phenotype of the *vcc* mutants. The segregation of these synergistic phenotypes in the progeny of our crosses suggested that the *eodl* modifier alleles are dominant. Our mapping-by-sequencing analyses pointed to an 800-kb candidate region at chromosome 3, which contained 55 genes with polymorphisms predicted to change their protein sequences. We further compared the transcriptomic profiles of the *vcc-2* single mutant and putative *vcc-2 eodl* double mutants using RNA-seq analysis. We identified 57 genes within the candidate region that were deregulated in the putative double mutants, but not in *vcc-2* mutants. By combining our mapping-by-sequencing and RNA-seq data, we narrowed down the list of candidate genes to 21. These genes represent a promising starting point for identifying *EODL*, as the nature of the *eodl* alleles may affect either the expression levels or the protein sequence. The identification of *EODL* could provide insight into the mechanisms that regulate leaf bilateral symmetry and the roles played by auxin and cytokinins in leaf margin morphogenesis (4).

(1) Roschzttardtz *et al.* (2014). *Plant Physiol* **166**, 889-902. (2) Yanagisawa *et al.* (2021). *Development* **148**, dev197210. (3) Wilson-Sánchez *et al.* (2018). *New Phytol* **217**, 1307-1321. (4) Navarro-Cartagena and Micol (2023). *Trends Plant Sci* **28**, 54-73.

This work was supported by grants from the Ministerio de Ciencia e Innovación of Spain (MCI; PID2021-127725NB-I00, EQC2018-005181-P and EQC2019-006592-P [MCI/AEI/FEDER, UE]) and the Generalitat Valenciana (GV; PROMETEO/2019/117, IDIFEDER/2020/019 and IDIFEDER/2021/033) to JLM. RO-V, SDL and SN-C held predoctoral fellowships from the GV (CIGRIS/2021/153, ACIF/2018/005 and ACIF/2017/163, respectively). CN-Q held a Technical Support Staff fellowship from the MCI and the European Social Fund (PTA2021-020575-I).

P2-13 Identification of novel Arabidopsis *DEN* genes that interact with *AS1* and *AS2*

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The *ASYMMETRIC LEAVES 1* (*AS1*) and *AS2* genes contribute to the specification of the dorsoventral axis of Arabidopsis rosette leaves (1, 2). Mutations in several genes encoding ribosomal proteins render a mild, pointed-leaf morphological phenotype; genetic combination of *as1* and *as2* alleles with alleles of genes that encode ribosomal proteins result in synergistic phenotypes characterized by a severe loss of leaf dorsoventral polarity (3). These findings suggest a morphogenetic role of the ribosome or an extraribosomal morphogenetic function of ribosomal proteins. The *denticulata* (*den*) mutants have small, serrated and pointed leaves, which suggest that they harbour mutations in genes encoding components of the translational apparatus (4). Using mapping-by-sequencing (5), we identified the *den1*, *den4*, *den7*, *den9*, *den11*, *den14*, *den15*, *den16* and *den17* recessive, loss-of function mutations, all of which cause synergistic phenotypes in double mutant combinations with *as1* or *as2* alleles. While some of the *den* mutations were found to map at genes encoding ribosomal proteins, others affected factors belonging to the translation machinery that are not structural components of the ribosome, such *DEN14*, which encodes a methyltransferase-like protein involved in pre-rRNA processing. In contrast, *den17* was found to be an allele of *ATP-BINDING CASETTE I10* (*ABCI10*), which is likely involved in metal ion uptake in chloroplasts and does not appear to be related to translation (6).

(1) Pinon *et al*. (2008). *Development* **135**, 1315-1324. (2) Yao *et a*l. (2008). *Development* **135**, 1325-1334. (3) Horiguchi *et al*. (2011). *Plant J* **65**, 724-736. (4) Berná *et al*. (1999). *Genetics* **152**, 729-742. (5) Lup *et al.* (2021). *Front Plant Sci* **12**, 655286. (6) Voith von Voithenberg *et al*. (2019). *Front Plant Sci* **10**, 1264.

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P2-14 Uncovering regulators of ribosome biogenesis in Arabidopsis

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In eukaryotic ribosome biogenesis, 45S ribosomal DNA (rDNA) transcription and 45S ribosomal RNA (rRNA) precursor processing have to be coupled for maturation of rRNAs. These processes have to be synchronized in a balanced manner with the transcription of genes encoding ribosomal proteins (RPs) and ribosome biogenesis factors (RBFs), and with the synthesis of RPs and RBFs in the cytoplasm, as well as their import to the nucleus. The required regulatory mechanisms, which seem to be kingdom-specific and have been studied in yeast and humans, must also exist in plants but still have not been uncovered. In fact, most plant genes encoding RBFs are annotated as such in Arabidopsis only based on structural similarity with their putative yeast or human orthologs (reviewed in 1). We are studying the roles that DNA POLYMERASE V (POL5), U3 SMALL NUCLEOLAR RNA-ASSOCIATED PROTEIN 18 (UTP18), UTP22, RIBOSOMAL RNA PROCESSING 36 (RRP36), and PLANT RNA HELICASE 75 (PRH75) play in Arabidopsis. We considered these proteins candidate to be both RBFs and regulators of 45S rDNA transcription and to coordinate the synthesis of ribosome structural components, as their yeast and human orthologs do. We obtained insertional alleles of these genes and found that loss of function of *POL5*, *UTP18* and *UTP22* cause embryonic or gametophytic recessive lethality. As an alternative, we are obtaining hypomorphic alleles by CRISPR/Cas9 mutagenesis, as well as constructing transgenes expressing artificial microRNAs (amiRNAs) to partially silence the expression of *POL5*, *UTP18*, *UTP22* and *RRP36* genes.

1. Sáez-Vásquez and Delseny (2019). *Plant Cell* **31**, 1945-1967.

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P2-15 Functional conservation of Arabidopsis VENOSA4 (VEN4) and human SAMHD1 in DNA repair

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Imbalance in the deoxyribonucleoside triphosphate (dNTP) pool due to an increase or decrease in the levels of any of the four dNTPs leads to increased DNA mutations, overloading DNA repair mechanisms. The human Sterile alpha motif and histidine-aspartate domain containing protein 1 (SAMHD1) is a nuclear protein that functions as a dNTPase to secure dNTP homeostasis, as well as in DNA repair. A dNTPase-independent role for human SAMHD1 in DNA double strand break (DSB) repair by homologous recombination (HR) has been described (1). Arabidopsis VENOSA4 (VEN4) and *Oryza sativa* STRIPE3 (ST3) are the putative orthologs of SAMHD1 that have been studied in plants, both of which are related to chloroplast and leaf development, stress responses, and dNTP metabolism (2-5). VEN4 hydrolyzes dGTP to 2′-deoxyguanosine (2′-dG) in vitro and positively regulates plant immunity (6). We found that *VEN4* expression was induced by hydroxyurea, a known inhibitor of dNTP synthesis that blocks progression of DNA replication and generates single and double strand breaks (DSBs). The *ven4* loss-of-function mutants were hypersensitive to hydroxyurea, and showed reduced DSB repair by HR. A metabolomic analysis of the *ven4* mutants detected high levels of fumarate, which is known to be required for DNA damage response (DDR) to DSBs, caused by ionizing radiation or hydroxyurea. In the absence of genotoxic stress, the *ven4* mutants showed increased DNA ploidy levels, reduced replication of the chloroplast genome, and deregulation of genes involved in DNA repair, which are signs of DNA damage accumulation. Our results reveal a high degree of cross-kingdom functional conservation between Arabidopsis VEN4 and human SAMHD1 in DNA repair, in addition to that previously known in dNTP metabolism.

1. Coggins *et al.* (2020). *Viruses* **12**, 382-410.
2. Yoshida *et al.* (2018). *Plant Physiol.* **178**, 3-6.
3. Xu *et al.* (2020). *Plant Physiol.* **182**, 721-729.
4. Wang *et al.* (2022). *Plant Sci.* **323**, 111395.
5. Sarmiento-Mañús *et al.* (2023). *bioRχiv* doi: 2023.03.30.534755v1.
6. Lu et al. (2022). *J. Adv. Res.* **46**, 1-15.

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P2-16 Analysis of quantitative expression and its location in gonadal tissue of genes *cyp19a* and *amh* on different maturing stages in both F1 and wild individuals of the senegalese sole (*Solea senegalensis*)

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In the aquaculture of *Solea senegalensis*, efficient reproduction in captivity is still difficult to achieve; hence the characterization of many sex- and reproduction-related genes in this species is of considerable scientific and commercial interest (1). The expression patterns of two of the relevant genes, anti-müllerian hormone (*amh*) and cytochrome P-450 aromatase (*cyp19a*), have been studied. Both genes are involved in the reproduction process and the male-female ratio of resulting larvae (2, 3 and 4); this is important in aquaculture because females grow faster than males. This study was carried out with wild and cultivated males and females, in various stages of maturation. The expression patterns were studied by qPCR analysis to determine relative quantitative expression and using riboprobes in gonadal tissue (*in situ* hybridization). Different expression patterns were observed in gonadal tissue depending on the maturation stage; and the expression was categorised as strong or diffuse depending on the sex and origin of the sample (wild or cultivated). Quantitative expression also showed differences between wild and cultivated individuals. In general, both genes are expressed in males and females, but the expression pattern of *cyp19a* showed differences between mature and immature males, measured by sperm production. In females *cyp19a* expression disappears during maturation. In the case of the *amh* gene, we observed that expression accumulates in the cytoplasm of oocytes. The study report will also include an analysis of genomic data of the genes. We consider that the results presented in the paper should be useful for a wide range of professionals concerned with improving reproduction of *Solea senegalensis* and with the aquaculture of flat fishes in general, given the many important cultivated species belonging to this taxon.

1. Merlo, M. A., Portela-Bens, S., Rodriguez, M. E., Garda-Angulo, A., Cross, I., Arias-Perez, A., Garda, E., & Rebordinos, L. (2021). A comprehensive integrated genetic map of the complete karyotype of solea senegalensis (Kaup 1858). Genes, 12(1), 1–12. https://doi.org/10.3390/genes12010049.
2. Halm, S., Rocha, A., Miura, T., Prat, F., & Zanuy, S. (2007). Anti-Müllerian hormone (AMH/AMH) in the European sea bass: Its gene structure, regulatory elements, and the
expression of alternatively-spliced isoforms. *Gene*, *388*(1-2), 148-158.
3. Guiguen, Y., Baroiller, J. F., Ricordel, M. J., Iseki, K., McMeel, O. M., Martin, S. A., & Fostier, A. (1999). Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). Mol Reprod Dev, 541, 54–162.
4. Portela Bens, S., Merlo, M. A., Rodríguez, M. E., Cross, I., Manchado, M., Kosyakova, N.,
Liehr, T., & Rebordinos, L. (2017). Integrated gene mapping and synteny studies give insights into the evolution of a sex proto-chromosome in Solea senegalensis. *Chromosoma*, *126*(2), 261–277. https://doi.org/10.1007/s00412-016-0589-2

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P2-17 Postembryonic developmental roles of the Arabidopsis *KEU* gene

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Cytokinesis in plant cells begins in the center of the cell division plane with the fusion of vesicles that transport cell wall material. This process generates the cell plate, which expands radially until it fuses with the parental cell wall at the preprophase band. Vesicle fusion in this process occurs in three steps: loose interaction or tethering of two membranes, followed by closer interaction or docking, and finally, membrane fusion. The docking step is carried out by SNARE trans-complexes, assisted by Sec1/Munc18 (SM) proteins through their interaction with the Qa-SNARE component of the complexes. In plant cytokinesis, the SNARE protein KNOLLE and the SM protein KEULE play a central role in vesicle membrane fusion (1, 2). We conducted a genetic screen for mutations induced by ethyl methanesulfonate, which resulted in the isolation of the *serrata4-1* (*sea4-1*) and *sea4-2* mutants in Arabidopsis (3). Both mutants have serrated rosette leaves and were found to carry novel recessive, hypomorphic alleles of the *KEULE* gene, known for its essential role in plant embryonic development. Through mapping-by-sequencing (4), we discovered that *sea4-1* carries a point mutation at the splice donor site of its 9th intron, which leads to a mixture of truncated and wild-type protein isoforms. The *sea4-2* mutation is predicted to cause a S57L substitution in the SNARE interaction domain of KEULE. Homozygous *sea4-1* and *sea4-2* plants are viable and fertile, but they have smaller rosettes and fewer leaves at bolting time than the wild type. The leaves of these mutants are serrated, small, and undulated, have a complex venation pattern, develop necrotic patches, and undergo premature senescence. By studying the *sea4* mutants, we were able to examine the roles of *KEULE* in postembryonic development, particularly in rosette leaf whole organ and margin patterning.

(1) Assaad *et al.* (1996). *Mol Gen Genet* **253**, 267-277. (2) Assaad *et al.* (2001). *J Cell Biol* **152**, 531-543. (3) Berná *et al.* (1999). *Genetics* **152**, 729-742. (4) Lup *et al.* (2021). *Front Plant Sci* **12**, 655286.

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P2-18 Gene sequencing and protein structure prediction of *Grapholita molesta* Busk (Lepidoptera; Tortricidae) cadherin: a candidate receptor for *Bacillus thuringiensis* Berliner Cry1A proteins

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Cadherins are a big family of transmembrane proteins involved in physiological processes such as cell structural integrity, migration, and signaling. The structure of these proteins comprises 3 domains: extracellular, transmembrane, and intracellular. A variable number of the called "cadherin repeats" (CRs) are located at the extracellular domain (1). In lepidopteran species, midgut cadherins have an important role as receptors of the insecticidal Cry proteins. These Cry toxins are produced by the entomopathogenic bacterium *Bacillus thuringiensis* (2). In the most accepted Cry toxins mode of action model, the cadherin-like receptors promote the oligomerization of the proteins, a step prior to the insertion of these structures in the epithelial cell membrane, leading to the disruption of gut integrity. Resistance to Cry proteins in lepidopteran populations has been associated, among other causes, with the presence of mutations in the cadherin-like genes (1), highlighting the important role of these receptors in different pest species. The main objective of the present study was to identify the putative cadherin-like receptor of Cry1A proteins in the stone-fruit crop pest *Grapholita molesta* Busk (Lepidoptera; Tortricidae). The cadherin of *Ostrinia nubilalis* Hübner (Lepidoptera; Crambidae, Acc. Number: AAY44392.1) (3) was used as a template to mine the *G. molesta* genome. As a result, a mRNA of 4839 base pairs on the positive strand was found, formed by 29 exons, coding for a 1612 amino acids protein. To verify the presence of this gene in *G. molesta,* insect larvae guts were dissected and their cDNA was synthesized, used as a template in PCR, sequenced and assembled. Regarding its structure, the *G. molesta* cadherin comprises 11 CRs, a transmembrane region, and a small intracellular part. Despite the low amino acid sequence identity amongst *G. molesta* and *O. nubilalis* cadherins (21%), the *in silico* 3-d structure showed highly similar conformations. This study describes for the first time the Cry1A cadherin-like candidate receptor in *G. molesta,* which will help in the study of the mode of action of Cry proteins and would provide tools for the early detection of resistance outbreaks.

1. Fabrick & Wu YiDong (2003). In: *Bt resistance: characterization and strategies for GM crops producing Bacillus thuringiensis toxins.* CABI Biotechnology Series.
2. Whalon & Wingerd (2003). Archives of Insect Biochemistry and Physiology 54, 200-211
3. Bel & Escriche (2006). Gene, 71-80.

This work was supported by EU grant No 861852, PROMETEO 2020/010, and PID2021-1229140B-100. D.T. was supported by MCIN PRE2019-089628 FPI grant.

P2-19 Subcellular localization of the Arabidopsis VCC protein and its interaction with PIN1

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The Arabidopsis *VASCULATURE COMPLEXITY AND CONNECTIVITY* (*VCC*) gene is involved in venation pattern formation in cotyledons (1) and bilateral symmetry in rosette leaves (2). Plants homozygous for *vcc* loss-of-function alleles show strongly reduced complexity of cotyledon venation pattern, leading to open areoles, free-ending veins, and other disconnections. The margins of *vcc* rosette leaves exhibit bilaterally asymmetric lobes. We found that the *$35S_{pro}$:VCC:CFP* transgene partially suppresses the defects on cotyledon venation patterning shown by the *vcc-2* mutant. The polarization of the auxin efflux carrier PIN-FORMED1 (PIN1) within the cell membrane contributes to auxin flow directionality and canalization, which in turn contributes to vascular cell fate specification (3). We already reported that PIN1 polarization is not altered in *vcc-1* leaf development (2), but a recent study showed that VCC colocalizes with and is required for PIN1 stability and polarization in cotyledon venation patterning (4). We found that PIN1 is properly polarized towards the root apex in *vcc-1* plants, suggesting that *VCC* loss of function does not perturb auxin flow in Arabidopsis roots. We previously found that the VCC protein localizes to the endoplasmic reticulum (1), but our transgenes and those of other authors did not fully restore the wild-type phenotype in the *vcc* mutants (1, 2, 4), hindering the proposal of robust conclusions. We re-examined the subcellular localization of VCC in *vcc-2 $35S_{pro}$:VCC:CFP* roots, and found that VCC does not colocalize with an endoplasmic reticulum marker. We are currently designing new transgenes that will allow a more precise subcellular localization of VCC, as well as re-analyzing the effect of *vcc* mutations on PIN1 polarization during leaf development.

(1) Roschzttardtz *et al*. (2014). *Plant Physiol* **166**, 889-902. (2) Wilson-Sánchez *et al*. (2018). *New Phytol* **217**, 1307-1321. (3) Ravichandran *et al*. (2020). *New Phytol* **227**, 1051-1059. (4) Yanagisawa *et al*. (2021). *Development* **148**, dev197210.

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P2-20 Subcellular localization of Arabidopsis ribosome biogenesis factors

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The ribosome is an essential supramolecular complex and its biogenesis is tightly regulated and consumes most of the energy resources of any cell. We are studying several Arabidopsis genes encoding putative ribosome biogenesis factors (RBFs): PLANT RNA HELICASE 75 (PRH75), U3 SMALL NUCLEOLAR RNA-ASSOCIATED PROTEIN 18 (UTP18), RIBOSOMAL RNA PROCESSING 36 (RRP36) and POLYMERASE V (POL5). The *Saccharomyces cerevisiae* likely orthologs of Arabidopsis UTP18, RRP36 and POL5 are part of the Small Subunit (SSU) processome, a large ribonucleoprotein complex that builds the 40S ribosomal subunit. Yeast Utp18 interacts with mRNA transport 4 (Mtr4), an RNA helicase that recruits the exosome to the 5′-ETS of 35S pre-rRNA (the primary transcript of the 35S rDNA genes), for its degradation (1). Yeast Rrp36 and Pol5 are regulators of 35S rDNA transcription (2,3). Although PRH75 is known to participate in 18S rRNA maturation (4), no functional studies have been published on UTP18, RRP36 and POL5. To determine the subcellular localization of these putative Arabidopsis RBFs, we constructed transgenes containing translational fusions of these genes and the GFP gene, driven by their endogenous promoters. We transferred the transgenes to *POL5/pol5-1*, *UTP18/utp18-1* and *prh75/prh75* plants and their respective wild types. The *PRH75*$_{pro}$*:PRH75:GFP* transgene did not rescue the morphological defects of the *prh75* mutant. However, we obtained viable, partially rescued *utp18-1/utp18-1 UTP18*$_{pro}$*UTP18:GFP* plants, which will be useful for the functional analysis of UTP18. We will determine the subcellular localization of the RBFs under study by Stimulated Emission Depletion (STED) nanoscopy analysis.

1. Vanden *et al*. (2022). *Curr. Opin. Struct. Biol.* **73**, 102331.
2. Gérus *et al*. (2010). *Mol. Cell. Biol.* **30**, 1130-1144.
3. Nadeem *et al*. (2006). *Mol. Genet. Genom.* **276**, 391-401.
4. Chun-Kai *et al*. (2016). *Plant Cell Physiol.* **47**, 174-191.

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SESIÓN 3: GENÉTICA DE MICROORGANISMOS

Ponencia invitada:

I3-01 Differences in vertical and horizontal transmission dynamics shape plasmid distribution in clinical enterobacteria

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Conjugative plasmids can transfer both vertically and horizontally in bacterial communities, playing a key role in the dissemination of antimicrobial resistance (AMR) genes across bacterial pathogens. AMR plasmids are widespread in clinical settings, but their distribution is not random, and certain associations between plasmids and bacterial clones are particularly successful. However, knowledge remains limited about the contribution made by vertical and horizontal transmission dynamics to plasmid distribution and maintenance in clinically relevant bacterial communities. In this study, we used a collection of wild type enterobacterial strains isolated from hospitalized patients to perform a comprehensive analysis of the transmission dynamics of the globally spread carbapenem resistance plasmid pOXA-48. We combined in vitro and in vivo experimental approaches to quantify key traits responsible for vertical (the level of AMR) and horizontal (conjugation frequency) plasmid transmission. Our results reveal significant variability in these traits across different bacterial hosts, with Klebsiella spp. strains showing higher pOXA-48-mediated AMR and conjugation frequencies than Escherichia coli strains. Using experimentally determined parameters, we developed a simple mathematical model to interrogate the contribution of vertical and horizontal transmission to plasmid distribution in bacterial communities. These simulations revealed that a small subset of clones, combining high vertical and horizontal plasmid transmission ability, play a critical role in stabilizing the plasmid in different polyclonal microbial communities. Our results indicate that strain-specific differences in plasmid transmission dynamics dictate successful associations between plasmids and bacterial clones, shaping AMR evolution.

Comunicaciones orales:

O3-01 Metagenomic data are compositional. The problem and its solution

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Metagenomic data are compositional data (i.e., non-negative variables expressed as proportions that add up to 1). Spurious correlations appear in compositional data. Furthermore, by extracting a subset of variables and re-expressing them as proportions, the relationships between the variables change and the correlations between them are no longer the same as in the original database; there is no *subcompositional coherence*. To avoid these problems, compositional data are analyzed as ratios (or as logarithm of the ratio to avoid percentages). With p compositional variables, the "full space" is defined by all $p(p-1)/2$ log-ratios, but since there are a huge number of variables, subsets of fewer dimensions are used: ALR, where variables are represented with respect to a reference variable: $\log(x_i / x_{REF})$; CLR, that uses the geometric mean G of the variables as a reference $\log(x_i/G)$; and ILR, that uses geometric means of subsets of variables $\log(Gi/Gk)$. CLR is isometric with respect to the "whole space", it preserves the distances between variables, but it does not have subcompositional coherence because each subset of variables has a different G. ILR is isometric and has subcompositional coherence, but it is very difficult to interpret. ALR it is not isometric and the result depends on the x_{REF}, but selecting an x_{REF} having very little variability between samples, comparing two $\log(x_i/x_{REF})$ is like comparing two $\log(x_i)$, somewhat easy to interpret; plus spurious correlations almost disappear (G of the CLR is much more variable). However, although ALR is not isometric, when choosing a x_{REF} with low variability, ALR is almost isometric. We have shown this by projecting the reduced space of the ALR on the "whole space", using Procrustes analysis, and showing that the correlation between the coordinates in both spaces is close to 1. In metagenomics, there are such a large number of variables that it is easy to find for x_{REF} a variable with low variability and high Procrustes correlation with the "whole space". We have taken several metagenomic databases, and we have found several possible x_{REF} allowing us to work in an approximately isometric space, but with variables that are much easier to interpret.

O3-02 New extracytoplasmic function (ECF) sigma/anti-sigma pairs involved in the complex *Myxococcus xanthus* response to copper

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In *Myxococcus xanthus*, photoexcitation of protoporphyrin IX generates highly reactive singlet oxygen ($^1O_2$) that, together with plasmalogen lipids, causes inactivation of the membrane-bound anti-sigma factor CarR by a still elusive mechanism to free the cognate extracytoplasmic function (ECF) sigma factor CarQ, thus enabling transcription of genes for carotenogenesis (1,2). Remarkably, in a manner that is independent of light and plasmalogens, the CarQ-CarR pair is also able to respond to copper, an important cofactor for redox active cuproenzymes, yet very toxic when in excess due to ROS formation and mismetallation. Our global transcriptomic analyses to decipher how CarQ-CarR senses light-induced $^1O_2$, and independently copper, reveals the latter to be even more intricate than previously reported (3), with a regulon comprising at least three other ECFsigma factors (*M. xanthus* has ~45 in total; 4). One of the three pairs has been studied in detail, as it presents several features that resemble the CarQ-CarR pair. In-depth analysis of this new ECFsigma/anti-sigma pair can provide insights on why it responds only to copper, whereas CarR can sense both light and copper. We will present our findings in this regard.

