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Comparative genomics of the putatively asexual powder lichens Lepraria adn other Lecanoromycete lichens

Bubba Pfeffer

Comparative genomics of the putatively asexual powder lichens *Lepraria* and other Lecanoromycete lichens.

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Bubba Pfeffer (they/them/theirs)

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Thesis of Bubba Pfeffer Approved By

_____ DATE _____

DR. JESSICA L. ALLEN, CHAIR, GRADUATE STUDY COMMITTEE