1. Pérez-Castaño et al. (2022). Environ Microbiol 24, 1865-1886.
2. Gallego-García et al. (2019). Science 366, 128-132.
3. Pérez J et al. (2018). Metallomics 10, 876-886.
4. Abellón-Ruiz et al. (2014). Environ Microbiol 16, 2475-2490.

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O3-03 Regulated expression of a CRISPR-Cas defense island and its role against attack by myxophages

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CRISPR (clustered regularly interspaced short palindromic repeats) genetic loci and their CRISPR-associated (*cas*) genes are adaptive, small RNA-guided 'immune' defense systems in bacteria and archaea that combat bacteriophages and invading genetic elements. Expression of CRISPR-Cas systems is a prerequisite for their defense functions, but much is unknown about how this crucial step is regulated and coordinated with sensing of the invasion signal. The bacterium *Myxococcus xanthus* has three of such CRISPR-Cas systems: a type I-C (CRISPR1-Cas), a type I-A (CRISPR2/3-Cas) and a type III-B (CRISPR4-Cas). We showed that the extracytoplasmic function (ECF) sigma factor DdvS and its cognate membrane-bound associated anti-sigma DdvA, together with a global regulatory complex, exert a novel multifactorial control on the expression of all the components of the CRISPR4-Cas system (1). Besides the CRISPR4-Cas system, the DdvS regulon comprises: the *ddvS-ddvA* gene pair itself and six genes that constitute two putative types of the newly identified cyclic oligonucleotide-based antiphage signaling systems (2). All these genes cluster in a large genomic segment, a so-called defense island, with four DdvS-dependent promoters and are expressed as a single polycistronic transcript. The use of an ECF-sigma/anti-sigma pair is an effective mechanism to coordinate signal sensing with controlled transcription of a CRISPR-Cas defense island. We have been investigating the role of this defense island against attack by known myxophages, as well as the molecular and structural basis of how DdvS and its anti-sigma DdvA regulate the expression of this CRISPR-Cas defense island. The findings from our ongoing studies will be presented.

1. Bernal-Bernal et al. (2018) Nucleic Acids Res 46, 6726-6745.
2. Millman et al. (2020). Nat Microbiol 5, 1608-1615.

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O3-04 Changes in the microbiota across life in a healthy Mediterranean cohort

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The human gut microbiota influences the health of its host. A good part, the mutualistic part, does have a beneficial role in developing the immune system, preventing infections, acquiring nutrients, and, probably, properly functioning the brain and nervous system. However, environmental factors can also alter the development of microbiota. Dysbiosis of the microbiota, conversely, consists of a relevant alteration of its composition and function compared to healthy people. Defining what constitutes a healthy microbiota makes it quite challenging, and today this remains a question that requires further research. Here, we have studied the intestinal microbiota of three age groups of healthy infants, adults, and the elderly of a Mediterranean population, from which we have periodically obtained stool samples and on which we have carried out the determination of 16S rRNA gene, metagenomes (MG), metatranscriptomes (MT). We have observed that the microbiota's stability differs with age; it is more unstable in infants than adults and elderly people. Regarding the analyses of the conserved microbiota across studied periods of the three groups, it is about 60%. In addition, we identified a core of microbial taxa that are, in our view, true mutualistic symbionts that are universally present in all three age groups studied; moreover, consequently, we found the presence throughout the host life of the Mediterranean population of bacterial genera that presumably have co-evolved with the human species, given support to the existence of a phylogenetic core of symbionts. MG and MT analyses showed a relatively lower number of significant differences between infants and adults (MG: 16 functions and 17 pathways in DESeq2 tests, and MT: 2 and 6, respectively) compared to that between adults and elders (MG: 20 functions and 42 pathways, and MT: 5 and 1, respectively) and especially in the comparison between the most extreme ages; that is, between infants and elders (MG: 57 functions and 92 pathways, and MT: 4 and 2, respectively). Overall, many of them are related to the aging process.

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Comunicaciones Flash Talk y póster:

FP3-01 Calculating genomic signature distances between phages and their bacterial host for distinguishing lytic and lysogenic phages

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Abstract.
Environmental impact of uncultured phages is shaped by their preferred life cycle (lytic or lysogenic), however, our ability to predict it is very limited. In recent years several studies have shown that Homology-free methods (genomic signature) can be useful for the classification of viral genomes (1) and for characterizing bacteriophages by comparing their genomic signature with that of their hosts to obtain host-phage relationships and determine their lifestyle (2). In this communication we present two approaches to discriminate lysogenic and lytic phages based on the comparison of the similarity of their genomic signatures with those of their hosts which may reflect their co-evolution; a) the Euclidean distance between the relative frequencies of short length k-mers, in our case k = 4 (k4freq) and b) alignment-free comparison based on exact k = 14 oligonucleotide matches (k14exact). In the first stage, we worked with a set of 5126 reference host strains, which had assigned phages in the NCBI Virus RefSeq database. In order to explore their genetic diversity, we characterized these strains by their genomic signature and clustered according to their similarity. Afterwards, the k4freq distance and k14exact similarity of each host to the 284 well-characterized phages was calculated. Our results suggest that there is an approximate threshold for distinguishing lysogenic and lytic phages by both oligonucleotide-based methods. The knowledge of the phage life cycle is very important for the interpretation of phage-bacteria links obtained by metagenomic studies, in which the bacteria cannot be cultured. For that reason, our two genomic signature based approaches have a large potential to be utilized in a variety of studies, ranging from biomedical to environmental research.

(1) Pride DT et al. (2006) Evidence of host-virus co-evolution in tetranucleotide usage patterns of bacteriophages and eukaryotic viruses. BMC Genomics 7, 8.
(2) Deschavanne P et al. (2010) The use of genomic signature distance between bacteriophages and their hosts displays evolutionary relationships and phage growth cycle determination. Virol J 7, 163 .

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FP3-02 Molecular characterization of *Wolbachia w*Mel in native populations of *Drosophila* for its application in biocontrol of the tiger mosquito in Valencia

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Biological control of insect pests has spread in recent times as an alternative to the use of insecticides. In this context, *Wolbachia pipientis*, an endosymbiotic alphaproteobacteria widely distributed among terrestrial arthropods, has been integrated into various vector control programs for mosquito management, taking advantage of the death of the embryos caused by cytoplasmic incompatibility between the gametes of parentals infected with strains obtained from different species (Incompatible Insect Technique, ITT) (1). Thus, the *w*Mel strain, isolated from *Drosophila melanogaster*, has been shown to have a blocking effect on fertility when male tiger mosquitoes *Aedes albopictus* that have been infected with this strain mate with females carrying the common *Wolbachia* strains in this species (*w*AlbA and *w*AlbB) (2). In this study we present the molecular characterization of *Wolbachia* from natural populations of *Drosophila melanogaster* captured in Requena (Valencia), and isolines obtained from the above. The ultimate goal is to identify the most suitable autochthonous strains to explore the effects of their introduction into males of *Ae. albopictus* previously cured of *Wolbachia*. Their controlled release would allow these males to mate with females from the natural population of tiger mosquitoes in the city of Valencia, which would be useful for reducing the population of this mosquito species, capable of transmitting up to 22 infectious diseases, including some caused by highly relevant arboviruses, such as Chikungunya, Dengue or Zika (3).

References:

1. Sicard et al. (2019). Curr Opin Insect Sci 34, 12-20. DOI: 10.1016/j.cois.2019.02.005
2. Blagrove et al. (2012). Proc Natl Acad Sci USA 109, 255-260. DOI: 10.1073/pnas.1112021108
3. Bueno-Marí et al. (2023). Rev Esp Salud Pública 2023, e202303017.

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FP3-03 Maternal microbial vertical transfer, before or after birth?

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Traditionally, the development of the human microbiome was considered to start at birth when the newborn comes into contact with the microbiota of the mother. From birth, the gut microbiota (GM) participates in important physiological functions. Therefore, microbial colonization in early life is a critical event which not only affects the health of newborns, but also is profound to long-term health. However, research carried out on the placenta, amniotic fluid and umbilical cord blood confirm that the first contact of the human being with the microbial environment is prior to the moment of birth, colonizing in the maternal womb. The mother's lifestyle as well as the mother's weight or the state of her GM, are key to the state of health of the newborn. For that, we collected rectal scraping samples from 67 mother and newborns pairings and vaginal swab samples of the mothers and we sequenced the 16S rRNA gene by *Illumina* technology. Only two mothers had normal weight, all others presented body mass indexes ≥ 25 (20 were overweight and 45 obese) and all were residents in South American countries. Interesting results showed that the study of beta diversity by ANCOM-BC analysis revealed that the abundance of pathogenic genera such as *Escherichia/Shigella* or *Enterococcus* were significantly higher in newborns. However, it is also remarkably that *Bifidobacterium*, a genus with beneficial properties, did not show statistical differences between mothers and newborns in rectal samples. These results, that are in concordance with other studies, could be interpreted as a prove of the maternal microbial vertical-transference and also about the crucial role that *Bifidobacterium* plays in the first stages of life. We hypothesize it might promote the development of infant-acquired immunity by protecting babies from these pathogens. On the other hand, the genus *Prevotella* in newborns was always detected in correlation with the presence of this genus in the mother's vaginal and rectal samples at the same time, or only in the rectal sample. Summarising, these preliminary results suggest once again that the first contact with the mother's GM happens previously to the child delivery.

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FP3-04 Exploring the Common Human Gut Eukaryotic Microbiota around the World

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In recent years numerous large-scale metagenomic studies have been carried out to characterise the complex consortium of microorganisms that constitute the human gut microbiome (1). Most meta-studies have focused primarily on the Bacterial component of the microbiome, generally leaving aside the role of eukaryotes in the human gut. In addition, most of the studies we can find are based on metabarcoding metagenomics (2, 3, 4), with very few meta-studies based on whole genome shotgun (5). However, microeukaryotes have a major impact on the physiology, host lifestyle and also on the diversity and composition of the wider symbiotic community of bacteria and viruses (6), and this should provide future motivation to investigate them. This study aims to explore the microbial eukaryotic core (a group of microbial eukaryotic taxa shared by most humans) across different human populations worldwide. To this end, a comprehensive collection of around 30 studies and meta-studies has been collected, including Human Microbiome Project and MetaHIT. In addition, we emphasise the use of novel pipelines: 1) Meta-Omics Dataset Curation Toolkit (developed by the group itself) a workflow designed to facilitate, accompany and guide the researcher during the manual curation process of metadata and FASTQ files associated with research projects hosted in the European Nucleotide Archive (ENA); and 2) EukDetect a state-of-the-art eukaryotic taxonomic profiler engineered to work with metagenomic whole genome shotgun data employing eukaryotic gene markers (7). Our study aims to provide some insights into the common eukaryotic members of the human gut microbiota across a large span of different human populations around the world.

1. Scholz et al. (2016). Nature methods, 13(5), 435-438.
2. Parfrey et al. (2014). Frontiers in microbiology, 5, 298.
3. Tito et al. (2019). Gut, 68(7), 1180-1189.
4. Scanlan & Marchesi (2008). The ISME journal, 2(12), 1183-1193.
5. Beghini et al. (2017). The ISME journal, 11(12), 2848-2863.
6. del Campo et al. (2020). Functional Ecology, 34(10), 2045-2054.
7. Lind & Pollard (2021). Microbiome, 9(1), 1-18.

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FP3-05 Genomic analysis of lytic myxophages and identification of *Myxococcus xanthus* genes conferring resistance

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The discovery and studies of CRISPR-Cas systems have spurred a resurgence in the identification and characterization of the many defense and resistance mechanisms employed by bacteria against bacteriophages. We have been investigating *M. xanthus* CRISPR-Cas systems (it has three of such systems; 1) and resistance to specific myxobacterial phages or myxophages. The availability of myxophages and their genomes is indispensable for such studies, but only about four myxophages have been isolated thus far (2), and the genome for just one of these was known until very recently. We have now sequenced two of the available lytic myxophages, Mx1 and Mx4, and an independent Mx4 genome announcement was made recently (3). Here, we will present an overview of our data focused on Mx1, together with our ongoing isolation of Mx1-resistant *M. xanthus* strains, identification of genes implicated in the resistance, and their analysis. This has required sequencing and assembly of the previously unavailable genome of the host strain DK1050, used in most studies in our group (4).

1. Bernal-Bernal D et al. (2018). Nucleic Acids Res 46, 6726-6745.
2. Vasse M, Wielgoss S. (2018). Viruses 10, 374.
3. Wielgoss S. (2021). Microbiol. Resour. Announc.10, e0095321.
4. López-Rojo et al. (2023). Microbiol. Resour. Announc. 12, 10.1128/mra.01020-22.

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FP3-06 Role of methionine sulfoxide reductases in the *Myxococcus xanthus* light response

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In the Gram-negative soil bacterium *Myxococcus xanthus*, blue light triggers carotenogenesis as a photoprotective response. Delving into how *M. xanthus* responds to light uncovered two parallel and novel light sensing and signaling mechanisms (1,2). In the more complex pathway, photoexcitation of protoporphyrin IX generates highly reactive singlet oxygen that, together with plasmalogen lipids, causes inactivation of the membrane-bound anti-σ factor CarR to free the cognate extracytoplasmic function sigma factor CarQ, thus enabling transcription of genes for carotenogenesis (1,2). Our transcriptomic analyses of the global response to light in *M. xanthus* has revealed that the CarQ-CarR regulon includes, besides genes involved in carotenogenesis, two putative periplasmic methionine sulfoxide reductases (or Msr): MXAN_6864, a bifunctional thiol-based MsrBA, and MXAN_6048/6047, a molybdenum-dependent MsrP/MsrQ system, both of which act on methionine sulfoxide S and R diastereoisomers. Chromatin immunoprecipitation and RT-qPCR confirmed that CarQ is required for and directly drives photoinducible expression of MXAN_6864 as well as MXAN_6048/6047. In most domains of life, Msr systems repair oxidized methionines and play central roles in protein quality control, oxidative stress resistance, and cell signaling (3). Thus, to probe the importance of the two Msr repair systems in the *M. xanthus* response to photooxidative/oxidative stress, we have deleted each system individually or together in a wild-type genetic background or in one with a transposon insertion at the carotenogenic operon to block photoprotection by carotenoids. Additionally, we have examined subcellular localization of both Msr systems, and performed bioinformatic analyses to detect possible periplasmic targets of methionine oxidation. To gain a broader vision of the evolutionary expanse of the *M. xanthus* light response, we have used comparative genomics to examine how the two Msr systems and their regulation by CarQ are conserved across myxobacteria.

5. Padmanabhan et al. (2021). Microorganisms 9, 1067.
6. Gallego-García et al. (2019). Science 366, 128-132.
7. Ezraty J et al. (2017). Nat. Rev. Microbiol. 15, 385–396.

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Comunicaciones póster:

P3-01 Microbiota changes associated with frailty in older adults

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Until recently, aging has been handled regarding longevity and life expectancy, however, it need not always be accompanied by a rise in healthspan. Frailty is an aging-associated geriatric syndrome characterized by losing muscle mass and strength, reduced endurance, decreased physiological reserves, and cognitive impairment (1). It is estimated that between 4 and 27% of elders suffer from frailty (2), being more prone to experience a series of clinical adverse events usually associated with a lower quality of life and a higher risk of death, hospitalization and institutionalization. During aging, the gut microbiome, also referred to as "the second genome", changes, and the presence of certain bacterial taxa and their associated metabolic functions could influence the health status and trigger physiological disorders (3). In the present study, we established a sub-cohort of 163 elder volunteers from the Seniors-ENRICA-2 cohort classified into frailty and non-frailty groups following the Rockwood frailty scale (4). We conducted a microbiome study of stool samples where the 16S rRNA gene and metagenomes were sequenced on an Illumina system. Significant differences were obtained when comparing the compositional bacterial genera between frailty and non-frailty groups. Beta diversity analyses at the genus level also showed statistically significant differences between both groups (CCA p.value=0.001). Furthermore, gender-associated differences were also observed in taxonomy. Such differences were even higher for orthologs genes (KOs) and KEGG metabolic pathways from metagenomes. While 761 KOs and 74 pathways were found statistically different between frailty and non-frailty men, only 3 KOs and one pathway showed differences comparing frailty and non-frailty women. In addition, compositional and functional differences were found between frailty men and women, but none between the non-frailty ones. These preliminary results suggest that frailty syndrome affects the microbiota differently depending on the gender, being greater once they are frailty, providing clues on putative-specific interventions.

1. Xu et al. (2021). Front. Cell. Infect. Microbiol. 11:696186.
2. Khan et al. (2019). Anesthesiol Clin. 37(3), 453-474.
3. Guo et al. (2022). Front. in Med. 9:827174.
4. Rockwood et al. (2005). CMAJ 173(5), 489-495.

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P3-02 Plasmalogen biosynthesis in the bacterium *Myxococcus xanthus*: parallels and variations with the mammalian pathway

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Plasmalogens are glycerophospholipids with a vinyl ether bond at the *sn*-1 position of the glycerol backbone (instead of the common ester bond), which are found exclusively in animals, some anaerobic bacteria and, among aerobic bacteria, in *Myxococcus xanthus* and closely related species. These lipids affect membrane fluidity and function, and have a proposed antioxidant role because the vinyl ether bond is prone to cleavage by reactive oxygen species. Human brain, heart and leukocytes are rich in plasmalogens, which occur in all subcellular membranes, and their deficiency or anomalous levels correlate with various disorders including cancer and Alzheimer's disease (1). The final step in their biosynthesis requires introduction of the characteristic vinyl bond into precursor ether lipids by plasmanyl-ethanolamine desaturase (PEDS1), whose oxygen-dependent activity in mammals was reported five decades ago. Nevertheless, the exact identity of PEDS1 remained unknown until our studies established that it corresponds to CarF in *M. xanthus* and to homologues called TMEM189 in animals (2,3). Whereas the pathway for plasmalogen biosynthesis in mammals, starting in the peroxisome and culminating in the endoplasmic reticulum where PEDS1 acts, is well-characterized, that in *M. xanthus* remains largely uncharted. Strikingly, our studies demonstrate that two parallel routes for plasmalogen biosynthesis, both converging at CarF, have evolved in *M. xanthus*. One depends on the multifunctional enzyme ElbD and the other on MXAN_1676, which shares a remarkable 46% sequence identity with the human enzyme that first introduces the ether bond in the peroxisome (2,4). Neither enzyme, however, suffices to generate the ether lipid precursor. We will present our ongoing genetic analyses to identify the other genes required and map the pathways in *M. xanthus*, and draw parallels as well as variations from that in mammals.

8. Braverman & Moser. (2012). Biochim Biophys Acta 1822, 1442-1452.
9. Gallego-García et al. (2019). Science 366, 128-132.
10. Padmanabhan et al. (2022). Front Cell Dev Biol 10, 884689.
11. Lorenzen et al. (2014). Nat Chem Biol 10, 425-427.

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P3-03 Trends of genome's complexity metrics in endosymbiont organisms and their free-living counterparts

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In the search for the assessment of a genome's complexity and its comparison between organisms, several complexity metrics have been proposed by the literature in recent years. Genomic Signature[1] (GS) is a k-mer-based metric, the value corresponding to the k-mer that maximises the difference between observed and expected equifrequent classes of mers. This metric is based on the relative abundances of short oligonucleotides and chaos game representation applied to genomes. BioBit[2] is also a k-mer-based metric based on the difference between the maximum entropy for a k-mer of a random genome of the same length as the genome under consideration and the entropy of that genome for such a k-mer. Finally, Sequence Compositional Complexity[3] (SCC) is another metric that increases with the number of parts (i.e., compositional domains) and the length and compositional differences found in a genome sequence by a segmentation algorithm, paralleling the concept of 'pure complexity' of McShea and Brandon. In this study, we measured those metrics in the context of a regressive evolutionary event in terms of complexity, the endosymbiosis, where we expect they show decreasing trends if they show complexity. For that, we selected a set of endosymbionts and also included some of their free-living counterparts to analyse the results within the context of the process of endosymbiosis. We observe a disparity in the trends of the metrics and provide an explanation to justify said disparity by studying the mathematical background of each metric and their biological implications in the case of study.

1. Moya, A., Oliver, J.L., Verdú, M. et al. Driven progressive evolution of genome sequence complexity in Cyanobacteria. Sci Rep 10, 19073 (2020). https://doi.org/10.1038/s41598-020-76014-4
2. Bonnici, V. and Manca, V. (2016) 'Informational laws of genome structures', Scientific reports, 6, p. 28840. https://doi.org/10.1038/srep28840
3.Román-Roldán, R., Bernaola-Galván, P. and Oliver, J.L. (1998) 'Sequence Compositional Complexity of DNA through an Entropic Segmentation Method', Physical Review Letters, pp. 1344–1347. https://doi.org/10.1103/physrevlett.80.1344

P3-04 MetRef, an auto-updatable database for reference / representative genomes and their complexity metrics

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In the search for the assessment of a genome's complexity and its comparison between organisms, several complexity metrics have been proposed by the literature in recent years. Genomic Signature[1] (GS) is a k-mer-based metric, the value corresponding to the k-mer that maximises the difference between observed and expected equifrequent classes of mers. This metric is based on the relative abundances of short oligonucleotides and chaos game representation applied to genomes. BioBit[2] is also a k-mer-based metric based on the difference between the maximum entropy for a k-mer of a random genome of the same length as the genome under consideration and the entropy of that genome for such a k-mer. Finally, Sequence Compositional Complexity[3] (SCC) is another metric that increases with the number of parts (i.e., compositional domains) and the length and compositional differences found in a genome sequence by a segmentation algorithm, paralleling the concept of 'pure complexity' of McShea and Brandon. The calculation of some of these metrics, specially in long genomes, can be quite resource demanding and time consuming. We present MetRef as a project to facilitate the study of reference and representative genomes within the context of the study of complexity. By retreiving the contents of RefSeq and with an auto-upgradable nature, we provide a framework so that groups working with these metrics can always rely on an updated database of reference genomes to use as support to their specific studies. The calculation of each of the metrics has been streamlined, resource and time-wise, in order to facilitate the update process of each version of the database.

1. Moya, A., Oliver, J.L., Verdú, M. et al. Driven progressive evolution of genome sequence complexity in Cyanobacteria. Sci Rep 10, 19073 (2020). https://doi.org/10.1038/s41598-020-76014-4
2. Bonnici, V. and Manca, V. (2016) 'Informational laws of genome structures', Scientific reports, 6, p. 28840. https://doi.org/10.1038/srep28840
3.Román-Roldán, R., Bernaola-Galván, P. and Oliver, J.L. (1998) 'Sequence Compositional Complexity of DNA through an Entropic Segmentation Method', Physical Review Letters, pp. 1344–1347. https://doi.org/10.1103/physrevlett.80.1344

P3-05 Microbial community associated with the larynx squamous cell carcinoma

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Given the potential relationship between larynx squamous cell carcinoma (LSCC) and microbial dysbiosis, we used a metagenomic approach and next-generation sequencing to profile the microbiome from faeces, saliva, and normal, peritumoral and tumoral tissue from patients diagnosed with LSCC, before and after treatment with radiotherapy. In addition, we took a case-control approach to study the interplay between oral/gut microbiota and LSCC patients, for that purpose, we collected faeces and saliva samples from 50 healthy volunteers. The goal of this study was to characterize the taxonomic and functional composition of the microbiome from samples collected before treatment, to analyse differences in the microbiome from different types of samples, and to compare with samples collected from the same patient after treatment to find biomarkers of LSCC. Besides, we compared the differences with healthy people, so that we can ultimately evaluate the role of the microbiome as a predictor of radiation sensitivity. We analysed 57 saliva samples (containing 13 post-treatment samples), 53 faeces samples (containing 13 post-treatment samples), 99 tissue samples from patients, and 50 saliva samples, 50 faeces samples from volunteers. We first described and compared the taxonomic and functional differences between the samples according to their origin and type, including alpha, beta-diversity and LEfSe analyses, focusing on those differentially present taxa and genes. Likewise, we analysed differences in saliva and faeces between samples before and after treatment and its impact on bacterial composition, abundance, and diversity. This work provides a foundation for future studies aimed at understanding the role of the microbiome in LSCC.

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SESIÓN 4: MEJORA GENÉTICA Y BIOTECNOLOGÍA

Ponencia invitada:

I4-01 Advances in Cucurbits Genetics and Breeding

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Cucurbitaceae is the second largest fruit and vegetable family, next only to Solanaceae. It encompasses many cultivated crops, such as melon, pumpkin, squash, bottle gourds, cucumber, watermelon, etc., Spain being the first producer and exporter in EU. Cucurbitaceae have high economic and social impact, as they are not only present in almost every arable region worldwide but are also cash crops for large commercial growers and staples for rural communities.

The last decade has witnessed a rapid development of genetic and genomic resources for cucurbits breeding. The COMAV´s Cucurbits Breeding group, in close collaboration with national and international researchers, has contributed to the development of draft genome assemblies, diverse transcriptomes, high throughput genotyping platforms, high-density genetic maps, new breeding populations, induced and spontaneous mutants by in vitro culture techniques, making it possible to accelerate molecular breeding of this family.

Using molecular tools and the impressive natural genetic diversity of the family, such as wild sources, landraces, old varieties or exotic germplasm (held at UPV Genebank and other national and international repositories), we have tackled a number of breeding programs aimed to overcome major constraints in cucurbits production worldwide. Resistance sources for viral and fungal diseases, which limit cucurbits cultivation, have been selected and used in breeding programs. Rootstocks with combined resistances are also developed. Mapping strategies have provided molecular markers linked to major genes and QTLs (resistances to ToLCNDV, WMV, CYSDV, ZYMV, Powdery mildew, charcoal rot caused by *Macrophomina phaseolina*, etc.,) which are useful for marker-assisted selection.

Candidate genes linked to resistance to viruses and fungi (DNA-primase and MLO genes) are being functionally validated by Virus-induced gene silencing (VIGs). Gene editing is also used to develop resistant varieties by adapting platforms for generating CRISPR/Cas vectors effective for these recalcitrant species of agronomic interest.

Harmful effects of abiotic stresses (high temperatures, drought, and salt stress) are increasing in the current climate change scenario, as cucurbits are mostly cultivated in areas specially affected by global warming. Digital imaging of root traits and aerial phenotyping using drones are being applied as new strategies for breeding against these stressful conditions.

Fruit quality is also a main breeding objective. Sugars, acids, and volatile compounds are main metabolites contributing to consumer acceptance whose modification must be studied in breeding programs. We used viral vectors to produce health promoting metabolites, such as carotenoids, in edible cucurbit tissues.

Their economic and social relevance, as well as their high variability and marker acceptability, place the cucurbits among the preferred vegetable crops for organic growers. Current challenges in cucurbits breeding imply the use of the available tools to develop new varieties adapted to organic production systems.

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Comunicaciones orales:

O4-01 Trait stability and its genetic basis in European Traditional Tomato

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Traditional tomato varieties have been selected by farmers given their adaptation to local growing conditions and culinary usage. However, trait stability using "multi-environment trials" (MET) in traditional tomato has been seldom addressed. Recently, we have characterized a European traditional tomato collection consisting of 1,489 genotypes (TRADITOM collection) to gain insights into genetic and phenotypic variation in traditional tomato. The large size of the TRADITOM collection made difficult to handle it for MET and for genotype-by-environment interaction analyses (GEI). To overcome this issue, we developed a multipurpose core collection (TCC), comprising 226 accessions which capture most of the genotypic, phenotypic and geographical diversity present in TRADITOM collection. MET analysis of TCC accessions and an additional panel of 39 modern varieties equivalent to the traditional tomato types, enabled us to study 33 agro-morphological traits and their response to the environment across four independent locations. Comparison of the traditional varieties with the modern reference panel revealed that some traditional varieties displayed excellent agronomic performance and high trait stability, as good as or better than that of their modern counterparts. In addition, genome-wide association and genome-wide environment interaction studies detected 199 quantitative trait loci (QTLs) in traditional tomato, being 137 of these novel. Out of those detected QTLs, 72 QTLs were associated with the phenotype mean (meanQTLs) and 127 with stability (stbQTLs) and QTL-by-environment interactions (QTIs). The large proportion of independent mean and stability loci in European traditional tomato germplasm indicated that, the stability gene regulatory model is the predominant, what would facilitate tomato adaptation and flexibility to different environments. Most QTLs displayed additive gene actions, with the exception of stbQTLs, which were mostly recessive and overdominant. Candidate genes for stbQTLs and QTIs had molecular functions involved in stress and hormone signalling, while developmental genes were more often associated to meanQTLs. Our study enhances the understanding of the genetic basis of valuable agronomic traits and opens avenues for the exploitation of the allelic diversity available within European traditional tomato germplasm.

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O4-02 Identification of *SmAPRR2* and *SmGLK2* genes as responsible for uniform chlorophyll distribution and netting in the eggplant fruit peel

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The distribution of chlorophylls in the eggplant fruit peel can either be uniform or irregular, with the latter being referred to as green netting (1,2). These traits are not only important for the fruit appearance, but also for fruit nutritional quality. Therefore, it is crucial for eggplant breeding to identify potential candidate genes underlying these traits. In this study, a total of 420 individuals from the first eggplant multi-parent advanced generation inter-cross (MAGIC) population were phenotyped and high-throughput genotyped using the eggplant single primer enrichment technology (SPET) platform (3). Through a genome-wide association study (GWAS), strong associations were found for both traits. A major peak on chromosome 8 was identified for the uniform green fruit pigmentation trait, while a significant peak was localized on chromosome 4 for the green fruit netting trait. The genomic candidate regions were narrowed down by screening hundreds of recombinants derived from introgression lines developed between contrasting parents for the traits studied leading to the identification of *SmAPRR2* gene as the best candidate for the uniform green fruit pigmentation trait and *SmGLK2* for the green netting. Furthermore, 277 accessions from the G2P-SOL eggplant core collection were analysed, and several allelic variants were identified as responsible for the disruption of *SmAPRR2* and *SmGLK2* genes, resulting in the absence of uniform fruit chlorophyll distribution and netting pattern, respectively. These mutations were also found to be geographically clustered, suggesting that these phenotypes may have arisen and been selected independently during domestication. This study identified the causative genes for two important breeding traits that will enhance eggplant breeding programs focused on fruit colour and nutritional quality.

1. Daunay *et al.* (2004). Eggplant (*Solanum melongena*) fruit colour: Pigments, measurements and genetics, 108–116.
2. Frary *et al.* (2014). Euphytica 197(2), 211–228.
3. Barchi *et al.* (2019). Scientific Reports 10, 1005.

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O4-03 Genomic landscape of wound-induced adventitious root formation in tomato

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Adventitious root (AR) regeneration in response to wounding is an essential process required for vegetative propagation in many cultivars of agronomic interest, and also an interesting mechanism to ensure adaptation to challenging environmental conditions [1]. Since only few regeneration studies have been performed with tomato as a model species [2, 3], the analysis of genetic variants from natural populations with contrasting architecture in the AR system is an interesting tool to understand the mechanisms that lead to this complex phenotype. In the present work, we studied wound-induced AR formation in a collection of 132 tomato accessions, including *Solanum pimpinellifolium*, *S. lycopersicum* var. *cerasiforme* and *S. lycopersicum* var. *lycopersicum*, representing the genetic and morphological variability of this species in its centers of origin and domestication [4]. We found a wide range of variations in the studied rooting traits that could be exploited later in tomato breeding. Genome-wide association (GWA) mapping performed with our data allowed us to identify several genomic regions involving more than ten single nucleotide polymorphisms (SNPs), including candidate genes related to the regulation of the auxin response or related to a member of cytochrome P450 enzymes, previously identified as a domestication marker for several agronomic traits in tomato [5]. Furthermore, the results obtained from the quantification of endogenous hormone levels in selected accessions with contrasting AR phenotypes showed that alteration of the key hormonal balance could explain the observed regeneration phenotypes. This will allow us to characterize the key regulatory pathways involved in AR regeneration in tomato, which could be useful to obtain cultivars with higher rooting capacity to increase production under challenging environmental conditions, such as drought or nutrient depletion.

1. Gonin et al. (2019). Plants 8, 240
2. Arikita et al. (2013). Plant Science 199, 121-130
3. Trujillo-Moya et al. (2011). BMC Plant Biology 11, 1-13
4. Mata-Nicolás et al. (2020). Horticulture Research 7, 66
5. Chakrabarti et al. (2013). PNAS 110, 17125-17130.

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O4-04 Towards a genetic-based toolkit for outdoor sex identification in aquaculture: an application in *Solea senegalensis*

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There is a growing interest in aquaculture and wildlife stock management to identify fish sex in a fast, easy, and accurate manner. Fish exhibit a wide variety of sex determination mechanisms, including genetic, environmental, and mixed systems that hampers a correct identification of the sex of individuals. For this reason, advances in molecular biology have led to the development of molecular markers that can accurately identify the sex of fish based on genetic differences between males and females even at early stages of development, without the need for sacrificing the fish or examining internal organs. However, these techniques require specialized equipment usually restricted to molecular biology laboratories. This constrain limits *in situ* sex identification and often implies results can only be obtained after several days since sampling. In this study we will show the application of an isothermal DNA amplification approach for sex identification in fishes. We will show the optimization procedure for the loop-mediated isothermal amplification (LAMP), a single-tube technique for the amplification of DNA that synthetizes DNA at a constant temperature and, therefore, it does not require thermal cyclers so it might be taken outdoors. We optimized LAMP to differentiate between males and females of the Senegalese sole (*Solea senegalensis*), a flatfish and economically important species whose females grow faster than males so obtaining all-female populations is an appealing strategy to increase farms productions. A portable DNA extraction protocol followed by a LAMP-based approach will allow to discriminate between sexes, although some optimization is still required to adapt this protocol outdoors. Future work in this direction will facilitate enormously the work at aquatic farms and that of marine wildlife managers and researchers.

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Comunicaciones Flash Talk y póster:

FP4-01 Relationship between gene sequence-based markers for glutamine synthetase loci and yield components in wheat

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Genetic improvement of wheat potential yield is one major breeding goal to face global food demands. This aim is commonly addressed through increasing main wheat yield components, i. e., kernel weight, kernels per spike and spike density. The activity level of glutamine synthetase (GS), a key enzyme in plant N assimilation and mobilization to the target organs, and genotype variation at GS loci have been shown to influence some of these traits (1). Therefore, wheat breeding programs may be benefited by molecular marker-assisted selection (MAS) of valuable GS genotypes. GS1 loci (located on homoeologous group-6 chromosomes) and GS2 loci (located on homoeologous group-2 chromosomes) encode, respectively, the main cytosolic and the plastidic isoforms of the enzyme in wheat (2). Based on the GS genes sequence polymorphisms found for the GS1 and GS2 homoeogenes in a panel of wheat varieties, our group has developed biallelic markers for GS1A, GS2A and GS2D (3). A collection of 187 Spanish bread wheat landraces has been genotyped for these markers. Data on thousand-kernel weight (TKW) and kernels per spike (KS), previously recorded for this collection (4), have been used to determine the reliability of these markers in MAS strategies aiming to improve wheat yield. The analyses have detected the beneficial influence of specific marker alleles at GS2A and GS2D on TKW. No epistatic interaction between these homoeloci has been detected, which supports additive effects of the allelic variants tested. It also suggests that pyramiding strategies of the marker alleles for these genes can be successful to increase TKW in wheat. On the other hand, one GS1A marker allele associated with increased KS values. For both traits, epistatic effects between allelic variants at non-homoeologous loci have also been demonstrated.

1. Thomsen et al. (2014). Trends in Plant Science 19, 656-663.
2. Bernard et al. (2008). Plant Molecular Biology 67, 89-105.
3. Pascual et al. (2023) Molecular Breeding 43, 8.
4. López-Fernández et al. (2021). Plants 10, 620.

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FP4-02 Role of Transcription Factors involved in the synthesis of wheat prolamins

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Celiac disease is one of the main pathologies related to the consumption of certain cereal grains. Developing wheat varieties that lack the proteins responsible for triggering adverse reactions to wheat is an attractive target for plant biotechnology, and both RNA interference (RNAi) and CRISPR/Cas9 were applied (1, 2). Transcriptomics research has shown changes in transcription factor expression related to the regulation and compensation of gluten and non-gluten proteins in wheat grains (3). Low-gluten RNAi lines (1) were studied using RNAseq to understand the process of protein compensation in the grain, whereby the total protein content remains constant despite the strong decrease in gliadins by RNAi. From this analysis, four transcription factors (TaGL9, DREB, NAC057, and NAC038) were identified as playing an important role in this compensation mechanism (3). The objective of this work was to determine the role that those four transcription factors have in the synthesis of prolamins in wheat grain. In this study, we aimed to generate knock-out plants using the CRISPR/Cas9 system. To that, guides RNA were designed and their efficiency validated *in vitro*. Later, plasmids containing the most efficient sgRNAs were assembled and delivered into immature wheat embryos by particle bombardment. The transformation efficiency ranged from 0.07% to 10%, depending on the bombardment. A total of 85, 16, 15 and 5 putative edited plants were obtained for plasmids pMGCNAC057, pMGCNAC038, pMGCDREB, and pMGCTaGL9, respectively. All of them are currently under analysis to estimate editing frequencies and their roles in the regulation of gluten and non-gluten proteins in wheat grains.

1. Gil-Humanes et al. (2008). J Cereal Sci, 48, 565–568
2. Sánchez-León et al. (2018). Plant Biotechnol. J. 16 902–910.
3. Marín-Sanz & Barro (2022). Front. Plant Sci, 13, 935851.

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FP4-03 Validation of heat tolerance QTLs in elite lines and pyramiding of QTLs related to abiotic stresses in tomato

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Heat and drought are among the most damaging stresses affecting crops. In previous works, we have identified several QTLs involved in the tolerance to these stresses. However the effects of those QTLs in different genetic background and the possible interaction between them have not been addressed. Therefore, we analysed the effect of heat tolerance QTLs into commercial elite lines and their interactions after QTL pyramiding. Seven elite lines from Enza-Zaden S.L. were crossed with two heat tolerant introgression lines, (*S. lycopersicum* var. MoneyMaker x *S. pimpinelifolium* TO-937) SP1-4 and SP12-5, and the heat tolerant tomato genotype CLN1621L (provided by NTW). Also, QTLs combinations were analyzed with crosses between SP1-4 x SP12-5 and SP1-4 x BIL1619A (drought tolerant; provided by HUJI). Plants were grown under a stepwise temperature increase at three temperature regimens: T1 (25ºC day/20ºC night; optimal); T2 (30ºC day/25ºC night; moderately high); T3 (35ºC day/30ºC night; extremely high). The fruit set proportion (FRS) was used as discriminating variable to assess tolerance to high temperatures. For the heat tolerance QTL introduction, the presence of the SP1-4 and CLN1621L alleles in the seven elite lines produced a positive effect improving their tolerance to high temperatures with significantly higher values for FRS at T2 and T3. On the other hand, only one hybrid between SP12-5 and one elite line showed higher heat tolerance. Likewise, heat and drought tolerance QTL pyramiding increased FRS at both moderate or extremely high temperatures. Moreover, the combination of QTLs for heat tolerance in the cross SP1-4 x SP12-5 also increased FRS at T2. These results confirm the favourable effect of the QTLs identified in SP1-4 and SP12-5 involved in heat tolerance in tomato and the benefits to combine multiple stress QTLs to improve the performance of tomato under adverse conditions.

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FP4-04 Using lentil wild relatives to identify genes related to *Ascochyta* blight resistance

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Lentil (*Lens culinaris* Medik.) is one of the most important legumes globally due to its high nutritional quality but also because this crop contributes to improve soil quality. The production of lentils has increased by 50% worldwide in the last two decades, being Canada the current first world producer and exporter; however, factors such as drought and the pathogen *Ascochyta lentis*, considerably reduce yield. The preferred cultivation of certain elite varieties by main producers has led to a decrease in lentil genetic diversity available to growers. Consequently, one of the approaches to obtain new resistant varieties is to exploit other gene pools. The lentil´s wild relatives *L. orientalis*, *L. tomentosus*, *L. odemensis*, *L. lamottei*, *L. ervoides*, and *L. nigricans* could be reservoirs of many desirable traits including *Ascochyta* resistance that are worth assessing. Recently, we have carried out the first draft of the lentil pan-transcriptome by evaluating one commercial cultivar and seven wild accessions (1). This first draft of the lentil pangenome confirmed the presence of transcripts enriched in NB-ARC domains characteristic of resistance genes. By aligning those transcripts using the lentil genome assembly v2.0 (2), we have recently obtained transcriptome assemblies from cultivated and wild relatives during both infection with *Ascochyta lentis* and control conditions. A differential expression analysis was performed, and infection resistance scores were employed to target differentially expressed genes (DEGs) shared among the most resistant accessions. We were able to verify the existence of differentially expressed genes in all the accessions. The GO analysis of these DEGs suggested their roles in the regulation of principal biological processes such as macromolecules catabolic processes or amino acid and protein biosynthesis. Importantly, there was an overrepresentation of genes involved in stress responses such as ethylene-induced responses, auxin-mediated pathways and regulation of systemic acquired resistance. Another representative GO category in these DEGs was "molecule transport" both intra and extracellular via vesicles transport and exocytosis. We hope that further analyses of the large amount of data obtained may lead to the discovery of the genes and key mechanisms of resistance to this pathogen and their subsequent aplication to the development of improved varieties.

1. Gutierrez-Gonzalez, J.J. et al. (2022) Agronomy 2022, 12, 1619. https://doi.org/10.3390/agronomy12071619
2. Ramsay L, et al. (2021). bioRxiv. 2021 Jul 24. https://doi.org/10.1101/2021.07.23.453237
Genome files retrieved from https://knowpulse.usask.ca/genome-assembly/Lcu.2RBY

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FP4-05 Start of a breeding program to introduce virus resistances in Flor de Baladre and Pimiento tomatoes (*Solanum lycopersicum* L.)

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Recent collaboration between the CIAGRO-UMH tomato breeding group, IMIDA and COMAV-UPV has led to the development of a breeding program with Flor de Baladre and Pimiento tomato varietal types, which are originally from Region of Murcia and selected from germplasm bank BAGERIM (IMIDA). The aim is to obtain breeding lines with virus resistances and traditional characteristics like the original varieties, that may be attractive to local farmers. This is a classic breeding program based on backcrossing and selection assisted by molecular markers (SNPs), using the line UMH1200 (CIAGRO-UMH) as donor parent of *Tm-2a*, *Ty-1* and *Sw-5* genes, which confer resistance to ToMV, TYLCV and TSWV, respectively. In every cycle, selection of the best backcrosses is done by the phenotypic evaluation of adult plants. The initial crossing was made in the second half of the year 2020 between the UMH1200 line and several Flor de Baladre and Pimiento accessions selected because their agronomic and quality qualities, and considering the genetic distance between them, to ensure the highest diversity as possible in the breeding process. In the first half of the year 2023, the fourth backcross plants (BC4) have been cultivated to obtain the BC5 generation. In current work, we show the results obtained to date.

1. Alonso et al. (2015). Actas de Horticultura 71: 521-524.
2. García-Martínez et al. (2011). HortScience 46(7):1054-1055.
3. Carbonell et al. (2022). Actas del III Congreso Universitario en Innovación y Sostenibilidad Agroalimentaria-2022. 12-19.

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FP4-06 Genetic analysis of Arabidopsis growth effect in bioreactors

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The growth of higher plant cells in bioreactors is a process whereby complete plant tissues are induced to form friable calli, and these serve as inoculum for bioreactors. Proper growth of plant cells in a bioreactor is considered important as both biomass and the production of biomolecules of interest are linked. While farming plants have undergone a strong genetic selection towards improved productivity, this has not been the case for bioreactor cell production. One type of the commonly used bioreactors to produce plant cells are Continuous Stirred Tank Reactors (CSTR). Their design is widely studied and standardized with advantages for scaling, correct mixing, temperature control and optimal oxygen transfer. There is ample knowledge showing the effects of process parameters and bioreactor design on cell growth. However, little is known about the genetic improvement for cell growth in bioreactors. In this work, we are taking a genetic approach using Arabidopsis thaliana to identify mutations affecting cell growth in bioreactors. The screening is based on an artificial vision system, starting with calli induction followed by a second step in liquid cell cultures. The aim of this study is to achieve higher yields in both biomass and the desired products in bioreactor cultures identifying the genes involved in high growth in unicellular growth conditions. These results show several independent molecular pathways involved in improved plant cell growth in bioreactors. We are editing the identified genes in the most used species in plant biotechnology to create high productive lines.

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Comunicaciones póster:

P4-01 Grafting as an alternative to fight soil stress in melon and watermelon crops

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Grafting is a very well-known technique used in organic farming to reduce production losses in cucurbit crops caused mainly by soil-borne pathogens, but also by abiotic stresses such as drought, salinity or the presence of heavy metals. It is an environmentally friendly and economical alternative to the use of chemicals. The election of the right rootstock is crucial as it will provide the resistances and will also help in yield stability. To study the effect of grafting on yield and fruit traits, four rootstocks were selected to graft three traditional melon (*Cucumis melo* L.) and three watermelon (*Citrullus lanatus*) landraces. The experimental hybrids *Cucumis ficifolius* x *Cucumis anguria* (UPV-FA) and *Cucurbita maxima* x *Cucurbita ecuadorensis* (F1ECU), as well as the commercial rootstock Shintoza (*Cucurbita maxima* x *Cucurbita moschata*, H.M. Clause Vegetable Seeds) were used as rootstock for the three melon accessions, 'Piel de Sapo', 'Rochet' and 'Amarillo' type. F1ECU and Shintoza were also assayed as rootstocks for watermelon, besides the *Citrullus amarus* accession BGV 5167. Three Spanish watermelon landraces were used as scion. These combinations were trialled in two different environments, in Comunidad Valenciana and Anadalucía, respectively. Mortality and behaviour of the plants and root system was studied, as well as morphological and quality traits of the fruit. There was significant effect of the genotype, the environment and their interaction. The results showed that rootstocks UPV-FA and Shintoza were promising for melon and Shintoza and F1ECU for watermelon, compared to the non-grafted control, both in terms of production and fruit morphology and quality. This research will be completed with a replication in 2023 with the aim identifying the best rootstock-scion combinations that can help local farmers.

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P4-02 Introgression breeding in eggplant unveils the genetic control of prickliness

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Eggplant, despite displaying a wide range of useful traits for breeding, has a narrow genetic base that limits genetic improvement. To address this challenge, the introgression breeding approach has been employed to incorporate natural variation from crop wild relatives (CWRs). The utilization of advanced backcrosses (ABs) and introgression lines (ILs) in eggplant presents a promising opportunity to develop improved cultivars with desirable traits, such as plasticity and resilience for adaptation to climate change, tolerance to biotic and abiotic stresses, and enhanced nutritional content. Furthermore, ABs and ILs serve as valuable resources for genetic studies. In this way, a set of ABs developed between *S. insanum,* a prickly common eggplant ancestor, and the recurrent non-prickly parent *S. melongena* has been used to dissect the genetic control of the prickliness. Prickles in eggplant act as a defense against herbivores, and a major dominant quantitative trait locus (QTL) for prickliness, designated as *Pl*, has been previously identified on chromosome 6 (1,2). To further narrow down the *Pl* locus, ABs with introgressions from chromosome 6 were used, leading to the identification of a genomic region of 96 kb. Notably, introgressions of the *Pl* locus from other phylogenetically distant "spiny" solanums, such as *S. dasyphyllum* and *S. elaeagnifolium* from secondary and tertiary gene pools, respectively, confirmed the evolutionary conservation of this locus in the subgenus *Leptostemonum*. Based on their putative biological function, three candidate genes within the *Pl* locus region were identified. The study of the genetic control of the prickliness trait in eggplant through the introgression breeding approach provides valuable insights into the evolutionary dynamics of the trait and offers potential candidate genes for future breeding programs.

1. Barchi et al. (2019). Sci. Rep. 9, 1005.
2. Miyatake et al. (2021). Breed. Sci. 70, 438–448.

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P4-03 Development of a genosensor for the detection of peanut in food

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The presence of certain allergenic ingredients in food, even at trace levels, must be declared on the label to prevent accidental consumption by the allergic population. This negative impact can be reduced by using reliable and specific tools capable of detecting traces of food allergens. Although protein-based assays are the most widely used for allergen detection, PCR-based methods have been proposed since DNA is more stable than proteins during food processing. As an alternative to real-time PCR, electrochemical biosensors based on nucleic acid detection (genosensors) could be useful for food allergen screening. The best regions of the genome from which to choose probes are those with interspecific variation that allow discrimination between closely related species. These may be single sequences or high copy number sequences from both nuclear and organellar genomes. The aim of this work was to develop a genosensor for the sensitive and specific detection of peanut in food matrices. The trnH-psbA region of the chloroplast was used as the target sequence. To meet the required sensitivity the use an express-PCR prior to detection with the amperometric genosensor was proposed. Different DNA extraction methods and PCR conditions were assessed. The system was set up using binary mixtures containing different proportions of defatted peanut flour (10%-0.001%) in spelt wheat flour. It was then used for the detection of peanut in food matrices. Data were compared with those obtained by quantitative PCR using the same target sequence. The results showed that our system can be considered as an effective tool for allergen detection, as the trnH-psbA sequence showed high specificity and a detection limit of 0.01% of peanut was achieved.

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P4-04 Evaluating the Impact of Coverage Depth on SNP Discovery in Eggplant using Skim Whole Genome Sequencing

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The technological evolution and cost reduction of sequencing methodology, combined with the advancement of bioinformatics tools, constitutes a great opportunity for the development of tools and resources for genetics and breeding. This boost fostered the development of new high-throughput genotyping strategies, being one of the most promising skim whole genome resequencing (SWGR), characterized by using low sequencing coverage. Five sequencing coverages (1X-5X), generated in silico from a whole genome resequencing at 5X of a *Solanum melongena* genotype, were evaluated. After mapping against the eggplant reference genome 67/3 and performing the SNP calling, the resulting VCF file was filtered using different minimum coverages (from 1 to 10F) to study the size of each set of SNPs. A comparison with a gold standard set, based on the resequencing of the same genotype at 20X, was also made to avoid possible false positives and artifacts due to the low sequencing coverage used. Previously, the gold standard dataset was filtered for maximum and minimum mapping coverage of 40F and 10F, respectively, in order to avoid overrepresented regions and to confidently call heterozygous variants, for a minimum frequency of the alternative allele at 0.3, and also variants in chromosome 0 were removed. Genetic variant identification increased with higher sequencing coverage, but decreased with stricter minimum coverage filters. The increase of the minimum coverage filter was also associated with a higher confidence in the remaining variants being true positives. Notably, there were no significant differences in the number of variants identified between sequencing at 1X or 2X coverage. Furthermore, lower sequencing coverages achieved higher concordance in variant calling when using low minimum coverage thresholds (1-3F), being 69% and 62% at 1X and 5X, respectively, while higher concordance was obtained with increased sequencing coverage at higher filtering thresholds (7-10F), being 43% and 54% at 1X and 5X, respectively. This study provides valuable recommendations for the design of high throughput genotyping pipelines, enabling fingerprinting a larger number of accessions at reduced costs and thus fostering genetics and breeding studies. However, the best combination of sequencing coverage and variant filtering should be evaluated on a case-by-case basis, taking into account available resources and study objectives.

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P4-05 The loss of function of the tomato homolog of *ROTUNDIFOLIA3* impairs leaf expansion and plant growth

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We characterized the tomato *2489etmm* recessive mutant, which belong to our T-DNA insertional mutant collection (1). Homozygous mutant plants showed slower growth rate at greenhouse conditions, as well as wrinkled leaves and enlarged stem. Despite these developmental defects, *2489etmm* mutant plants were able to develop flowers and yield seed-bearing fruits after almost ten months growing under optimal conditions, in contrast to the three months required by the wild-type plants. By combining mapping-by-sequencing and CRISPR/ Cas9 genome editing methods, we proved that a 2-bp deletion in the tomato homolog of the Arabidopsis *ROT3* gene (*SlROT3*) was responsible for the phenotype observed in the *2489etmm* mutant. We engineered knockout mutations at the *SlROT3* locus by using the CRISPR/Cas9 system with a single guide RNA. Independent first-generation CRISPR lines homozygous or biallelic for edited mutant alleles showed slower growth, wrinkled leaves, and enlarged stem, a phenotype resembling that observed in the *2489etmm* mutant. The *ROTUNDIFOLIA3 (ROT3)* gene encodes a cytochrome P450 family protein involved in regulating leaf length of the model species *Arabidopsis thaliana*. Specifically, *ROT3* is required for the conversion of typhasterol to castasterone in the early C6-oxidation pathway of brassinosteroid biosynthesis (2). Brassinosteroids are a group of plant steroid hormones and playing a key function during plant growth and development. Together, our results suggest that *SlROT3* may act regulating tomato leaf expansion in a similar manner as occurs in Arabidopsis, by probably participating in brassinosteroid biosynthesis pathway.

1. Pérez-Martín et al. (2017). Plant Biotechnology Journal, 15: 1439-1452.
2. Ohnishi et al. (2006). The Plant Cell 18, 3275–3288.

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P4-06 Breeding program for the introgression of genetic resistance to viral and fungal diseases in melon landraces

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Abiotic stresses cause important economic losses in melon (*Cucumis melo* L.) cultivation. To date, out of the 59 viruses reported infected cucurbits at least 28 of them have been detected in the Mediterranean basin. The *Potyviruses Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV), and the *Cucumovirus Cucumber mosaic virus* (CMV), all of them aphid-transmitted, are among the most widespread in open fields in the Mediterranean countries. The whitefly-transmitted *Geminivirus Tomato leaf curl New Delhi virus* (ToLCNDV) and the *Crinivirus Cucurbit yellow stunting disorder virus* (CYSDV) are also considered very damaging in cucurbits. Among the aerial fungal diseases affecting cucurbits, powdery mildew, mainly caused by *Podosphaera xanthii* in warmer countries, is the most limiting in melon. The introgression of genetic resistance to these pathogens is one of the main breeding objectives, especially for cultivation under organic agricultural systems, where protection is lower than in conventional farming. The breeding program here presented exploits different sources for the introgession of resistance into melon traditional landraces. The traditional melon types included in the program are 'Piel de Sapo', 'Blanco', 'Amarillo', 'Rochet' and the snake melon 'Alficoz'. The resistance sources used as donors are: the African accession TGR-1551 (resistant to WMV, CYSDV and powdery mildew), the Indian accessions PI 414723 (resistant to ToLCNDV, WMV and ZYMV) and WM-7 (resistant to ToLCNDV) and PI 161375 'Shongwan Charmi' (resistance to CMV). The availability of molecular markers tightly linked to the resistance genes derived from these sources has allowed the advance in the program. BC3 or BC4 generations are available for some of the traditional landraces. The quality profiles for the most advanced generations are similar to the profiles of the recurrent parents. The generations available will be the basis for the development of varieties incorporating the resistances.

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P4-07 Evolutionary conservation of *ENO* function in regulating floral meristem activity within the Solanaceae dry and fleshy fruit species

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The balance between floral stem-cell proliferation and differentiation is crucial to the development of optimally sized flowers with a determined organ number. This balance is tightly regulated by a complex transcription factor network involving *EXCESSIVE NUMBER OF FLORAL ORGANS (ENO)*, a novel regulator of the tomato fruit size that encodes a member of the APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) superfamily. ENO regulates the expression domains of the *WUSCHEL* stem cell identity gene in a flower-specific manner. Thus, *eno* mutant plants display enlarged floral meristems and fasciated flowers with a higher number of floral organs, which in turn give rise to larger fruits as a consequence of additional carpels (1). To examine whether the *ENO* gene function is evolutionary conserved within Solanaceae, we studied the *ENO* ortholog gene (*NbENO*) in *Nicotiana benthamiana*, a wild tobacco species yielding dry and dehiscent fruits. To understand the *NbENO* function, we first monitored its spatio-temporal expression pattern throughout development by *in situ* hybridization, which revealed that *NbENO* is expressed in the central zone of the shoot apical meristems and in the outermost cell layers of floral meristems. In addition, we used CRISPR/Cas9 technology to obtain knockout lines of the *NbENO* gene. Vegetative growth of the *NbENO* CRISPR/Cas9 lines was indistinguishable from that of the wild-type ones. In contrast, significant differences were detected in flowers and fruit development. Whereas the fourth whorl is occupied by a bicarpelar gynoecium in the wild-type flowers, the *NbENO* CRISPR/Cas9 lines developed three-carpellate flowers resulting in dry capsules with six dehiscence zones. However, this increased number of carpels showed incomplete penetrance and variable expressivity, as flowers with two or three carpels were found in the same *NbENO* CRISPR/Cas9 plant. Taken together, our results exposed a potentially evolutionary conservation of *ENO* function in controlling floral meristem activity within the Solanaceae dry and fleshy fruit species.

1. Yuste-Lisbona et al. (2020). Proc. Natl. Acad. Sci. USA 117, 8187-8195.

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P4-08 A new library of introgression lines from wild melon into 'Piel de Sapo' to study domestication traits

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A collection of 22 introgression lines (ILs) has been developed from the wild accession Ames 24294 Trigonus (TRI) into a 'Piel de Sapo' (PS) genetic background. Each IL carried an average of 1.4 chromosomal fragments from TRI, and those introgressions represented 75% of the whole wild TRI genome. The IL collection was evaluated in greenhouses (Málaga and Meliana) and the field (Alcásser), to study, mainly, traits related to domestication syndrome, such us fruit weight (FW) and flesh content (FFP). TRI produces small fruits (20-50 g) without edible flesh, while PS produces large (1,500-2,000 g), oval-shaped fruits (length/diameter ratio around 1.3) with 70% of FFP. The IL collection showed an impressive variation in FW (ranging from 500 to 3,700 g). Twelve ILs decreased the value of FW, while one IL increased it. Regarding FFP, two ILs decreased its value, while one IL increased it. Genes in theses introgressions are candidate for having been involved in melon domestication. Variation was also observed in other quality traits as fruit shape, color, soluble solid content, ripening behaviour and flesh firmness. These first results confirm that the TRI IL collection is a very powerful tool to map traits of agronomic interest in melon and, specifically, to better understand the domestication process of this crop.

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P4-09 K-seq, an affordable, reliable, and open Klenow NGS-based genotyping technology

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K-seq(1), is a new genotyping methodology based on the amplification of genomic regions using Klenow polymerase amplification with short oligonucleotides followed by PCR, and Illumina sequencing. The methodology has been already tested in several plant species as different as tomato, potato, pepper, petuniaxhibrida, asparagus, chickpea, wheat and, also, in a mammal species, dog. Efficiency when compared with GBS was tested by analyzing the same samples. Both methodologies shown similar results, although K-seq had the advantage of finding more SNPs for the same number of Illumina reads, due to a better mapping performance. The reproducibility was checked with two independent experiments of the tomato samples, the correlation coefficient of the SNP coverages between samples was 0.8 and the genotype match was above 94%. K-seq has been already used to genotype F2 tomato populations, allowing to make an ultra-dense genetic map with 147,326 SNP markers with an average distance between markers of 0.2 cM(2). The genotyping of hexaploid wheat samples generated specific markers for all subgenomes and the SNPs generated from the diploid ancestors were located in the expected subgenome with accuracies above 80%. In dog, the genetic distances obtained with K-seq data are similar to the WGS based ones. The short oligonucleotides could be adapted to each species easily to improve the specific results. K-seq is an open, offered unencumbered of any patent, easy to set up, cost effective and reliable technology ready to be used by any molecular biology without special equipment in many genetic studies.

1. Ziarsolo P, Hasing T, Hilario R, et al. K-seq, an affordable, reliable, and open Klenow NGS-based genotyping technology. Plant Methods. 2021;17(1):30. Published 2021 Mar 25. doi:10.1186/s13007-021-00733-6
2. Mata-Nicolás E, Montero-Pau J, Gimeno-Paez E, et al. Discovery of a Major QTL Controlling Trichome IV Density in Tomato Using K-Seq Genotyping. *Genes (Basel)*. 2021;12(2):243. Published 2021 Feb 8. doi:10.3390/genes12020243

P4-10 Evaluation of a collection of 'De Penjar' tomato accessions under conventional and organic cultivation conditions

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The 'De Penjar' tomato (*Solanum lycopersicum* L.) is a local varietal type of the Valencian Community. Its evolution under low-input management practices has led to the selection of resistant/tolerant genotypes to adverse conditions, such as water stress. In this work we have evaluated a collection of 44 'De Penjar' tomato accessions with 31 morpho-agronomic descriptors (27 quantitative and 4 qualitative) and 4 quality characteristics under two cultivation conditions, organic and conventional. Of each accession, 3 blocks of 4 plants were cultivated in a farm in the town of Alcalà de Xivert. (Castelló). The evaluated collection displayed a high variability for characters such as the color and shape of the fruit. Thus, we found accessions of red, pink, red-orange and pink-orange coloured fruit. On the other hand, it presents fruits with morphologies from flattened, slightly flattened, rounded, rectangular, ellipsoid, heart-shaped, obovoid to pear-shaped. This distribution of colors and fruit morphologies remained stable in the two culture conditions. Regarding the other quantitative characters, significant differences were observed among accessions for 18 and 26 out of the 27 characters evaluated under organic and conventional farming, respectively. In organic cultivation, no differences were observed for the characters leaf in inflorescence, fruit setting sequence, incidence of pests and diseases in leaf and fruit, plant vigor, fruit hollowing, and yield. However, in the case of conventional cultivation there were only no significant differences for the incidence of diseases in fruit. The same trend was observed in terms of quality characteristics (1). The cultivation of the collection used in conventional culture conditions resulted in a higher level of variability in the materials than in organic culture conditions. This has made it possible to have a large number of accessions well adapted to this last cultivation system and, on the other hand, opens the possibility of selecting the best materials, especially in terms of vigor, fruit set sequence and production for conventional cultivation systems. In any case, the collection used constitutes a very valuable germplasm in the genetic improvement of this type of tomato.

1.Figàs, M.R. et al.2018. Scientia Horticulturae, 238: 107.

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P4-11 A saturated *Cucurbita* inter-specific genetic map for ZYMV resistance dissection

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Cucurbita genus comprises several species of squashes, pumpkins and gourds with economic importance due to its edible seeds and fruits, but also because of the increasing need of development of new rootstocks, apart from ornamental purposes and cattle consumption. Potyviruses such as *Zucchini yellow mosaic virus* (ZYMV) cause great damage on cucurbit crops. Both screening for new resistant resources and the understanding of mechanisms of resistance, including identification and mapping of resistant genes, are essential to control diseases, especially insect-borne diseases, via genetic breeding. Previous studies identified resistance in an accession of wild *C. ecuadorensis* that can be introgressed in cultivated *Cucurbita* spp. In the present work, an F_2 population derived from PI 432443 (*C. ecuadorensis*) and the *C. maxima* accession SUD-CU-6 maintained by the COMAV's Cucurbit Breeding Group has been assessed based on symptoms score during 3 weeks after mechanical inoculation and genotyped using the NGS platform DArT (Diversity Arrays Technology)-seq in order to map the genomic regions involved in the resistance. About 13,000 markers were called with this technology, but a strict selection was performed to discard the ones polymorphic within parentals, with high missing data, or with distorted segregation. Finally, an inter-specific genetic map with 2,063 SNPs, physically located in the *C. maxima* assembly v.1.1, was constructed at minimum LOD 5 (21 linkage groups and 2,331 cM) enabling the detection of a major QTL on chromosome 3 located around 7 Mbp, using CIM methodology. Relative viral accumulation by quantitative-PCR has also been carried out. Future analyses of recombinants and will help to better define the QTL and possibly narrow down the region for identification of candidate genes.

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P4-12 *In vitro* selection of melon genotypes tolerant to water stress

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The scarcity of water, even more pronounced by climate change, has accelerated the need to develop and select varieties adapted to rainfed growing conditions. The African melon accession TGR-1551 (*C. melo* ssp *agrestis*) shows tolerance to drought stress. A set of recombinant inbred lines (RILs) dervived form this source has been developed in the genetic background of the 'Amarillo' type melon 'Bola de Oro' (*C. melo* ssp. *melo*). The objective of the work here presented was to test *in vitro* culture methods to evaluate melon germplasm for tolerance to water stress. The genotypes used in the assays were both parental lines of the RILS, TGR-1551 and 'Bola de Oro', as well as the F1 hybrid between them. The evaluation was carried out in media supplemented with different concentrations of polyethylene glycol (PEG), which causes osmotic stress and simulates water stress. Internodes were grown in *in vitro* culture media with PEG concentrations ranging from 0-2.5%. The results after 30 days of cultivation showed that the most tolerant parent had a higher percentage of rooted plants and more developed roots. This behavior was also observed in the F1 hybrid, which also presented, in the control medium, greater growth compared to the two parents. Evaluations in media with PEG at the cellular level have also been initiated and are in progress. Once consistence is confirmed between the results obtained in this *in vitro* culture media and the tolerance level of the different genotypes, this methodology will be used to evaluate the TGR-1551-derived RILs for their tolerance to drought stress. The selection of the tolerant lines will be useful *per se* and also to identify the genomic regions that contribute/provide tolerance to water stress.

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P4-13 Morphological and agronomic characterization of a collection of 'Tomata Valenciana' accessions under organic cultivation conditions

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The 'Tomata Valenciana' is a Valencian traditional tomato variety that presents fruits with small locules distributed irregularly around a large circular heart, which gives it great firmness and fleshy texture. This traditional variety has a very important economic projection at the local level, with an annual marketed production of over $1{\cdot}10^6$ kg. In this work, the morphological and agronomic characterization of a collection of 51 'Tomata Valenciana' accessions grown under organic farming conditions has been carried out. These accessions, from the Association of Producers and Commercializers of the Valencian Tomato, were cultivated in an organic farm in Catarroja. Thirty plant and 25 fruit characters were evaluated. All accessions show the same type of leaf. Significant differences have been observed between the evaluated accessions for 24 plant characters. Regarding the fruit characters, significant differences were detected for 20 characters. The results obtained reveal great variability in most of the characters evaluated. Thus, there are accessions with an average weight of the fruit from 200 g up to over 750 g The same occurs with traits such as yield (between 2.5 kg/plant and 6.5 kg/plant). The principal components analysis of the traits evaluated allowed grouping the accessions of 'Tomata Valenciana' according to the intensity of the green shoulder of the fruit, as well as by its pointing. Thus, there are accessions with intense green shoulders and more pointed fruits called the 'Masclet' type. We have classified other accessions, more flattened, with less marked shoulders and a greater number of locules, as 'Blanca' type. Some accessions display intermediate fruit characteristics between these two mentioned types. The use of phenomic tools could help in the distinction of these different varietal subtypes (1). This work reveals the existing variability at the plant and fruit level of the 'Tomata Valenciana' type and allows the future selection of variants of this traditional variety depending on the preferences of both farmers and consumers, as well as their inclusion in programs selection and genetic improvement.

1.Figàs, M.R. et al. 2015. Genet Resour Crop Evol, 62:189.

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P4-14 Effect of water stress on growth and biochemical responses in a set of eggplant (*Solanum melongena*) lines with *S. incanum* introgressions

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In recent years, agricultural production has been affected by abiotic stresses, which have been exacerbated by climate change. In this sense, the reduced availability of water for irrigation makes it essential to generate new varieties tolerant to water deficit. In this trial, a set of eggplant lines with introgressions from the wild parental *S. incanum* were evaluated for traits that confer an increased ability to grow under conditions of low irrigation availability. Plants with five true leaves from nine introgression lines (ILs) and their parents were irrigated daily at 100% or 30% of field capacity to simulate drought conditions. After 14 days of treatment, growth and biochemical characteristics were assessed. Water stress resulted in reduced plant growth and increased water use efficiency, proline and malondialdehyde contents. Although the ILs had a higher biomass than the wild parent under stress conditions, none was higher than the cultivated parent. Nevertheless, several QTLs detected that may be associated with higher drought tolerance. For growth, two QTLs were identified, one associated with root biomass (*dwr6%*) and one with stem biomass (*dwt8*). A QTL associated with higher leaf water content (*lwc12%*) and another associated with higher water use efficiency (*wue1*) were also found. Finally, two QTLs associated with higher chlorophyll content under stress (*chl2* and *chl8%*) were detected on chromosomes 2 and 8. These results suggest that eggplant lines with introgressions from drought-tolerant wild relatives could be a practical approach to improve drought tolerance. However, fine-mapping of tolerance QTLs and minimisation of linkage drag are essential for significantly improving drought tolerance through introgression breeding.

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P4-15 Genome-Wide Association Mapping for resistance to downy mildew and agronomic, commercial, and quality traits in quinoa

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Quinoa (*Chenopodium quinoa* Willd.) was first domesticated by ancient Andean civilizations in the region surrounding the Bolivian and Peruvian Altiplano. Nowadays, quinoa is gaining worldwide attention due to its outstanding nutritional value and its ability to grow in various stress conditions, such as drought, salinity and frost. Quinoa remains as an important food crop in South America, but, in order to meet its increasing demand, its cultivation has expanded, being currently grown in more than 95 countries. Unfortunately, as this crop was only recently expanded worldwide, there is limited scientific knowledge about relevant aspects for quinoa breeding, such as the inheritance of desirable agronomic and quality traits, or the identification of molecular markers linked to them that could be used in marker assisted selection. In Spain quinoa was first introduced in Andalucía region ten years ago. Current cultivars grown in Spain have been developed in agroclimatic conditions differing from the Spanish ones (Peru, Netherlands and Denmark). Therefore, a quinoa breeding program has been established at the Institute for Sustainable Agriculture (Córdoba, Spain) with the goal of developing quinoa varieties showing a better adaptation to Spanish field conditions. As part of this breeding program, a collection of 212 quinoa accessions from different countries was screened for resistance to downy mildew and different agronomic (yield, earliness, plant height, days to maturity), commercial (seed size, seed colour) and seed quality traits (saponins, protein, fatty acids and tocopherols content), during two years, under rainfed conditions, in two locations. For all traits, substantial phenotypic variation among accessions was found. This collection was sequenced using DArTSeq technology and sequenced reads were mapped to the quinoa reference genome. As a result, 17,238 SNPs and 15,249 SilicoDArTs markers were identified. A GWAS analysis is in progress in order to identify the genes controlling the traits scored. Relevant outcomes of this analysis will be presented in the congress.

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P4-16 Initial development of a set of melon introgression lines derived from the *Macrophomina phaseolina*-resistant accession PI185111

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The pathogenic soil fungus *Macrophomina phaseolina* (Mp) causes charcoal stem and rot disease in melon (*Cucumis melo* L.) leading to significant crop losses due to plant wilting. Resistance to Mp has been reported in different exotic melons. Accession PI185111 (wild *Agrestis* group, Ghana origin) showed high levels of resistance (1), even with high temperatures (2). The breeding program for the introgression of the resistance in commercial backgrounds was initiated by crossing PI185111 with the melon variety Piñonet (Piel de Sapo, *Ibericus* group, origin Spain). Our preliminary genetic studies suggested that resistance is controlled by at least three different genomic regions. In order to continue with the introgression program and to facilitate the genetic dissection of the resistance to Mp derived from PI185111 a set of introgression lines (ILs) is being developed in the genetic background of Piñonet. The second backcross generation has been genotyped with SNPs covering the entire melon genome, implemented in the Agena Bioscience iPLEX® Gold MassARRAY medium throughput platform. A total of 76 plants were selected prioritizing that their introgressions represented the whole genome of PI185111, but also that each of the plants had a low proportion of the genome of the wild parent. The third backcross generation of these plants will be generated in the spring season to continue with the generation of the ILs. Selfing offspring of these plants will be obtained in order to further analyze the genome regions linked to the resistance to Mp. Moreover, controlled inoculation assays using the toothpick method are being carried out with 50 plants of the second backcross generation. These plants were selected for their introgressions of the resistant parent that represent the entire chromosomes putatively linked with the resistance to Mp. These results altogether will allow the advance in the breeding program and in the development of the set of ILs.

1. Ambrósio et al (2015). Euphytica 206, 287-300.
2. de Sousa Linhares et al (2020). European Journal of Plant Pathology 158, 431-441.

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P4-17 *Bartonella quintana* in Synthetic Biology: Effects of selective media at a proteomic level

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The use of microorganisms with naturally reduced genomes to obtain a suitable cell chassis is frequent in Synthetic Biology. To this end, Obligatory endosymbiotic organisms, whether parasitic or mutualistic, tend to have reduced genomes compared to their free-living relatives, because of the evolutionary process called 'genomic reduction syndrome'. Yet, these genomes present a group of genes that are essential to life and constitute what is called a minimal genome. Their characterization, as well as the possibility of optimizing them, by eliminating non-essential genes or by adding genes to complete impaired metabolic pathways, is highly relevant in the field of synthetic biology. However, most endosymbionts are not cultivable, which makes handling difficult. *Bartonella quintana* str Toulouse is a facultative endosymbiont that has the capacity to infect mammalian cells, making this bacterium a good model to design a chassis for potential biomedical applications. However, it has a very slow growth rate due to its complex nutritional requirements. Our objective is to define the ideal medium composition that improves growth efficiency, which will also have an impact on the ease of performing genomic manipulation experiments for a better characterization of the model prior of its use as an endosymbiont chassis. First, we generated a draft of a metabolic model of B. quintana from genomic data from GenBank using ModelSEED. Then, we manually curated the model using databases like BRENDA, KEGG or BlastP and, through flux balance analysis (FBA), we determined which compounds are limiting factors for *Bartonella*'s growth, comparing the results with the composition of commercial media. Next, we established a protocol for culturing this bacterium using media supplemented with these compounds in different concentrations and measured its growth impact using optical density at 600nm (OD600). Finally, we have performed SWATH comparative proteomics studies among different supplemented media to determine changes related to changes in media composition.

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P4-18 Development of multiple sets of eggplant (*Solanum melongena*) introgression lines with crop wild relatives

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Crop wild relatives (CWRs) are a valuable and largely unexploited genetic resource for breeding. We are developing four sets of new eggplant introgression lines (ILs) using as donor parents CWRs from the primary, *Solanum insanum* (INS), secondary, *S. incanum* (INC), and *S. dasyphyllum* (DAS), and tertiary genepool, *S. elaeagnifolium* (ELE). These CWRs were selected for their potential to adapt to stress conditions resulting from climate change with the aim of developing pre-breeding materials ready to be incorporated into breeding pipelines, along with the dissection of the genetics of climate-related and morpho agronomic traits. The first to be developed and the most advanced is the INC ILs set (1), where 93% of the donor genome is covered by 23 pure immortals. These fixed INC ILs together with advanced backcross materials (ABs) allowed the identification of several QTLs for relevant traits (2, 3). Even though the development of the other three ILs sets started together in 2015, their progress is uneven due to the increased complexity of handling phylogenetically more distant crosses. The most advanced is the INS set, where the whole donor genome is covered by 31 pure lines, which represent almost 87% of the INS genome, and five ABs. Regarding the DAS set, 90% of the donor genome is covered by 25 ABs and 15 pure ILs. Similarly, for the ELE set, 11 ILs and 22 ABs also cover almost 90% of the CWR genome. The latter is one of the most distant crosses achieved in the eggplant gene pool and required embryo rescue, representing an extraordinarily valuable pre-breeding material (4). This systematic introgression breeding represents a major contribution to developing resilient eggplant materials with new and improved traits readily available by breeders, while constituting invaluable tools for plant geneticists.

1. Gramazio et al. (2017). Front. Plant Sci. 8:1477
2. Mangino et al. (2020). Agronomy 10(4), 467
3. Rosa-Martínez et al. (2022). Horticultural Plant Journal
4. García-Fortea et al. (2019). Scientia Horticulturae 246:563-573

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P4-19 Raising specific antibodies against *SmMYB113* and *SmGLK2* gene products in *Solanum melongena*

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The accumulation of anthocyanin coupled with a uniform distribution of chlorophylls in the eggplant (*Solanum melongena*) fruit peel, are desirable, as they guarantee homogeneous and dark colouring of the fruits. We identified two candidate QTL, one in chromosome 10 (anthocyanin-related trait) and the other one in chromosome 4 (green fruit netting trait), in a recombinant MAGIC population. As a result, *SmMYB113* and *SmGLK2* genes were selected to raise peptide-based antibodies. The immunogenic peptides for *SmGLK2* (N-terminal) and *SmMYB113* (C-terminal) were designed in the variable protein sequence of the respective gene products. The respective antibodies were developed in rabbit (five immunizations) and purified by affinity chromatography. Finally, the antibodies specificity was tested by western blot and confirmed by LC/MSMS analysis using as positive control the *SmMYB113* and *SmGLK2* proteins from eggplant expressed in *Escherichia coli* and purified with Strep-tag.

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P4-20 A bioinformatic workflow for InDel analysis in the wheat multi-copy α-gliadin gene family engineered with CRISPR/Cas9

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The viscoelastic characteristics of wheat flour are given by gluten proteins, which include monomeric gliadins and polymeric glutenins (1). However, gluten proteins also activate the immune response in certain human pathologies such as celiac disease (CD). The gene editing technology known as CRISPR/Cas has been successfully implemented for the knock-out of α-gliadin genes in wheat (2), and bread and durum wheat lines with low gluten and low stimulatory capacity to trigger CD have been developed. Nevertheless, these genes have multiple, highly homologous copies that are arranged in tandem in the A, B, and D subgenomes, making the mutation analysis of these genes challenging. In this work, we developed an NGS amplicon sequencing-based bioinformatic pipeline for the analysis of insertions and deletions (InDels) in the α-gliadin genes targeted with two single guides RNA (sgRNA) (3). This method enables the identification of mutations and the analysis of InDels by comparing the mutated amplicons to the most similar wild-type parental sequence. In order to study the prevalence of each InDel over successive generations and observe the effects of the segregation of the Cas9 coding sequence in various lines, TMM normalization was carried out for inter-sample comparisons. This workflow enables a fast characterization of mutations in multiple genotypes and guides RNAs, and in several generations, for a multi-copy gene family.

1. García-Molina et al. (2019) Nutrients, 11, 487.
2. Sánchez-León et al. (2018) Plant Biotechnol. J. 16, 902–910.
3. Guzmán-López et al. (2021) Int. J. Mol. Sci. 22 (23), 13076.

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P4-21 Evaluation of heterosis for agronomic and quality traits in Spanish peppers (*Capsicum annuum* L.)

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Capsicum annuum L. is valued for its organoleptic properties and for being an interesting source of biochemical compounds (1,2). Due to the great diversity of ecotypes available in Spain, four experimental hybrids were produced combining the Spanish cultivars Bierzo, Najerano, BGV5126 and BGV10582 with a breeding line that acts as a donor of *L4* and *Tsw* resistance genes against the Pepper mild mottle virus (PMMoV) and Tomato spotted wilt virus (TSWV), respectively. The phenomena of heterosis (i.e. midparent value as reference) and heterobeltiosis (i.e. best performing parent as reference) were evaluated for yield, precocity as well as ascorbic acid (AsA) and total soluble sugars (TSS) contents under organic farming in Murcia, Spain. The hybrid BGV10582x275 showed significant values of heterosis (52.1%) and heterobeltiosis (46.6%) for yield. Precocity also revealed the highest heterosis (123.8%) and heterobeltiosis (112.2%) values in BGV10582x275, though Najerano showed a significant precocity respect to its hybrid, that revealed an heterobeltiosis value of -58.6%. Regarding AsA levels, significant heterosis and heterobeltiosis values of 24.8% and 21.8% were observed in BGV5126x275; whereas 39.4% and 24.1% were detected, respectively, in BGV10582x275. However, TSS did not reveal significant positive values of heterosis or heterobeltiosis in any hybrid compared to the corresponding parent lines. These results show the potential of Spanish ecotypes to be introduced in Capsicum breeding programs for exploiting the genetic phenomena of heterosis and/or heterobeltiosis under low input conditions.

1. Jadczak et al. (2010). Journal of Elementology 15, 509–515.
2. Materska et al. (2005). Journal of Agricultural and Food Chemistry 53, 1750–1756.

This work was supported by the Agencia Estatal de Investigación (AEI) through the project PID2019-110221RR-C32 and the grant CIPROM/2021/020 funded by Generalitat Valenciana (GVA). M. Jiménez thanks the Ministry of Universities for her grant FPU20/03486.

P4-22 Complete genome sequence of *Passiflora chlorosis virus* from passion fruit

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We present the first complete genome sequence of an isolate of *Passiflora chlorosis virus* (PaCV) identified in passion fruit (*Passiflora edulis* Sims) plants grown in Israel. PaCV is a member of the *Potyviridae* family whose assembled genome comprises 9672 nucleotides and encodes a polyprotein of 3084 amino acids predicted to be proteolytically cleaved into 10 mature peptides. PaCV genome sequence shares a 68.5% nucleotide sequence identity and a 71.5% amino acid sequence identity with isolates of the bean common mosaic necrosis virus (BCMNV), the most closely related virus within the *Potyvirus* genus. Using the genome sequence, we designed oligonucleotides that have allowed us to detect the virus in samples from different tissues of infected passion fruit plants using quantitative RT-PCR. The availability of this complete sequence has allowed us to gain insight into the genome structure and phylogenetic relationships of this virus, which demonstrate that PaCV is correctly classified as a distinct species in the genus *Potyvirus*.

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P4-23 Epitranscriptomic modifications in *hairplus*, a trichome mutant affected in a genome-wide methylation regulatory gene

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Trichomes are specialized epidermal cells developed on the aerial surface of almost all terrestrial plants. These structures constitute true biofactories that produce complex molecules toxic to the pests (glandular trichomes) and act as physical barriers that prevent pest dissemination (non-glandular trichomes). One of the main regulators of tomato (*Solanum lycopersicum* L.) glandular trichomes formation is the *HAIRPLUS* (*HAP*) gene, whose loss of function gives rise to modifications in the epigenome of mutant plants which causes an increase in the density of type I glandular trichomes (1). Epitranscriptomic changes consist in post-transcriptional base modifications in messenger RNA (mRNA), and the most common, reversible epitranscriptomic marks, is adenine methylation to form N6-methyladenosine (m6A). The epitranscriptome regulate various aspects of RNA metabolism such as translation and degradation, and in some plant species they have shown to be responsible for alterations in developmental processes such as trichome branching and morphology in *Arabidopsis thaliana* L. and *Populus spp*. (2). In this work, we have studied the transcriptomic and epitranscriptomic modifications caused by the *hap* mutation in two mutant backgrounds and two organs (leaves and floral stems) with different trichome densities. The epitranscriptomic analysis was carried out on RNA isolated from three biological replicates of each genotype and was performed by direct RNA sequencing using Oxford Nanopore Technologies on a MinION sequencer. Sequencing analysis was carried out using the MinKNOW software with a protocol specific for direct reads of whole mRNA molecules (3). The results obtained demonstrate that *hap* loss of function alleles show epitranscriptomic changes in genes of several metabolic networks and will contribute to shed light on the complex molecular mechanism that uses *HAP* to regulates glandular trichome formation in tomato.

(1) Fonseca, R., Capel, C., Yuste-Lisbona, F. J., Quispe, J. L., Gómez-Martín, C., Lebrón, R., ... & Capel, J. (2022). Functional characterization of the tomato HAIRPLUS gene reveals the implication of the epigenome in the control of glandular trichome formation. *Horticulture Research*, *9*.
(2) Shoaib, Y., Usman, B., Kang, H., & Jung, K. H. (2022). Epitranscriptomics: An Additional Regulatory Layer in Plants' Development and Stress Response. *Plants*, *11*(8), 1033.
(3) Parker, M. T., Knop, K., Sherwood, A. V., Schurch, N. J., Mackinnon, K., Gould, P. D., ... & Simpson, G. G. (2020). Nanopore direct RNA sequencing maps the complexity of Arabidopsis mRNA processing and m6A modification. *Elife*, *9*, e49658.

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P4-24 Introgression of the resistances derived from TGR-1551 in a "Piel de Sapo" genetic background

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Watermelon mosaic virus (WMV), *Cucurbit yellow stunting disorder virus* (CYSDV) and powdery mildew (*Podosphaera xanthii* (Castagne) Braun) are three of the most important pathogens affecting melon (*Cucumis melo* L.) cultivation. The African accession TGR-1551 has been described as a reliable resistance source against all of them [1–3]. The availability of molecular markers tightly linked to the resistance genes derived from TGR-1551 has allowed the beginning of a breeding program to introgress the three resistances into a "Piel de sapo" (PS) genetic background. Two BC_4 plants derived from an initial cross between TGR-1551 and the yellow melon type "Bola de oro", that had the introgressions associated with the resistances, were selected as donor parents and crossed to the PS accession BGCM-126. To date, BC_3 plants have been obtained and, for each offspring, molecular marker assisted selection has been carried out. Fruits from the selected F_1, BC_1 and BC_2 plants, carrying the resistances, showed good morphological characteristics and organoleptic quality. Moreover, throughout the studied generations, a significant progress can be observed in the recovery of the fruit characteristics and the profiles of acids and sugars of the recurrent parental line. Indeed, the studied BC_2 fruits had fully recovered the organoleptic quality of BGCM-126, reaching a higher sugar content. The available offspring will be the basis for the development of quality commercial PS varieties incorporating TGR-1551 derived resistances.

1. Pérez-De-castro et al. (2020) Int J Mol Sci 21, 5970.
2. Pérez-de-Castro et al. (2019) Mol Breed. 39, 1-15
3. López-Martín et al (2022) Int J Mol Sci. 23, 12553.

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P4-25 Phenotyping of reproductive characters in a new dwarf variety of *Solanum melongena*

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Common eggplant (*Solanum melongena*) is an important Solanaceous crop. In the last years, its production has increased worldwide, becoming the third most cultivated Solanaceous plant after potato and tomato. However, compared with other Solanaceous species, research at the molecular level is less developed. Among other reasons, eggplant plants are large and take several months to reach the reproductive phase. Therefore, it is difficult to grow them in growing chambers to achieve the level of environmental control required for certain molecular biology experiments.

Recently in our lab, a dwarf variety of eggplant was obtained resulting from a cross between *Solanum melongena* L. and *Solanum anguivi* Lam. Beyond its reduced size, this variety displays early flowering with respect to normal-sized eggplants and other characteristics that make it more amenable for molecular studies.

In this work, we have analyzed its flowering behavior (number of flower clusters, number of flowers per cluster) and floral characters related with fertility (style length and pollen/ovule fertility) in control and heat stress conditions, to evaluate its usefulness for reproductive and abiotic stress studies at the molecular level in eggplant. We have found that this new variety holds potential as a model plant for the evaluation of reproductive characters in eggplant.

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P4-26 Genetic analysis of the interspecific reproductive barriers in *Cucumis* L.

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Pre- and post-zygotic interspecific reproductive barriers (IRBs) contribute to reproductive isolation in *Cucumis* L. by acting sequentially (1). Moreover, IRBs in this genus constitute a complex network that may include incompatibility and incongruity overlapping processes. So while wild *Cucumis* species have interesting traits for breeding of cultivated ones (e.g. melon and cucumber), IRBs prevent genetic exchange between them. Dissection of the genetic basis underlying IRBs in *Cucumis* may eventually facilitate overcoming of those barriers and the use of wild germplasm for breeding. With this purpose, we generated a segregating backcross population derived from an interspecific cross between two wild species, *C. dipsaceus* Ehrenb. ex Spach and *C. pustulatus* Hook.f., showing unilateral cross-incompatibility that consist of 88 individuals. Then, a Genotyping by Sequencing (GBS) approach was followed to produce a set of 1.384 filtered SNP markers that were used to develop a high saturated linkage map for this population. Phenotyping of pre- and post-zygotic IRBs was performed by recording pollen tube growth using fluorescence microscopy and by evaluating fruit and seed set, respectively. Preliminary results show the presence of genomic rearrangements, including inversions, and segregating distortion regions that may be related to IRBs. Notwithstanding, further research will be necessary to confirm these associations.

1. Ferriol et al. (2023) Plants 12: 926

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P4-27 Unraveling the celiac disease-related immunogenic complexes in a set of wheat and tritordeum genotypes: implications for low-gluten precision breeding in cereal crops

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The development of low-gluten immunogenic cereal varieties is a suitable way to fight the increment of pathologies associated with the consumption of cereals. Although RNAi and CRISPR/Cas technologies were effective in providing low-gluten wheat (1,2), the regulatory framework, particularly in the European Union, is an obstacle to the short- or medium-term implementation of such lines. In the present work, we carried out a high throughput amplicon sequencing of two highly immunogenic complexes of wheat gliadins in a set of bread and durum wheat, and tritordeum genotypes. Bread wheat genotypes harboring the 1BL/1RS translocation were included in the analysis and their amplicons successfully identified. The number of Celiac Disease (CD) epitopes and their abundances were determined in the alpha- and gamma-gliadin amplicons, including 40k-γ-secalin ones. Bread wheat genotypes not containing the 1BL/1RS translocation showed a higher average number of both alpha- and gamma-gliadin epitopes than those containing such translocation. Interestingly, alpha-gliadin amplicons not containing CD epitopes accounted for the highest abundance (around 53%), belonging mainly to the B-subgenome. The alpha- and gamma-gliadin amplicons with the highest number of epitopes were present in the D-subgenome. Although the durum wheat and tritordeum genotypes showed the lowest number of alpha- and gamma-gliadin CD epitopes, two bread wheat genotypes presented low immunogenic potential scores, and one of them lacked the 33-mer, a highly immunogenic peptide in CD (3). Our results allow progress in unraveling the immunogenic complexes of alpha- and gamma-gliadins and can contribute to the development of low-immunogenic varieties within precision breeding programs, by crossing or by CRISPR/Cas gene editing.

(1) Sánchez-León, S., Gil-Humanes, J., Ozuna, C. V., Giménez, M. J., Sousa, C., Voytas, D. F., & Barro, F. (2018). Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant biotechnology journal, 16(4), 902-910.
(2) Gil-Humanes, J., Pistón, F., Tollefsen, S., Sollid, L. M., & Barro, F. (2010). Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. Proceedings of the National Academy of Sciences, 107(39), 17023-17028.
(3) Shan, L., Molberg, Ø., Parrot, I., Hausch, F., Filiz, F., Gray, G. M., Sollid, L. M., & Khosla, C. (2002). Structural basis for gluten intolerance in celiac sprue. *Science*, *297*(5590), 2275-2279.

We acknowledge Instituto Nacional de Tecnología Agropecuaria (INTA) for providing the Argentinian wheat varieties. This project was funded by the Spanish Agencia Estatal de Investigación (PID2019-110847RB-I00 and TED2021-129733B-I00, MCIN /AEI / 10.13039/501100011033), European Union ("NextGenerationEU"/PRTR), and Junta de Andalucía (P20_01005).

P4-28 A New Dwarf Eggplant Line Derived from an Interspecific Backcross Introgression Programme as a Potential Model Plant

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Eggplant (*Solanum melongena*) is a crop of great global importance, ranking fourth in vegetable production (1). Unlike in tomato (*S. lycopersicum*), in which a dwarf tomato variety (Micro-Tom) is available (2), no suitable miniature eggplant cultivar has been developed to be used as a research model plant in this crop. In this study, during the process of introgression breeding of a white-fruited eggplant with the wild eggplant relative *S. anguivi* species, a miniature dwarf plant was identified in the second backcross generation. The selfing of this individual plant was performed and the resulting generation underwent selection for the dwarf phenotype. Subsequently, several rounds of self-fertilization and selection were performed. Eventually, a stabilized miniature eggplant line was selected. This novel line presents distinct morphological and agronomic traits that qualify it as a suitable candidate as a model plant for eggplant research. More specifically, it is characterized, apart from its compact architecture, by semi-determinate and rapid growth, short internodes, multiple inflorescences, and numerous small (2-3 cm long) white fruits. The variety is an early bloomer and is well adapted to growth in small pots (0.5-1.5 L). It flowers under a diverse array of conditions, including full sun or shaded areas, and it can be cultivated either in pots or soil. Due to its characteristics, apart from being a valuable plant model for research on eggplant genetics and physiology, it holds potential as an ornamental crop.

1. FAOSTAT (2021).
2. Scott and Harbaugh (1989). *Florida Agr. Expt. Sta. Circ*. S-370.

This work was supported by PID2021-128148OB-I00 (MCIN/AEI/ 10.13039/501100011033 and "ERDF A way of making Europe"), PDC2022-133513-I00 (MCIN/AEI /10.13039/501100011033 and European Union NextGenerationEU/ PRTR), CIPROM/2021/020 (Generalitat Valenciana, Spain), FPU21/02288 (to MM-L; Spanish Ministerio de Universidades), and RYC2021-031999-I (to PG; MCIN/AEI /10.13039/501100011033 and the European Union through NextGenerationEU/ PRTR).

P4-29 Characterization of novel Cas nucleases from the deep ocean

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The nematode *C. elegans* is an animal model convenient for rapid CRISPR-Cas genome editing experiments[1]. Among other advantages: a single microinjection in the germline can expose dozens of germ cells to CRISPR-Cas9 reagents, the life cycle is short (3 days from embryo to adult), and the hermaphroditism facilitates the generation of gene edits in homozygosis in two weeks. Exploiting these features, we validated the use of Cas9 variants of minimal PAM in animals[2] (in collaboration with Miguel A. Moreno-Mateos lab, Zebrafish's experts).

Next, novel CRISPR-Cas systems were identified by analysing over 100 marine microbial metagenomes collected during Malaspina 2010 Circumnavigation Expedition. After identifying the tracrRNAs for seven novel nucleases, we chose two from the unexplored bathypelagic deep ocean for further characterization. These new enzymes efficiently cut DNA *in vitro* and in human cell lines. At the meeting, we will discuss their enzymatic properties, and their nuclease activity and specificity in editing the *C. elegans* genome.

References:
[1]Vicencio and Cerón (2021) CRISPR Journal. Feb;4(1):32-42.
[2]Vicencio et al, (2022). Nature Communications. May 12;13(1):2601.

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P4-30 FITONET, the social network for plant biodiversity

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The FITONET Operational Group brings together relevant companies (Bullsoft Solutions, UPA, DANUNHA, el Obrador de Creative, Agrosa), foundations (Cellbitech, Cajamar), platforms (BIOVEGEN), and Spanish research centers (PTI AGROFOR from CSIC) to develop tools that will allow the agri-food sector to valorize plant biodiversity. The objective of FITONET is to implement an information transfer system based on collections developed and/or maintained in public centers, increase the genetic diversity of crops, develop a dynamic professional social network, and generate living labs/pilot experiences that demonstrate to farmers, the agri-food sector, and society in general the importance of using biodiversity to obtain sustainable, high-quality, and profitable crops and products. Access to plant genetic resources and improved varieties will be facilitated, promoting greater involvement by farmers, associations, and companies. Demonstrative trials of cereal varieties (maize, rye, spelt), for general consumption and with low gluten content, producing flour and bread from them, vegetables (melon, tomato, and pumpkin), and legumes will be conducted. An IT environment will be developed to support the digital ecosystem, including web and mobile apps and their integration into the NOAH Plant & Breeding platform, implementation of the NOAH Plant & Breeding solution for the characterization of plant materials and the monitoring of agronomic trials. Further information and updates on project progress can be found at https://gofitonet.es/.

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P4-31 Physiological and biochemical differential response of two landraces of *Solanum melongena* to different NaCl concentrations

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Food production is being limited worldwide by increasing abiotic stresses such as soil salinity which affects the most economically important crops (1). New genotypes of these species are being studied for different stresses to find genetic resources related to tolerance to abiotic stresses. Eggplant (*Solanum melongena*) shows a vast variability within the species, and, due to its potential crossability, related varieties could be an interesting tolerance gene resource for new breeding programs (2). In this work, young plants of two eggplant landraces (MEL1, and "Berenjena de Almagro") have been grown to assess their physiological and biochemical responses under salt stress. Growth parameters such as plant height, leaf surface, root length and fresh weight and biochemical parameters such as chlorophylls, ions and proline have been measured in plants irrigated for 21 days with control, 200 mM and 400 mM NaCl. Both genotypes showed a reduction of leaf and root fresh weight, number of leaves, and height with salinity stress. However, 'Berenjena de Almagro' reduction of leaf surface was lower than MEL1 at 200 mM, completely difference than root length at 200 mM, which was maintained by MEL1 at 200 mM. Interestingly, regarding photosynthetic pigments, both genotypes performance was significantly different for all conditions tested. Regarding ion content, MEL1 and 'Berenjena de Almagro' accumulated similar Cl^- levels for leaves and roots, but significantly different levels of K^+ and Na^+. For stress responding osmolytes as Proline, H_2O_2 and Total sugar content, 'Berenjena de Almagro' showed higher accumulation than MEL1. These results show significant differences between the two eggplant genotypes based on a different response to salt. Further studies would be necessary to establish the genetic base of this diversity. This work compares physiological and biochemical parameters of two cultivated eggplant landraces (MEL1 and 'Berenjena de Almagro') under salinity stress to find available genetic resources for future breeding programs which aim to cope with a dynamic environment related to climate change.

1. FAO (2022). Available online: https://www.fao.org/statistics/es/.
2. Plazas et al. (2016) J. Amer. Soc. Hort. Sci. 141(1):34–44.

This work is supported by project AICO/2020/174 and CIPROM/2021/020 funded by Conselleria d'Innovació, Universitats, Ciència i Societat Digital (Generalitat Valenciana, Spain), and contract FPU19/04080 funded by Ministerio de Educación y Formación Profesional (Gobierno de España). The researchers acknowledge the plant material provided by the group of Dr. Prof. Jaime Prohens.

P4-32 Identification of a tomato insertional mutant (*Sed-2702*) that exhibits increased salinity tolerance

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Increasing the resilience of crops is a priority and urgent challenge in the current climate crisis. One of the main challenges in this regard is to obtain crops that are more tolerant to abiotic stresses, but to make significant progress, it is necessary to find more tolerant mutants and identify the genes that determine greater tolerance. Genetic analysis of mutants is one of the most successful strategies for identifying and analyzing new gene functions. Indeed, the characterization of a mutant provides valuable information on the function of the mutated gene and can be the preliminary step to cloning and functional analysis of that gene. In our laboratory, we have identified a mutant, named *Senescence delay-2702* (*Sed-2702*), that exhibits increased tolerance to salt stress. Co-segregation analysis between the T-DNA and the mutant phenotype allowed us to determine that the *Sed-2702* mutant is insertional. *In vitro*, we saw that hemizygous plants for the mutation were identical to WT, while those homozygous exhibited more drastic phenotyping, characterized by loss of apical dominance and uncontrolled axillary bud development. These results suggest that T-DNA promotes additive-type effects. We also found that hemizygous plants exhibited hardly any leaf senescence symptoms after a long period of *in vitro* culture. Both traits, that is, the senescence delayed of the hemizygous plants and excess axillary development of the homozygotes, suggest that T-DNA alters some tomato regulatory gene that modulates cytokinin synthesis. Indeed, endogenous hormone level analysis determined that the *Sed-2702* mutant exhibits significant increases in the content of 3 major cytokinins, namely dihydroxysizeatin with 425% increase, isopentenyladenine with 516.67% increase and transzeatin with 236.84% increase. Since cytokinins play an essential role in plant adaptation under saline conditions (1), we evaluated the behavior of hemizygous and WT plants under salt conditions and found that the hemizygotes showed higher tolerance to salinity. Therefore, the results of this work suggest that the higher endogenous cytokinin level of the mutant determines a better performance under certain stress conditions. Molecular identification of the mutation could allow the study of a new pathway related to cytokinin modulation and tolerance to abiotic stresses.

1. Yu et al (2022). Journal of Plant Growth Regulation 41:2279–2291.

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P4-33 Genomic tools to accelerate apricot breeding programs

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Apricot breeding, like for other stone fruits, is a challenging task due to the specific physiology of this species. As for other fruit trees, this process is hampered by the prolonged juvenile period that increases the length of the breeding cycle, the high land requirements and the influence of environmental factors, among others. During last decades, to cope with Sharka disease, same few North American resistant varieties have been used as donors in all apricot breeding programs worldwide (1). However, along with the viral resistance, these cultivars also provide other less desirable traits, such as self-incompatibility or poor adaptation to temperate climates. Subsequently, it is essential to develop genomic tools in order to accelerate the breeding programs. For this purpose, we are combining genomic and phenotypic data in order to detect QTLs/genes controlling important traits for breeding and to develop markers for assisted selection (MAS), following a similar strategy to the case of PPV resistance *ParPMC* (2,3) and the self-compatibility *ParMDO* genes (4). First, we made a genome-wide SNP identification using the Genotyping-by-Sequencing technique in order to construct a high density map in apricot. The mapping population ('GxCa') derives from a cross between the North American cultivar 'Goldrich' and the traditional Spanish cultivar 'Canino'. In total, about 4.6M SNPs were detected in the 126 individuals sequenced, using the peach genome (v2.0a) as reference (5). After an exhaustive filtering process, a set of 1,891 high quality SNPs were firstly selected for mapping. Finally, 582 and 569 SNPs were mapped into eight linkage groups in two separate maps for 'Goldrich' and 'Canino', spanning 600.45 and 772.18 cM, respectively. Regarding the phenotypes, 'GxCa' population segregates for several agronomic traits that could have a direct impact on productivity. In this sense, we are screening some reproductive-related traits and also others related with tree architecture with the use of images to increase the high-throughput and detailed precision of the phenotypes obtained. Finally, combination of both data will pave the way to the identification of QTLs/genes and the implementation of MAS in order to improve apricot breeding efficiency.

1. Martínez-Gómez et al. (2000) Agronomie, 20, 407-422.
2. Zuriaga et al. (2018) BMC Plant Biology, 18:25.
3. Polo-Oltra et al. (2020) Agronomy, 10, 1292.
4. Muñoz-Sanz et al. (2017) Journal of Experimental Botany, 68, 18.
5. Verde et al. (2017) BMC Genomics, 18:225.

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P4-34 Determination of the volatile compounds profile in several *Brassica oleracea* materials

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The cultivation of *Brassica oleracea*, which includes cabbage, cauliflower, and broccoli, is an important part of traditional European agriculture. The acceptance of the new varieties of these products by consumers depends to a large extent on their specific taste and flavor, which is determined by the volatile metabolites produced by the plant (1). Furthermore, many of these volatile metabolites have been associated with benefits to human health. However, the link between plant species/varieties and their volatile phytochemical emission profiles remains largely unknown (2, 3). To address this issue, this study focuses on the determination and characterization of the profile of volatile compounds in 23 Brassica materials, both commercial and pre-commercial, using the HS-SPME (headspace-solid phase microextraction) technique and gas chromatography (GC-MS). The results obtained show a wide variation in the profiles of volatile compounds among the studied materials. Furthermore, principal component analysis (PCA) showed that the profiles of these compounds can clearly distinguish between most of the 23 Brassicaceae genotypes and between varietal groups. A group of volatile compounds that present significant differences between genotypes was also identified, thanks to multivariate analysis. These results extend our knowledge about the profile of phytochemicals present in this group of plants, which can be used for a better valorization of them.

1. Bell et al. (2017). Food Chemistry 218, 181-191
2. Guijarro-Real et al. (2019). Food Research International 121, 765-775
3. Wei et al. (2021). Food Chemistry 340, 128-166

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P4-35 Virus-induced gene silencing of *MLO* genes in melon

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Powdery mildew (PM) is one of the main limiting factors of melon (*Cucumis melo* L.) cultivation worldwide. *Podosphaera xanthii* is the most common causal agent of PM in warmer conuntries. One of the current breeding objectives is to obtain cultivars resistant to this pathogen (1). Resistance has been identified in different genetic sources (2). Moreover, *Mildew Locus O* (*MLO*) genes have been reported as susceptibility factors in PM disease. In this study, virus-induced gene silencing (VIGS) has been used to down-regulate two *MLO* genes, MELO3C012438 and MELO3C005044, located in chromosomes 10 and 12, respectively. Two resistant accessions, PI 414723 and PI 482420, and two susceptible, 'Piel de Sapo' and 'Amarillo' types, were included in the assay. The vector for the VIGS analysis was based in a mild isolate of watermelon mosaic virus. A construct expressing a phytoene synthase that induces yellow pigmentation was used as control. Plants were agroinoculated with the VIGS vector and seven days later they were inoculated with *Podsophaera xanthii* race 3.5 severe. Symptoms were evaluated 7 and 14 days after inoculation with the fungus. Expression of the two *MLO* genes was measured by RT-qPCR before agroinoculation (T0), before inoculation with the fungus (T1) and at 7 (T2) and 14 (T3) days after inoculation. The two resistant accessions remained asymptomatic in all treatments. Symptoms in the "Piel de Sapo' melon were lower than in the control for the plants in which the VGIS vector targeted the *MLO* gene in chromosome 12 (MELO3C005044), but not for those targeting the *MLO* in chromosome 10 (MELO3C012438). The symptoms in the susceptible accession 'Amarillo' type agroinoculated with VIGS vectors to target both *MLO* genes were lower than in the control. Analysis of the expression of the *MLO* genes in the different genotypes and treatments suggested that silencing of *MLO* in chromosome 10 was associated with a reduction in symptoms in the 'Amarillo' melon.

1. Cui, L., et al. (2022). Horticultural Plant Journal, 8(5), 545–561.
2. López-Martín, M., et al. (2022). International Journal of Molecular Sciences, 23(20), 12553.

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P4-36 Characterization of an allelic series reveals a novel role for *FALSIFLORA* as a regulator of floral determinacy in tomato

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Tomato (*Solanum lycopersicum* L.) has a sympodial growth pattern, so that after floral transition, the shoot apical meristem becomes determined to form a vegetative sympodial meristem (SYM) and an inflorescence meristem (IM), and from the latter one the floral meristem (FM) is differentiated. Once a set number of floral organs have been initiated in the FM, stem cell activity is arrested to form a fully developed flower, a process known as FM determinacy (1). Prior to flower development, the tomato ortholog of the Arabidopsis *LEAFY* gene, *FALSIFLORA* (*FA*), promotes floral transition and determines FM identity (2). To better understand the *FA* functional role in reproductive development, we performed genetic and molecular characterization of an allelic series at the *FA* locus. All *fa* mutants exhibited delayed flowering and highly branched inflorescences, but they also displayed phenotypic variations in floral organ development. The *fa-1* knockout mutant inflorescences only developed vegetative meristems leading to leaf primordia. In contrast, the inflorescences of *fa-3* and *fa-3754* mutants, which bore non-synonymous variants, developed flowers with a reduced number of organs, displaying varying degrees of homeotic conversions. Despite these alterations, both *fa-3* and *fa-3754* mutants occasionally produced fruits, although they showed determinate and indeterminate growth pattern, respectively. Therefore, FM activity in *fa-3754* was not arrested as in wild-type plants, rather an ectopic SYM emerged from inside of the indeterminate fruit, resulting in the development of new leaves and inflorescences. We used CRISPR/Cas9 technology to generate diverse *cis*-regulatory alleles by editing the *FA* promoter sequence. As a result, CRISPR/Cas9 lines with reduced *FA* expression levels bearing deletions in the promoter region were identified. These variants developed highly branched inflorescences with flowers containing a reduced number of organs and homeotic conversions. Remarkably, in some of these edited flowers a new SYM is formed at the base of sepals, a phenotype that differed from that observed in plants bearing mutations at the *FA* coding sequence. Together, results indicate a novel role of *FA* in FM determinacy contributing to arrest stem cell activity, which highlight the importance of allelic series for characterizing gene function in specific developmental pathways.

1. Lozano et al. (2009). The International Journal of Developmental Biology 53, 1635–1648.
2. Molinero-Rosales et al. (1999). The Plant Journal 20, 685–693.

This work was supported by the PID2019-110833RB-C31 (MICI/AEI/FEDER, UE) research project.

P4-37 Genotyping of a 'Valenciana' tomato collection with SPET markers

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Single Primer Enrichment Technology (SPET) is a targeted genotyping strategy that relies on the sequencing of primer-flanking regions. The sequencing of regions nearby SNPs previously selected enables finding new closely linked SNPs that are of interest for performing diversity studies and allow the identification of redundancies in genebanks, among other interesting applications (1). Using this technology, a collection of 51 landraces of 'Valenciana' tomato from across the Comunitat Valenciana (Spain) territory were analyzed to determine the genetic variability. A first insight into the genetic variability amongst the whole collection indicates that at a genetic level, the main differences amongst the landraces are determined by the presence of introgressed resistance genes. This clearly generates two differentiated groups in a Principal Component Analysis (PCA); the first one includes the traditional varieties with some controls, while the second encompasses a group of improved landraces of the 'El Perelló' subtype, that has been subjected to breeding for introduction of genes of resistance. By zooming into the traditional varieties group it is possible to detect variability, associated to morphological differences. A new PCA with SPET data in this group showed that varieties group according to shape and color. The results provide insight into the genetic diversity of the tomato landraces of the Comunitat Valenciana and has implications for the conservation and enhancement of these local tomato materials.

References:
1. Barchi et al. (2019). Front. Plant Sci. 10:1005

This study forms part of the AGROALNEXT programme (AGROALNEXT/2022/006; MERESTOV project) and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana.

P4-38 Typification and differentiation of the pepper (*Capsicum annuum*) local variety 'Piparra Dolça d'Anna'

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Pepper (*Capsicum annuum*) is one of the most economically important vegetables in our country. In recent years, traditional varieties of vegetables have gained importance, increasingly valued by consumers based on their organoleptic and quality characteristics. In this sense, the variety of "guindilla" pepper 'Piparra Dolça d'Anna', a local variety from the municipality of Anna (València) is increasingly cultivated in the Valencian county of La Canal de Navarrés. Currently about 3 hectares are cultivated with a production of about 150 t. In the present work we performed the typification of the 'Piparra Dolça d'Anna' by means of its characterization compared with other four well-known varieties of "guindilla" type pepper. For this, the five varieties of pepper have been evaluated with 35 morphological/agronomic and 16 composition descriptors. Of each variety, 3 blocks of 4 plants were cultivated in a farm in the town of Anna. Out of the 35 morphological/agronomic characters, no significant differences were observed for 20 of them. Significant differences were found in the height of the plant and the length of the stem, with 'Piparra Dolça d'Anna' displaying values similar to the variety 'Florian'. There were also significant differences regarding tillering, flower position and stigmatic exertion. However, the most important differences at the fruit level were for color (parameter L and b). Also, it is remarkable that 'Piparra Dolça d'Anna', 'Florián' and 'Guindilla Dulce' were not pungent, while 'Ibarroria' and 'Guindilla Piparra Ibarra' had some pungency. Regarding the composition characteristics, there were significant differences in 6 of the 16 characters evaluated. The most important differences were for of sucrose content and especially flavonoids, compounds with high antioxidant power (1), for which our 'Piparra Dolça d'Anna' presented the highest levels. In conclusion, the local variety 'Piparra Dolça d'Anna' is differentiated from other varieties of the "guindilla" type and is characterized by having plants with a high level of fruit set and yield, fruits with a characteristic greenish hue, lack of pungency and a high content of sucrose and especially flavonoids. This last characteristic can help a lot in its positioning as a local pepper variety of great interest due to its compositional characteristics.

1. Cho, S.H. et al. 2020. Antioxidants, 9:986.

Acknowledgements: CIPROM/2021/020 (Generalitat Valenciana, Spain).

P4-39 New approaches to investigate *de novo organ* formation in tomato explants

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The cultivated tomato (*Solanum lycopersicum*) is one of the most important horticultural crops in the world. The optimization of culture media for callus formation and tissue regeneration of different tomato genotypes presents numerous biotechnological applications. In this work, we have analysed the effect of different concentrations of zeatin and indole-3-acetic acid on the regeneration of explants in 'M82' and 'Micro-Tom' tomato cultivars. We evaluated regeneration parameters such as the percentage of callus formation, the area of the formed calluses, as well as the initiation percentage and the number of adventitious stems. The best hormonal combination generates globular structures after two weeks. We observed the formation of leaf primordia from these structures at about three weeks. After passing the regenerating micro-stems to a specific growth medium, it was possible to obtain whole seedlings between four and six weeks. This procedure has been applied to characterize regeneration in eight additional genotypes of *S. lycopersicum*, including other commercial varieties, mutants and ancestral tomato varieties. Our method is suitable for obtaining many seedlings of different tomato genotypes from cotyledon explants in a very short period of time, with direct applications for transformation with *Agrobacterium tumefaciens* and the use of gene editing techniques.

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SESIÓN 5: GENÉTICA DE POBLACIONES Y EVOLUCIÓN

Ponencia invitada:

I5-01 The population and evolutionary genomics of bacteria

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The population and evolutionary genetics field has experienced two major revolutions in the past decades: the development of coalescence theory and the implementation of high-throughput sequencing (HTS) techniques. Both developments have represented a game changer to understand the current distribution of genetic variation in bacterial populations and relate them to epidemiological and evolutionary events in the recent and distant past. This has been a major goal of our laboratory in the past decades. For this, we have used HTS for obtaining whole genome sequences (WGS) of clinical and environmental isolates of an array of bacteria and, along with information downloaded from public databases, we have combined population genomics and evolutionary inference to fundamental problems (what has been the role of recombination/horizontal gene transfer in the evolution of a particular pathogen?, how important is selection along the evolution of a new species?, when can we say that the pangenome of a species is closed or open?) and applied questions (can we use WGS of bacteria in a forensic problem?, can we understand what has driven the spread of antimicrobial resistance in a particular location?) Despite their smaller size, the analysis of bacterial genomes is not free of problems and difficulties and the sheer number of sequences available make many of these even harder to solve. If we ignore them, our conclusions will likely be strongly affected. Here, I will present some of the results we have obtained in the analysis of bacterial pathogens, ranging from ancient genomes of *Treponema pallidum*, the causative agent of syphilis, to modern lineages of *Klebsiella pneumoniae*, a major driver in the current pandemic of antimicrobial resistance, along with forensic analysis of the sexually transmitted *Neisseria gonorrhoeae* and nosocomial outbreaks of *Pseudomonas aeruginosa*. Altogether, the methods and principles used in these and other analyses are being transformed and implemented in genomic surveillance of pathogens, a game changer approach for the current and future threats of infectious diseases. Our results have provided a better understanding of the processes driving population and evolutionary change at the genome level of bacteria and they have resulted in actionable information for public health.

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Comunicaciones orales:

O5-01 Distinct transcriptional responses to acute and chronic oxidative stress in *Saccharomyces cerevisiae*

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By-products of aerobic respiration, like H_2O_2 or reactive oxygen species, can generate oxidative stress to which organisms have developed different response strategies. An organism responds to stress to maintain the redox homeostasis within the cell, hence, the cellular response to acute and chronic stress may not be the same. In this work, we set out to determine the transcriptional response that the yeast, *Saccharomyces cerevisiae* Y06240, has to this common stress of oxidation both during acute and chronic exposure. We determine the transcriptional response (RNAseq) and fitness effects of *S. cerevisiae* populations to oxidative stress (induced by hydrogen peroxide supplemented medium) compared to normal growth conditions (in YPD). The chronic response is obtained by evolving populations of *S. cerevisiae* under continuous exposure to oxidative stress for 66 generations (10 passages of 10% bottlenecks), whereas the acute response was determined under a short induction with hydrogen peroxide (24h, 6.6 generations). In previous studies, we have observed that anciently duplicated genes (small-scale and whole-genome duplicates) are particularly transcriptional plastic and are often at the centre of stress responses (osmotic and acidic stress, coupled with alternative non-fermentative carbon sources), showing a core of altered genes linked to this general oxidative stress response. We, therefore, have a special interest in duplicated genes. Overall, our analyses show that there is a clear difference in the transcriptional response to acute and chronic oxidative stress, with duplicated genes playing a bigger role in acute than in chronic response.

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O5-02 Understanding the genomic basis of adaptation: Lessons from the island radiation of the spider genus *Dysdera*

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Spiders of the genus Dysdera have undergone a spectacular colonization and recent species diversification in the Canary Islands, where we can find up to 60 endemic species. This rapid radiation has been associated with repeated events of dietary shifts related to different preferences on woodlice. Currently, we have morphological, behavioral, metabolic and transcriptomic evidence that this process is adaptive (1). However, until recently, the genomic basis is largely unexplored. To gain insight into the molecular basis of this candidate adaptive radiation, we performed a comparative genomic analysis across high-quality complete genome sequences (three at the chromosome-level) of six different species, comprising five Canary islands endemic plus one continental species. Remarkably, and despite belonging to the same genus, endemic species have a much smaller genome (1.7 Gb) compared to the species from the continent (3.3 Gb), likely as a result of a reduction of their genome size (2,3). Here, we have investigated the role of different genomic elements (protein-coding genes and non-coding regulatory regions, TEs and other repetitive elements, and gene families) in this extraordinary spider radiation. Since this insular radiation is concomitant with dietary shifts in dietary preferences, we predict an impact on gene family size and sequence evolution of the members of chemosensory gene families in these species (4). In this context, the chromosome-scale assemblies and accurate genome annotations generated in this study will improve our knowledge about the role of the chemosensory gene families and other repetitive elements in oceanic island radiations.

1. Vizueta et al. (2019). Mol. Ecol. 28, 4028-4045.
2. Sánchez-Herrero et al. (2019). GigaScience 8, 1-9.
3. Escuer et al. (2022). Mol. Ecol. Res. 22, 375-390.
4. Vizueta et al. (2020). Mol. Biol. Evol. 37, 3601-3615.

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O5-03 Conservation genomics of the Balearic shearwater (*Puffinus mauretanicus*)

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The Balearic shearwater (*Puffinus mauretanicus*) is the most threatened endemic bird species in Europe, with only 3,200 breeding pairs and annual population declines of 7-14%. *P. mauretanicus* and its sister species *P. yelkouan* differ in plumage, size and migratory behavior, but they show little nuclear genetic differentiation. The taxonomic status of these two species is not fully resolved, and it has been hypothesised that they hybridize in the island of Menorca. We generated the first reference genome for *P. mauretanicus*, and analysed whole-genome sequencing data of 36 individuals of both species. We found that *P. mauretanicus* shows stronger population structure than *P. yelkouan*, despite the much broader breeding range of *P. yelkouan*. We also found that the putative hybrid Menorcan population clusters with *P. yelkouan*. Globally, our results uncover a complex evolutionary history, where *P. yelkouan* would have originated from an eastward expansion of *P. mauretanicus*, with extensive gene flow between them. We also identified a gradual decline in the effective population size of *P. mauretanicus* since the colonization of the Balearic Islands by humans (~4 Kya). Nevertheless, the elevated genome-wide heterozygosity levels suggest that this decline has not yet reduced the species' genetic diversity. Finally, genome-wide scans uncovered a large haplotype block (~500kb) that might be involved in the morphological and ecological differentiation between the two species. Overall, our work highlights the application of population genomic data to assess the conservation status of *P. mauretanicus* and provides knowledge which can be translated into evidence-based action plans.

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O5-04 Genetic Insights of Pawpaw (*Asimina triloba* [L.] Dunal), the North American Forgotten Fruit

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Asimina triloba [L.] Dunal (Pawpaw) is a member of the Annonaceae family (the Soursop family), the most diverse family in the early-divergent Magnoliid clade. The family is comprised of around 130 genera and more than 2500 species distributed globally in tropical to subtropical regions. A single exception to the tropical distribution is the *Asimina* genus which is by-far the most northerly representative of the family. The *Asimina* genus is unique in the Soursop family as it is native to temperate regions of North America. *A. triloba* is the most widespread species of the Asimina genus; found in 26 states covering the entire eastern coast of the United States of America. Indeed, pawpaw can even be found growing in wild patches as far north as southern Ontario, Canada, where it the only species in the entire Annonaceae family that is known to survive annual minimum temperatures as low as -28.8^{o}C. The pawpaw fruit is very aromatic with ripe fruit having a creamy texture and a flavour combination that is a mixture of banana, mango, cherimoya, and pineapple. They were consumed by native East American tribes. It has been hypothesised that the northward migration of pawpaw has been entirely down to human activity. To test this hypothesis, we have generated a high-quality reference genome (0.89 Gb anchored into 8 pseudomolecules) as well as we have genotyped more than 81 populations (329 individuals) across 20 states. The sampling was possible thanks to a citizen science initiative for which we asked pawpaw afficionados to send us pawpaw leave samples. We have obtained more than 5,000 Single Nucleotide Polymorphisms (SNPs) using Genotyping-By-Sequencing technology (GBS). The population structure analysis has revealed two major clusters (East and West) divided by the Appalachian Mountains. We have not found any significant evidence that the pawpaw population structure has been influenced by the human activities, but we did find some evidence that the river basin are weak drivers of the structure.

Comunicaciones Flash Talk y póster:

FP5-01 Genetic diversity, structure, and dynamics of the European polecat (*Mustela putorius*) in the Iberian Peninsula

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The European polecat (*Mustela putorius*) is a small carnivore that is one of the most unknown mammals of the European fauna. Polecat populations are declining in several regions -including some Spanish and Portuguese- for several reasons. In relation to it, the study of population genetics must be a priority in conservation of threatened and declining species because it provides essential information for their management. However, the genetic characteristics and dynamics of the European polecat in the Iberian Peninsula has never been studied so far, leaving an enormous gap in the knowledge of such species. In this work, we analysed a complete panel of microsatellite loci in more than 200 samples, obtained from road-killed individuals across its whole natural distribution across Spain. Results include, for the first time, the description of its genetic population structure and variability, the gene flow among them, as well as bottleneck and inbreeding presence.

This work has been supported by the Santander-UCM project ref. PR44/21-29923.

FP5-02 DNA-based species delimitation analyses reveal extensive lineage diversity in *Haploginglymus*, a groundwater amphipod genus endemic to the Iberian Peninsula

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Groundwater ecosystems remain mostly unexplored and are inhabited by a rich but poorly known stygofauna among which the amphipods stand out as one of the most abundant, widespread and taxonomically diverse groups (1). The amphipod genus *Haploginglymus* is endemic to the fresh inland subterranean waters of the Iberian Peninsula (Spain and Portugal) where it dwells in caves, wells and the interstitial medium associated to riverbanks (2). Despite its almost generalized distribution across Iberia, only six *Haploginglymus* species have been formally described thus far (3-8). In this study we have sampled *Haploginglymus* representatives from the main river basins of the Iberian territory to characterize the phylogenetic diversity of the group. We have inferred their phylogenetic history and performed molecular species delimitation analyses based on cytochrome c oxidase subunit I mtDNA sequences under three different approaches: mPTP (9), GMYC (10), and ABGD (11). The results reveal an unexpected number of undescribed *Haploginglymus* species ranging from 14 to 15 molecular inferred entities by mPTP and ABGD, respectively, to 35 species according to the GMYC analyses. Our findings reveal the existence of a hidden diversity of *Haploginglymus* lineages in the Iberian groundwater systems where virtually each sampled locality hosts a new species.

1. Holsinger (1994). Hydrobiologia 287, 131-145.
2. Notenboom (1990). Limnetica 6, 165-176.
3. Mateus & Oliveira-Mateus (1958). Publicaçoes do Instituto de Zoologia 'Dr. Augusto Nobre' 59, 5-15.
4. Stock (1980). Bulletin Zoologisch Museum Universiteit van Amsterdam 7, 141-144.
5. Karaman (1986). Poljepriveda i šumarstvo 32, 75-90.
6. Pretus & Sabater (1990). Stygologia 5, 143-152.
7. Iannilli et al. (2009). Bollettino del Museo Civico di Storia Naturale di Verona Botanica Zoologia 33, 105-112.
8. Jurado-Rivera et al. (2017). Contributions to Zoology 86, 239-260.
9. Zhang et al. (2013). Bioinformatics 29, 2869-2876.
10. Fujisawa & Barraclough (2013). Systematic Biology 62, 707-724.
11. Puillandre et al. (2012). Molecular Ecology 21, 1864-1877.

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FP5-03 Mitophylogenomics support the placement of the enigmatic crustacean order Thermosbaenacea within the peracarida

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Thermosbaenaceans are eyeless, unpigmented crustaceans comprising a limited number of species from subterranean waters, most of them reported from the anchialine environment associated to marine coastal areas (1). Their current taxonomic placement within the crustacean superorder Peracarida is debatable since thermosbaenaceans carry their offspring in a dorsal brood pouch, in contrast with the thoracic ventral marsupium displayed by the rest of peracaridans (1,2). To shed light on this issue, we have used high-throughput DNA sequencing methods to characterize the complete mitochondrial genomes of two Thermosbaenacea representatives: *Tethysbaena scabra* (endemic to the Balearic Islands) and *Tulumella grandis* (endemic to the Bahamas). In addition, for comparative purposes we have retrieved 96 representative mitogenomes of several key crustacean taxonomic groups available in GenBank, including Eucarida, Hoplocarida, and Peracarida (orders Amphipoda, Cumacea, Isopoda, Mysidacea and Tanaidacea). Both Maximum Likelihood and Bayesian phylogenetic hypotheses derived from the analysis of the 13 mitochondrial protein-coding genes and the two rRNA genes retrieved the two thermosbaenacean species as a monophyletic clade, and sister to the rest of peracarid orders. The approximately unbiased test (3) to evaluate alternative topologies implemented in IQTREE2 (4) rejected any other possible taxonomic placement of Thermosbaenacea except a sister relationship with Amphipoda. Our study represents the first molecular analysis of the systematic position of thermosbaenaceans based on mitogenomes, supporting the taxonomic placement of this enigmatic group of groundwater crustaceans within the order Peracarida.

1. Jaume (2008). Hydrobiologia 595, 219-224.
2. Richter & Scholtz (2001). Journal of Zoological Systematics and Evolutionary Research 39, 113-136.
3. Shimodaira (2002). Systematic Biology 51, 492-508.
4. Minh et al. (2020). Molecular Biology and Evolution 37, 1530-1534.

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FP5-04 Temporal incongruence between demographic and genetic metrics in fisheries assessment: the European hake case study

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Current analyses aimed to dissect the influence of fishing intensity on relative estimates of the effective genetic population size (*LDNe*) of the southern hake fishery. Demographic data were drawn from official records of ICES while genetic data were obtained from a lustrum-based sampling series between 1975 and 2014. Despite *LDNe* has shown incertitude on the upper limit of some confidence intervals it required a single population sample and incorporated a correction for the downward *Ne* bias caused by low sample sizes (1). Opposite to neutral microsatellites, EST - microsatellites were suboptimal at detecting genetic erosion at the maximum population overharvest in lustrum 1996 – 2000 (2), a phenomenon also confirmed with the molecular variation of the mtDNA cyt *b* gene. We show that the maximum population mortality Z_N_{SSB} (1986 - 1990) predated both, the maximum genetic effective mortality *Z_LDNe* (1991 - 1995) and the maximum cohort-based mortality *Z_ICES* (1996 - 2000). Such temporal uncoupling between demographic and genetic metrics indicates that official cohort analyses are insufficient to assess the real biological status of the southern hake fishery.

1. Waples et al. (2010). Evolutionary Applications, 3(3), 244-262.
2. Pita et al. (2022). Animals 2022, 12(11), 1462.

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FP5-05 Evolution and recovery of the gut microbiota during and after rifampicin treatment in *Blattella germanica*

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The German cockroach *Blattella germanica* is an omnivorous insect in which two symbiotic systems coexist: the obligate endosymbiont *Blattabacterium cuenotii*, which is key in essential amino acid production and nitrogen recycling, and the rich and complex gut microbiota that must play an important role in host physiology. The mode of transmission is different in both systems: *Blattabacterium* is transmitted vertically from the mother to the oocytes, while the gut microbiota is acquired horizontally. In this work, we have developed an experimental design involving one control population and two treated populations during 10 days with 0.02% rifampicin, one of which was supplemented with faeces from the beginning. First, we dissected the hindgut of females every 2 days. Then, rifampicin was removed and the experiment continued to check the recovery of the gut microbiota without antibiotic. To assess the role of coprophagy in restoring the composition of a healthy gut microbiota, the rifampicin-only treated population was divided in two sub-populations, one of which was now supplemented with faeces. We dissected the hindguts of cockroach every 10 days until 60. We analysed bacterial abundances and diversity to study changes in the gut microbiota and its recovery once the antibiotic was removed. Rifampicin treatment altered gut microbiota's alpha and beta-diversity. Furthermore, faeces supplementation from the beginning of the experiment only delayed for two days the effect of rifampicin. However, the supplementation with faeces after removing the antibiotic treatment helped to restore the normal microbiota. Coprophagy thus, seemed to be important but not essential for the complete recovery of the microbiota.

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FP5-06 The role of extracellular vesicles in host-symbiont communication mediated by sRNA in *Blattella germanica*

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Organisms do not live in isolation but rather interact with individuals from other species. Yet, mutualistic stable relationships require an "entente cordiale" among the partners leading to a better fitted life form in which all of them benefit. Symbioses are widespread in eukaryotes, especially in insects. Many of them live in obligate relationship with different ecto- and endosymbiotic bacteria needed to maintain host fitness (1). It is the case of the cockroach *Blattella germanica*, with two symbiotic systems in separated compartments: the endosymbiont *Blattabacterium* in specialized bacteriocytes located in the fat body, and a complex microbiota in the gut lumen. The presence of small RNA molecules (sRNA) has been systematically reported in extracellular environments, including extracellular vesicles (EVs), and has been associated to cell-to-cell communication, as an additional layer of regulatory complexity (2,3). Our goal is to investigate whether sRNAs produced by the insect and/or by the endosymbiont exert a regulatory role related to endosymbiosis, either locally (in the bacteriocyte) or in distant target tissues, reached through the hemolymph inside EVs. In this sense, we have isolated EVs from insect's hemolymph and characterized them by Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy (TEM). Current studies of their cargo by -omics technology will be also presented and their possible role in bacteria-insect communication will be discussed.

References:

4. Baldo et al. (2021). *Genes (Basel)* 12. DOI: 10.3390/genes12030327
5. Wang et al. (2012). *Nature Communications* 9, 2205. DOI: 10.1038/s41467-018-04504-1
6. Bernal et al. (2014). *Journal of Proteomics* 105, 232–241. DOI: 10.1016/j.jprot.2014.02.012

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Comunicaciones póster:

P5-01 Genetic diversity as a tool to evaluate the conservation status of the Comber *Serranus cabrilla* in a Fishery Protection Zone and surrounding fishing areas

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The exploitation of fish species can produce declines in population sizes, resulting in loss of genetic diversity (1). This has led an increase in the number of areas in which the fishing is restricted. In this sense, the Balearic Islands has recently implemented some management measures to protect essential habitats to maintain the biodiversity and conservation of fisheries resources. In 2014, the Menorca Channel (MC) were declared Site of Community Importance (SCI) and in 2016, some areas of the MC were catalogued as Fisheries Protection Zone (FPZ) which implied the prohibition of trawling to protect maërl seabed (2). The Comber *Serranus cabrilla* (Linnaeus 1758) is a demersal species that inhabits maërl, rocky and sandy seabeds (3) and is one of the main species captured by trawler fishing in the Balearic Islands. The objective of this study was to estimate and compare the levels of genetic diversity of *S. cabrilla* between a FPZ and two surrounding fishing areas (SCI and ADJ; adjacent area), as well as to know the connectivity between FPZ and surrounding fishing areas in Balearic Islands, together with other Mediterranean and Atlantic zones. To do that, we used the Cytochrome C Oxidase subunit I (COI; DNA barcode) (4) from 92 individuals collected from three areas during CANAL_04_2022 survey: FPZ (34), SCI (34), and ADJ (24). In addition, 47 samples from other locations of the Balearic Islands, as well as Genbank sequences (95) from other Mediterranean and Atlantic populations were analysed (5-7). In general, nucleotide diversity (COI π) was high in the three areas, being slightly higher in FPZ (π=0.00986) followed by SCI (π=0.00927) and ADJ (π=0.00932). The individuals of *S. cabrilla* from the MC were represented by a total of 18 haplotypes, with 76% of individuals falling into four main haplotypes with no geographical differences. This lack of population genetic structure among areas indicates that there is a high gene flow between the study areas. The high genetic diversity values and the lack of genetic structure confirm the key role of connectivity in maintaining the local population supply, reinforced by the specific function of a Fishery Protection Zone.

Key words: COI, Genetic diversity, mtDNA, Menorca Channel, *Serranus cabrilla*

P5-02 Identification of *Bombus terrestris* subspecies and hybrids in Central and Southeastern Spain

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The buff-tailed bumble bee, *Bombus terrestris,* is a pollinator of crops and wild plants of great ecological and economic importance. In Spain, the native Iberian subspecies *Bombus terrestis lusitanicus* (*BTL*) is widely distributed, whereas *Bombus terrestris terrestris* (*BTT*) is naturally restricted to the Eastern Pyrenees (1). The positive impact of *B. terrestris* on crop yields has prompted an increase in the global trade of domesticated bumble bee colonies used for crop pollination, being *BTT* the most commonly marketed subspecies in Spanish tomato and berry greenhouses. However, it has been demonstrated that *BTT* from managed hives can spillover into nearby natural habitats and breed with native *BTL* populations, leading to local hybridization (2), which poses a threat to biodiversity. The aim of this work was the identification of colonies of these two subspecies and hybrids in natural habitats of Central and Southeastern Spain by morphological characterization and PCR amplification of a fragment of the mitochondrial 16S gene in queens and workers. To carry out this study we have used individuals from wild colonies established in the laboratory from queens captured in the field (Madrid and Almeria provinces). This methodology has allowed us to verify the presence of *BTT* and hybrids in localities at Southeastern Spain where *BTT* commercial colonies are present, indicating that they are colonizing areas outside of their natural distribution. In addition, hybrids between both subspecies were also found in Central Spain, suggesting that local hybridization is spreading to other areas. Remarkably, the determination of the maternal lineage of queens and workers, by the mitochondrial 16S rRNA gene, has allowed us to identify which colonies contained hybrids in cases where morphological identification did not yield a clear result. In order to improve hybrid identification, nuclear markers based on microsatellite amplification are being developed.

1. Ornosa and Ortiz-Sánchez (2004). Fauna Ibérica 23, 556.
2. Seabra et al. (2019). Evolutionary Applications 12, 679-691.

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P5-03 Genetic diversity of a population of *Capra pyrenaica* reintroduced in a protected environment

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Management and conservation of wild animal species are often affected by processes such fragmentation or reintroduction of low number of individuals which might reduce genetic variability at critical levels. In addition, few highly variable DNA sequences are available to develop species specific PCR assays for wild species to be used to evaluate genetic diversity. This is the case of the *Capra pyrenaica,* the Iberian ibex*,* affected by a fragmented pattern of distribution or by their reintroduction in certain locations, as it occurred in National Park Sierra de Guadarrama (PNSG) in the 1990s using a quite limited number of individuals. Few studies have been carried out on these populations using informative fragments of nuclear *drb1* gene, from the Major Histocompatibility Complex (MHC) and the mitochondrial *cytochrome oxidase subunit I* (COI). The results we obtained from a previous study with a representative sample of individuals from PNSG using these two DNA assays had revealed very low level of genetic variability. In the present study we tested two additional genetic analysis on a wider sample of individuals from the same location: an already described mitochondrial *cytochrome b* assay and a new one based on a genomic pseudegene sequence, possibly originated from the functional mitochondrial *cytb* gene (numt). The results indicated that *drb1* and COI markers were the most sensitive for genetic diversity detection. But using any of the available assays, the PNSG population showed lower genetic diversity values than the relict populations of the southern Iberian Peninsula, and a similar genetic structure to the Gredos populations from which the reintroduction in the PNSG took place.

P5-04 Measurement of genomic complexity in Chordata using different genome metrics

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The tendency toward increasing complexity in biological evolution is controversial in biology. Having a complexity measure can help with its resolution. We suggest appealing to genomes to measure complexity because they store information about the biotic and environmental interactions of species in their evolutionary history. The phylum Chordata contains a broad number of genomes completely sequenced, and its phylogeny is established. For this reason, we have focused on this phylum to apply the complexity metrics: Biobit (BB), Genomic Signature (GS), and Sequence Compositional Complexity (SCC)(1). The results were different according to the metrics. The genomic metrics were calculated and correlated with genome size. Biobit appears to correlate positively with genome size at the phylum level. However, the metric has an upward limit related to the genome size. The Genomic Signature presents a positive correlation with the genomic size. This metric clusters the genomes according to the class, although there is a high intra-phyla heterogeneity. In SCC, the results are more elusive. It suggests that this metric is greatly affected by the particular ecology and evolution of the species.

1. Moya, A., Oliver, J.L., Verdú, M. et al. Driven progressive evolution of genome sequence complexity in Cyanobacteria. Sci Rep 10, 19073 (2020). https://doi.org/10.1038/s41598-020-76014-4

CIPROM/2021/042 from Generalitat Valenciana

P5-05 Could Fishery Protection Zones be affecting the genetic diversity of the striped red mullet *Mullus surmuletus*?

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Genetic diversity plays a key role for natural selection allowing species to adapt to new environmental or anthropic conditions, therefore, its loss will decrease species adaptive potential. Because of this, genetic approaches to assess the genetic diversity has been developed, as the sequencing of the mitochondrial Cytochrome C oxidase subunit I (COI) (1), known as "DNA barcode". Different studies demonstrated that the nucleotide diversity of this gene can be used as a proxy for species' conservation status (2,3). Along these lines, the objective of the present study is to evaluate if a Fishery Protection Zone (FPZ) can affect the genetic diversity of the striped red mullet *Mullus surmuletus,* which is one of the most important target species in the trawl fishery developed in Balearic Islands. To do that, the Menorca Channel (MC) was selected as study area. In 2014, this area was declared a Site of Community Importance (SCI) given the number of habitats and consequent biodiversity presented (4). Two years later, some areas of the channel were banned from trawling and were declared Fisheries Protected Zones (FPZ). To compare genetic diversity, 34 and 33 samples were collected from SCI and FPZ, respectively, and also 27 samples form an adjacent zone (ADJ) were studied. In addition, the results from these areas were compared with 99 samples from the Atlantic and Mediterranean locations (48 from Genbank database). Nucleotide diversity value in FPZ (π=0.0033) was the highest in MC, statistically significantly different with SCI area (π=0.0021). Considering the total of the samples, thirty-two haplotypes have been found. Two of these haplotypes contain the majority of samples (69.7% of the diversity in FPZ, 88.2% in SCI and, 66.6% in ADJ). The network suggests that there is no population substructure neither in the Mediterranean area nor including the Atlantic samples indicating high gene flow between studied areas. These results would support the key role of connectivity in maintaining local population status that could have been enhanced by the specific function of a Fisheries Protection Zone (5).

Keywords: COI, *Mullus surmuletus*, nucleotide diversity, Menorca Channel, Balearic Islands

1. Ivanova et al. (2007). Molecular Ecology Notes, 7(4), 544–548
2. Petit-Marty et al. (2021). Conservation Letters, 14(1), e12756.
3. Petit-Marty et a. (2022). Frontiers in Marine Science, 9(May), 1–11.
4. Moranta et al. (2014). Instituto Español de Oceanografía-COB (Palma). Fundación Biodiversidad, Palma.
5. Sahyoun et al. (2016). PLoS ONE, 11(12), 1–15.

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P5-06 Risk of non-adaptedness based on the genetic diversity of *Abies marocana* and *Abies pinsapo* relict forests as warms the world

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The Mediterranean basin is one of the most important biodiversity hotspots, harbouring high rates of endemic plants species (1). Among them, the Moroccan (*Abies marocana* Trab.) and Spanish (*Abies pinsapo* Boiss.) firs are examples of relict trees, endemic from north Morocco and south Spain, respectively (2, 3). Climate change is currently threatening this biodiversity due to global temperature rise and increasing frequency and intensity of extreme drought events (4). As a consequence, the development of reliable conservation strategies is nowadays mandatory to ensure their persistence (5, 6). Genetic studies focused on these conifers may provide a better understanding about the molecular basis of their adaptive capacity. Extensive sampling of tree-level genetic diversity was carried out within the main populations of *A. marocana* and *A. pinsapo* to obtain a genetic matrix based on single nucleotide polymorphisms (SNPs). Methods included the assembly, SNP-calling, and several filtration steps of the sequences obtained using double digestion RAD-seq (ddRAD-seq). The results supported significant genetic differences between *A. marocana* and *A. pinsapo*. Genome-environment association (GEA) and risk of non-adaptedness (RONA) analyses support an impending vulnerability of both tree species, mainly based on their warming sensitivity. Our results highlight urgent challenges for research, management, and policy-making communities to maintain these iconic species.

1. Myers (2000). Nature 403, 853–858.
2. Linares (2011). Journal of Biogeography 38, 619–630.
3. Ben-Said (2022). Mediterranean Botany 43, e71201.
4. Climate Change (2022). IPCC Sixth Assessment Report.
5. Arista (2011). IUCN.
6. Alaoui (2011). IUCN.

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P5-07 Genetic vulnerability and adaptation of silver fir populations in the Spanish Pyrenees to climate change

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Climate change is compromising the survival of a wide variety of drought-sensitive trees across the world (1). Silver fir (*Abies alba* Mill.) is among the species showing signs of forest dieback and increased mortality rates as drought events have worsen in frequency and severity. Decline and mortality have been reported in populations of silver fir since the 80s in the Pyrenees, coinciding with a succession of dry years (2). In addition, regional climate predictions point to more frequent and intense droughts in the Mediterranean basin (3), concurrent to global warming (4), which will challenge the species' ability to cope with water shortage. Here, we present a multidisciplinary study which combines genetic information, in the form of molecular markers (SNPs), with environmental factors and dendrochronology to provide a comprehensive vision of how the silver fir genome is shaped by its interactions with the environment, and the extent to which it impacts growth patterns. The high number of genome-environment associations that were found supports a strong influence of the environment on this species' genome. Genotype-dendrophenotype associations were also detected, showing the importance of tree-level genetic variability to cope with changing climates. Finally, we provide some insight into the future of silver fir populations in the Pyrenees, using a machine learning approach, namely Gradient Forest, to calculate the genetic offset caused by climate change. These predictions suggest increased vulnerability to climate change within the western Pyrenees silver fir populations, in line with current field observation (2).

1. Allen *et al.* (2010). Forest Ecology and Management 259(4), 660–684.
2. Camarero *et al.* (2011). Forest Ecology and Management 262, 759–769.
3. Ozturk *et al.* (2015). International Journal of Climatology 35, 4276–4292.
4. IPCC (2022).

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P5-08 Effect of multi-antibiotic exposure in Blattella germanica: microbiota and fitness

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Gut microbiota is one of the most important microbial communities associated with animals. In insects, it is involved in different features of the host metabolism, such as vitamin supply, protections against pathogens and food digestion, among others. Furthermore, it can be altered by different factors such as diet, lifestyle or the ingestion of antibiotics. The abuse of antibiotics in our society is a serious economical and medical problem since it increases the abundance of multi-resistant pathogens than can cause nosocomial infections. The German cockroach is an ubiquitous hemimetabolous insect and it is carrying two different symbiotic systems, a complex gut microbiota and the endosymbiont (*Blattabacterium cuenotii*). In this work, we used three antibiotic (AB) combinations: rifampicin-kanamycin (RK), vancomycin-kanamycin (VK), vancomycin-rifampicin (VR). We treated three replica populations with one of the described AB combinations from day 5 to day 10 of the experiment, and then we removed the AB until day 70. In order to have a clear picture of the effect of the AB combinations over the gut microbiota composition, we sampled the hindgut of cockroaches in six time-points. The first sampling time was performed at day 5, then, during the AB treatment at days 7 and 10 and the other three were performed at days 30, 50 and 70. We also studied the fitness of the cockroaches in treated and control conditions. The parameters studied were weight, first oothecae appearance, number of oothecae, first hatching and number of nymphs. We studied diversity of AB treated populations compared to control and found no significant differences in alpha diversity in any condition. Regarding beta diversity, we found significant differences in every condition. Regarding fitness, we found that antibiotics affected the weight of cockroaches during exposure time. RK condition showed a delay in the first oothecae appearance, while VK and VR conditions showed an early appearance. Finally, the number of nymphs was significantly lower in the RK condition compared to control.

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P5-09 *COI* gene molecular variability of *Liocarcinus depurator* marine crab in the Atlantic-Mediterranean populations: data from 2021 and 2022

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Liocarcinus depurator is a marine crab that inhabits the muddy sea bottoms of the continental shelf and upper continental slope, from shallow continental shelf waters to over 300 m depth. Its larval stages are epipelagic and freely disperse due to marine currents. However, these currents can also produce eddies and gyres generating oceanographic fronts, limiting genetic connectivity between populations. For this reason, this is an excellent model species to study the effect of marine discontinuities affecting the gene flow between populations. For this purpose, the molecular variability of a fragment of *COI* (*Cytochrome Oxidase subunit I*) mitochondrial gene was analysed in *L. depurator* populations of the Atlantic-Mediterranean transition. In this area, the effect of Gibraltar Strait, Almeria-Oran Front and Ibiza Channel was evaluated. In 2021, despite the difficulties derived from the COVID-19 pandemics, it was possible to analyse the following populations: West of Alboran Sea, Alacant, Valencia, Ebro Delta and North Catalonia. A significant effect of the Almeria-Oran Front was detected, but not for Ibiza Channel. Unfortunately, the Gibraltar strait could not be studied because it was not possible to obtain a sample from Cadiz gulf. Interestingly, a previously haplotype detected in Greek coasts was observed for the first time in the West Mediterranean (North Catalonia population). In 2022, as the sanitarian situation clearly improved, more populations could be sampled: Cadiz, West of Alboran Sea, East of Alboran Sea, Alacant, Valencia, Ebro Delta and North Catalonia. With them, it will be possible to ascertain the effect of Gibraltar Strait, Almeria Oran Front and Ibiza Channel. Currently, we are analysing the results of 2022 sampling which will be presented in the congress.

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P5-10 Similar quasispecies of RGNNV are associated with low virulent phenotypes in sea bass and sea bream

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European sea bass (*Dicentrarchus labrax*), farmed in the Mediterranean Sea, can be affected by viral nervous necrosis disease (VNN) resulting in large economic losses. VNN is caused by nervous necrosis virus (NNV, *Betanodavirus* genus, *Nodaviridae* family), with a bisegmented (+) ssRNA genome, RNA1 coding for viral polymerase and RNA2 for capsid protein (CP). The most prevalent NNV species in sea bass is red-spotted grouper nervous necrosis virus (RGNNV), causing high mortality in larvae and juveniles (1). Reverse genetics studies have associated CP with RGNNV virulence in sea bass (2). The present work is focused on the intrahost genetic variability of RGNNV in relation to virulence across hosts. This study has been performed by NGS analysis of whole genome quasispecies (3) of two viruse with different virulence to sea bass and sea bream (*Sparus aurata*). A recombinant RGNNV (rDl965), highly virulent to sea bass, was used to infect juvenile sea bass and sea bream. In addition, a less virulent recombinant virus carrying a mutation in amino acid 270 of CP (Mut270Dl965), was used to infect only sea bass. At 1 and 5 days post-infection (dpi), viral RNA1 and RNA2 copies in the low virulence virus-host systems (Mut270Dl965 in sea bass and rDl965 in sea bream) was 1000 times lower than in the high virulence system (rDl965 in sea bass). Furthermore, RNA2 mutant spectra of the less virulent phenotypes exhibited similar Ts/Tv ratio, recombination frequency and quasispecies heterogeneity. Principal component analysis revealed that RNA2 of the less virulent virus-host systems clustered according to the frequency of substitutions and transitions, haplotype number, and Shannon index. In addition, characteristic recombination patterns at the 5' and 3' ends of RNA1, consistent with circular RNA, were observed in all viral populations. Our results suggest that a single amino acid change in CP may lead to modifications of RGNNV quasispecies in sea bass, resulting in mutant spectra like those of rDl965 in sea bream, both associated with a less virulent phenotype. This is the first report suggesting that changes in the quasispecies genetic structure *in vivo* of a bisegmented virus may underlie differences in virulence across hosts.

1. Bandín, I., & Souto, S. (2020). Betanodavirus and VER Disease: A 30-year Research Review. *Pathogens*, *9*(2), 106. https://doi.org/10.3390/pathogens9020106
2. Moreno, P., Souto, S., Leiva-Rebollo, R., Borrego, J. J., Bandín, I., & Alonso, M. C. (2019). Capsid amino acids at positions 247 and 270 are involved in the virulence of betanodaviruses to European sea bass. *Scientific Reports*, *9*(1), 14068. https://doi.org/10.1038/s41598-019-50622-1
3. Seoane, P., Díaz-Martínez, L., Viguera, E., Claros, M. G., & Grande-Pérez, A. (2022). *QuasiFlow: A bioinformatic tool for genetic variability analysis from next generation sequencing data* (p. 2022.04.05.487169). bioRxiv. https://doi.org/10.1101/2022.04.05.487169

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SESIÓN 6: GENÉTICA HUMANA

Ponencia invitada:

I6-01 A global catalog of genome diversity across the primate radiation

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Despite great advances in sequencing technologies, we are just starting to understand the genetic diversity and recent adaptations of our evolutionary close's relatives, the primates. Despite 60% of the world's primate species are currently threatened with extinction, only a few of them have been studied in depth, and still lack genomic resources. To this end, we have compiled high coverage whole genome sequences of >800 individuals including > 230 distinct species of primates representing all 16 extant families and thus dramatically increase the number of species with available genomic resources. This resource is providing a new view across different fields, including conservation genomics, evolutionary biology and a better understanding of the human genome, including novel algorithms to rank deleterious mutations in human diseases or to find evolutionary nucleotide conservation in all primates with associations to particular traits.

Comunicaciones orales:

O6-01 Knocking-out *USP48* in human iPSCs using CRISPR/Cas9 to generate 3D retinal organoids as a retinal disease model

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Ciliopathies are a broad group of heterogeneous inherited disorders associated with dysfunction of the cilium, a ubiquitous microtubule-based organelle that translates extracellular stimuli into cellular responses. The retina is one of the most affected tissues by mutations in ciliary genes due to the highly specialised neurosensory cilium that photoreceptors display, also known as outer segment, where photoreception and phototransduction occurs. To date, mutations in more than 100 ciliary genes have been associated with retinal degeneration, accounting for almost 25% of inherited retinal dystrophy (IRD) cases. Proteins related to the ubiquitin-proteasome system play an important role during retinal differentiation and ciliogenesis of photoreceptor cells. Mutations in several genes involved in ubiquitination and proteostasis have been identified as causative of IRDs and ciliopathies. USP48 is a deubiquitinating enzyme whose role in the retina is still unexplored although previous reports indicate its relevance for neurosensory organs since dominant mutations in this gene are causative of hearing loss. Our group has demonstrated that USP48 is highly expressed in cones, in agreement with the ChIP-seq peak of CRX – a photoreceptor-specific transcription factor crucial for photoreceptor differentiation – observed in its promoter and previous RNA-seq data from mouse retinas at different developmental stages. Furthermore, by means of several complementary biochemical assays – immunocytochemistry, co-immunoprecipitation, proteomics and western blotting – we describe that USP48 is a basal body-associated protein that interacts with ARL3 and UNC119a, causative of IRDs, using different domains and mechanisms. To further understand the role of USP48 in retinal development and homeostasis, we have generated a *USP48* knockout isogenic line in induced pluripotent stem cells (iPSCs) using CRISPR/Cas9 and started differentiation into 3D retinal organoids (ROs) as a human retinal disease model to characterise the retinal and cilium alterations during different stages of development of the retina caused by USP48 absence. Our results suggest that USP48 may act in the regulation of key ciliary proteins for photoreceptor function, the modulation of intracellular protein transport, and ciliary trafficking to the photoreceptor outer segment, highlighting *USP48* as a good candidate gene for inherited retinal dystrophies.

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O6-02 Overexpression of Myocilin in Transgenic Adult Zebrafish Results in Retinal Alterations and Variable Ocular Anterior Segment Defects Associated with Extracellular Matrix Abnormalities

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Myocilin is a is a secreted glycoprotein whose gain-of-function mutations are involved in glaucoma; however, its biological role remains still unknown. To investigate its normal function, we generated the first zebrafish line stably overexpressing myocilin [Tg(actb1:myoc-2A-mCherry)] using transposon-mediated transgenesis. Expression analysis showed an approximately four-fold increased transcription in transgenic zebrafish embryos (144 hpf). Variable and age-dependent ocular anterior segment alterations was observed in adult (13 months old) transgenic animals. Almost 60% of two-year-old male, but not female, transgenic zebrafish developed enlarged eyes with severe asymmetrical and variable abnormalities in the anterior segment, characterized by corneal limbus hypertrophy, and thickening of the cornea, iris, annular ligament and lens capsule. Small or absent ocular anterior chamber and pupils represented the most severe phenotype, due to iris overgrowth along with dysplastic retinal growth and optic nerve hypertrophy. Most altered ocular tissues of adult transgenic animals showed an increased expression of myocilin in the immunochemistry analysis. Signs of retinal gliosis and expanded ganglion cells and nerve fibers was also found. All senile male transgenic zebrafish demonstrated visual impairment. Transcriptomic analysis of the abnormal transgenic eyes revealed disrupted expression of genes involved in lens, muscular and extracellular matrix activities, among other processes. Altogether these results support the role of zebrafish myocilin in ocular anterior segment and retinal biology, through the influence of extracellular matrix organization and cellular proliferation.

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O6-03 Update of the genetic diagnosis of albinism in Spain

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Albinism is a rare genetic condition (1:17,000 newborns) featuring a severe visual impairment associated with variable hypopigmentation (1). To date we know 22 types of this rare disease, associated to mutations in 21 genes and one chromosomal region (2). The most common of these in the Western population is oculocutaneous albinism type 1 (OCA1), caused by mutations in the *TYR* gene (3). Within this type of albinism we find hypomorphic variants p.S192Y and p.R402Q, whose pathogenicity has been highly controversial until recent and whose combination could give rise to a pathogenic haplotype, being this relatively mild (4,5). These variants are commonly found in a significant number of patients with albinism, hence the need to clarify their involvement in albinism. The additional challenge for diagnosing these cases is the coexistence of healthy and affected individuals with the same genotype. A possible explanation is the existence of additional mutations within the regulatory non-coding region that can modify the effect of these hypomorphic variants (6)

Our laboratory launched in 2010, in collaboration with colleagues within CIBERER, an attempt for a universal genetic diagnosis system, the "albinochip" (initially based on the sequenom technology, nowadays no longer used), to detect the most frequent mutations in the genes involved in albinism. To date, we have diagnosed a large percentage of our families. In approximately 40% of cases we find the two mutations that cause albinism and in about 20% only one, and thereafter we find the second mutation by other diagnostic techniques.

Our current challenge is to solve the approximately 30% of undiagnosed cases whose mutations can be either found in other genes not yet identified or in non-coding regions of known genes. This is why we have selected candidate genes with a possible involvement in albinism, to design a wider panel of genes to clarify those cases that lack diagnosis. In this presentation we will review our genetic diagnosis efforts of albinism in Spain.

(1) Bakker R, et al. Prog Retin Eye Res. 2022 Nov;91:101091..
(2) Garrido G, et al.. Pigment Cell Melanoma Res. 2021 Jan;34(1):10-12.
(3) Fernández A, et al. Pigment Cell Melanoma Res. 2021 Jul;34(4):786-799.
(4) Grønskov K, et al. Sci Rep. 2019 Jan 24;9(1):645.
(5) Norman CS, et al. Sci Rep. 2017 Jun 30;7(1):4415.
(6) Michaud V et al. Nat Commun. 2022 Jul 8;13(1):3939.

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O6-04 Referral criteria to clinical genetics from Primary Care: Consensus document

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Introduction: Primary care (PC) is the first contact between the patient and the doctor, so it is essential to be clear about the criteria for suspecting a genetic disease and where it should be referred for study. Material and methods: Four scientific societies: the Spanish Society of Family and Community Medicine (semFYC), the Spanish Association of Human Genetics (AEGH), the Spanish Association of Pediatrics (AEP) and the Spanish Society of Medical Oncology (SEOM), have reviewed the criteria for referral to the clinical genetics services of the different published guidelines with the purpose of define the recommendations for PC. Conclusions: With this consensus document, the PC doctor and pediatrician will know when, how and where to refer their patients with hereditary and/or genetic pathology to clinical genetics services.

1. Ejarque Doménech I, et al. Aten Prim 54 (2022) 102501.

This work has not conflicts of interest.

Comunicaciones Flash Talk y póster:

FP6-01 Locating the α-dystroglycan *O*-mannosylglycosylation pathway in mouse retinal cells

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Dystroglycan (DG) is a glycoprotein composed of two subunits: α-DG, which is extracellular and heavily *O*-mannosylglycosylated, and β-DG, an integral transmembrane polypeptide. α-DG is responsible for providing an *O*-glycosylation-dependent link for cells to their extracellular matrix and the establishment of functional synapses in the CNS. Deficiencies in the α-DG *O*-mannosylglycosylation pathway cause dystroglycanopathies (DGPs), which are clinically and genetically heterogeneous neuromuscular dystrophies offering a shortened life expectancy and whose severe symptoms extend to the retina. These diseases include the Walker-Warburg syndrome, muscle-eye-brain disease and Fukuyama congenital muscular dystrophy, amongst other milder disorders. Their causative genes, 22 identified so far, mostly encode glycosyltransferases involved in the *O*-glycosylation of α-DG residing in the endoplasmic reticulum (ER) or the Golgi complex in the brain and muscle. However, the knowledge on these DGP-associated proteins' expression and localization in mammalian retinal cells is fairly scarce. In our group we have determined that all known DGP-associated genes are expressed in the adult human retina. Among them, we have validated the presence of *POMT1*, *POMT2*, *POMGNT1*, *POMGNT2*, *FKTN* and *FKRP* mRNA transcripts and/or their protein products in the neural retina of a number of mammalian species. In the present work we have focused on analyzing the subcellular-compartment location of proteins involved in the α-DG *O*-glycosylation pathway, namely POMT1/2, POMGNT1/2, fukutin, FKRP, B4GAT1 and LARGE1, in mouse retinal cells. We have used immunohistochemical and immunocytochemical techniques in order to characterize their expression pattern in mouse retinal sections and the 661W photoreceptor cell line, respectively, by means of colabeling for molecular markers of the ER, such as KDEL, or the Golgi complex, such as GM130. Confocal fluorescence microscopy observations revealed that POMT1/2 and fukutin were located in the ER, and POMGNT1, FKRP and LARGE1 in the Golgi of retinal neurons and/or 661W cells. By contrast, POMGNT2 and B4GAT1 were found in both organelles in mouse retinal cells. These results are indicative of the location in the ER and/or the Golgi complex of DGP-associated proteins, not only in the brain and muscle but also in mouse retinal cells, where they should play a relevant role in α-DG *O*-mannosylglycosylation.

FP6-02 Generation and functional analysis of a zebrafish knockout line for the congenital glaucoma gene *CYP1B1*

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Loss of function of *CYP1B1* is the main genetic alteration related to primary congenital glaucoma (PCG), an infrequent pathology characterized by delayed embryonic development of the iridocorneal angle of the eye, but whose underlying molecular mechanisms are still poorly understood. To improve the understanding of the role of this gene in PCG development, we have developed a cyp1b1 knockout zebrafish line using CRISPR/Cas9 genomic editing. The frameshift mutation c.535_667 resulted in a 72% reduction of mRNA and the predicted production of an inactive truncated protein (p.(His179Glyfs*6)) from the residual mRNA. The appearance of craniofacial alterations (microphthalmia and defects in mandibular development) was observed in 23% of the somatic F0 mutant larvae (144hpf). Although these alterations were absent in F3 homozygous larvae (144 hpf), 27% of the resulting adult fishes (4 months) showed uni or bilateral craniofacial alterations that suggest the existence of incomplete penetrance and variable expressivity phenomena in the phenotypic manifestation. These phenotypes increased up to 86% in the adult offspring of the inbred parents that showed the alterations, suggesting that the main component in these phenomena is of genetic origin. Transcriptomic analysis of larvae homozygous for the mutation (7 dpf) showed functional enrichment of differentially expressed genes related to extracellular matrix and cell adhesion, cell growth and proliferation, lipid metabolism (retinoids, steroids, and fatty acids), redox processes including genes of cytochromes P450 and inflammatory processes. This study shows the complexity of the phenotypes and molecular pathways associated with the loss of function of cyp1b1 and evidences the implication of the deregulation of the expression of extracellular matrix genes as one of the mechanisms underlying the pathogenicity associated with the loss of function. from cyp1b1.

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FP6-03 Functional interaction between zebrafish *adamtsl4* and *cpamd8* matrix metalloproteinase-related genes and its implication in early-onset glaucoma

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Glaucoma is a progressive and irreversible disease of the optic nerve, presenting high clinical and genetic heterogeneity. Congenital and juvenile glaucoma are part of the group of early-onset or childhood glaucomas, characterized by their low prevalence, for being an important cause of visual impairment, and poorly understood genetic mechanisms. It has been proposed that alterations in the development of the extracellular matrix of the anterior segment of the eye participate in the genesis of this type of glaucoma. Preliminary studies carried out in our laboratory revealed a high mutational burden in extracellular matrix metalloproteinase-related genes, in a cohort of Spanish childhood glaucoma patients in whom no monogenic disease-causing alterations have been identified. Among the genes that contribute to the high mutational load are *ADAMTSL4* and *CPAMD8*. Based on these results, we hypothesize the existence of a complex inheritance of this type of glaucoma, in which additive partial functional disruption of different extracellular matrix genes might contribute to this disease pathogenesis. To evaluate the hypothesis, we carried out gene expression analyses and functional studies in zebrafish of two of the genes that contribute to this mutational burden (*ADAMTSL4* and *CPAMD8*). Immunohistochemical analysis showed the presence of ADAMTSL4 and CPAMD8 proteins in tissues of the anterior segment of the adult human eye related to glaucoma. Zebrafish orthologous proteins were also detected in the anterior segment and retina of larvae (6 dpf). On the other hand, the heterozygous loss-of-function of these orthologous genes resulted in wild type-like ocular phenotypes. However, the combined heterozygous loss-of-function of the two zebrafish orthologous genes (*adamtsl4* and *cpamd8*) led to development alterations of the anterior segment of the eye that manifested as reduced ocular anterior chamber volume. This result supports the existence of a functional interaction of these two genes in zebrafish and suggests that it could also be present in the human eye. Furthermore, these findings support that the combined effect of rare variants of the *ADAMTSL4* and *CPAMD8* genes, along with those of other related genes, might contribute to the genetic susceptibility to early-onset glaucoma.

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FP6-04 Genetic interaction between liver enzyme levels and the risk of developing diabetes mellitus 2 (T2D) over time in a Spanish population

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T2D is associated with several liver abnormalities, including elevated blood levels of liver enzymes[1]. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT) are considered markers of liver injury and indicators of liver metabolic function. On the other hand, transaminases have also been found to be predictors of T2D and hepatic insulin sensitivity. Thus, patients diagnosed with liver disease by liver enzyme quantification are at increased risk of developing T2D[2,3]. Our aim was to study the associations between 97 SNPs, previously selected from data obtained by exome sequencing in a case-control study, and liver enzymes for the development of T2D in a follow-up period.

Three thousand adults from the Di@bet.es study (a general population study focusing on the prevalence of T2D in Spain) who had enzyme measurements at baseline and at seven years follow-up were included in the statistical analysis. Genotyped SNPs were studied by targeted next-generation sequencing (NGS) using a generalized linear mixed model (GLM) for data statistical analysis to determine whether there was an association between liver enzyme levels at baseline and presence of T2D at baseline and after the follow-up period.

Significant interactions were found between pancreatic enzyme levels and the risk to develop T2D through time. The most prominent polymorphisms were: rs114886261 (CPNE9, $p<10^{-16}$), rs74378191 (RDH14, $p<10^{-16}$) and rs3797192 (C5orf49, $p<10^{-16}$). Those SNPs are located in genes involved in biological, metabolic and inflammatory processes.

Liver enzymes levels modulate the contribution of specific genetic markers to the development of T2D through time. Further studies are required to establish this interaction mechanisms and the role of many other genetic variations.

References

1. Ahamed F, Karim MR, Haque MA, et al. Study on Alanine Aminotransferase in Patients of Type 2 Diabetes Mellitus. *Mymensingh Med J*. 2021;30(2):343-350. Accessed March 20, 2023. https://europepmc.org/article/med/33830112
2. Sookoian S, Pirola CJ. Liver enzymes, metabolomics and genome-wide association studies: From systems biology to the personalized medicine. *World J Gastroenterol*. 2015;21(3):711. doi:10.3748/WJG.V21.I3.711
3. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol*. 2016;31(5):936-944. doi:10.1111/JGH.13264

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FP6-05 Truncated MAGEL2 and its novel subcellular localisation in Schaaf-Yang syndrome

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Schaaf-Yang syndrome (SYS) is an ultra-rare neurodevelopmental disorder caused by truncating mutations in *MAGEL2*. SYS patients show a clinical phenotype which includes intellectual disability, neonatal hypotonia, sleep disorders, joint contractures and facial dysmorphism (1). MAGEL2 contributes to the regulation of retrograde transport, circadian rhythm and RNA metabolic processes (2-4). In a recently published study, we transfected cell lines with vectors encoding wild-type (WT) or truncated MAGEL2 to assess the subcellular localisation of the mutated protein (5). Immunocytochemistry assays showed that in cells transfected with the WT construct (MAGEL2-HA), MAGEL2 was mainly located in the cytoplasm, while there was a shift towards the nucleus in the truncated protein (MAGEL2-Gln638*-HA). In this work, to ensure that the results are a common feature of *MAGEL2* truncating variants, we have generated additional plasmids carrying mutations found in five distinct SYS patients. Furthermore, to avoid potential tag-specific outcomes, the current vectors feature a FLAG motif at the N-terminal region of the protein. Once more, our assays reveal that the heterologously expressed truncated proteins are predominantly found inside the nucleus, in contrast with the cytoplasmic localisation of WT MAGEL2. Importantly, this phenomenon is observed consistently across different cell lines, underscoring the robustness of the observations. While the lack of a properly functioning MAGEL2 antibody hampers the validations of these findings in endogenous MAGEL2, our results point to a pathogenic neomorphic effect of the truncated protein in the nucleus. We propose that the pathogenicity of SYS-associated mutations may be explained by both the loss of MAGEL2 function and a new role of the truncated form in the nucleus.

1. McCarthy J, Lupo PJ, Kovar E, et al. (2018). Am J Med Genet A. 176(12):2564–2574.
2. Hao Y-H et al. (2015). Mol Cell. 59(6):956–69.
3. Carias KV et al. (2020). PLoS One. 15(4):e0230874.
4. Sanderson MR et al. (2021). J Biol Chem. 297(2):100959.
5. Castilla-Vallmanya L, Centeno-Pla M, Serrano M et al. (2023.) J Med Genet. 60:406–415.

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FP6-06 Circulating cell free *DNA (ccfDNA)* as a biomarker of biological age in humans

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Aging is a natural and progressive process characterized by the generalized deterioration of the functions of the organism. Nevertheless, chronological age fails to be an accurate indicator of the health of each individual or his/her remaining lifespan. Therefore, there is a need to identify biomarkers of aging that can be used to determine the biological age or the rate of aging of an individual. The basis of the overall deterioration associated with aging lies in the establishment of a chronic oxidative and inflammatory stress that causes damage to various cellular components, affecting the normal functioning of cells and increasing cell apoptosis. Due to cell apoptosis, DNA fragments (both nuclear and mitochondrial) are released out of the cell into the bloodstream (1).

This cell-free DNA (ccfDNA) has gained increasing interest as a promising candidate non-invasive biomarker in multiple fields. The aim of the present study was to analyse ccfDNA levels in plasma from 150 healthy men and women aged 23-80 years as a potential biomarker of biological age. For this purpose, three genes were selected: one of nuclear origin (β-globin) and two mitochondrial sequences (a region of the CoxIII and NADH genes) and the plasma levels were quantified by real-time PCR. The biological age of each individual was calculated with the Immunity Clock, a mathematical model based on some immunological functions defined as markers of the aging rate (2).

We found an age-related increase in the levels of the three investigated DNA fragments in plasma both in men and women, increasing from the 50s onwards. Moreover, the levels of β-globin were found to positively correlate with the biological age of each individual calculated with the Immunity Clock.

Besides, the level of each fragment was also analysed in plasma from Alzheimer (AD) and Parkinson (PD) disease patients, as both were characterized by a proinflammatory state, immune system dysfunction and oxidative imbalance that affects cellular integrity. Results showed an increased concentration of circulating Cox III in plasma associated with both AD and PD. This seems to support the idea that plasma β-globin fragments have the potential to be used as a marker of biological age. Interestingly, CoxIII may be useful as a biomarker of neurodegenerative diseases.

1. Kananen L et al. (2023) Geroscience 45(1), 85-103.
2. Martínez de Toda I, et al (2021). The Journals of Gerontology A 76, 11, 1939–1945.

SESIÓN 7: DOCENCIA DE LA GENÉTICA

Comunicación oral:

O7-01 The phylogenetic map of genetics teaching in Spanish Universities

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Genetics is a very broad discipline comprising transmission genetics, molecular, evolutionary, or developmental genetics. Traditionally, Genetics was taught as a single course in Biology in a single year course. The evolution of Genetics as a field and the increase in the number of degrees has rendered a new map of the Genetics curriculum. In this presentation we show the current map of different degrees where Genetics is, or could be present, and discuss the different curricular requirements. Future actions may need to take into account the new legal framework regarding areas, fields and scopes of research and lecturing.

Comunicaciones póster:

P7-01 Teaching Genetics to Secondary School Students

Belén Méndez*, Nadia Fernández*, Isabel García*, Pilar Arana, José Barrios, Cesar Benito, Beatriz Beroiz, Carmen Callejas, Rocío Codes, Ana Figueiras, Félix Gil, Mónica González, Nicole Gorfinkiel, José L Hórreo, Carolina Llanos, Carmen Moreno, Marta Muñoz, Alicia de la Peña, Pedro Perdiguero, Marcela Rosato, Mónica Pradillo, F Javier Gallego, F Javier Espino, Isabel Ballesteros, Rosario Linacero

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Bringing scientific culture closer to society is one of our goals as university teachers. We need to communicate to society that science solves social needs and is one of the necessary pillars to achieve the Sustainable Development Goals.

Genetics is one of the most transversal fields of biology and has the greatest social impact; today, more than ever, we find applications of genetics in our daily lives. However, the actual knowledge that society has about these topics is usually very limited. Secondary school students, who can act as transmitters in their family and social environment, are probably the best niche for society to promote scientific education.

In today's world of education, there is an increasing emphasis on improving learning through new ways of acquiring knowledge and involving students in their own learning. The approach to genetics for high school students can be seen as an innovation in education, using Inquiry-Based Science Teaching, or as a service to society through a process of service-learning.

In the Department of Genetics at the Complutense University of Madrid, we have developed both approaches, educational innovation and service-learning projects, in which students acquire knowledge of genetics through the realisation of a case study in which genetic tools are used to solve problems of interest to society, such as food fraud and biodiversity assessment. In this way, the following objectives are intended to be achieved

1.- Create a scientific culture in our society in order to provide tools for understanding scientific progress.
2.- Generate scientific vocations in students by integrating the learning of our undergraduate, masters and doctoral students with service to secondary education centres as the basis of citizen science.

05 ÍNDICE DE PONENTES

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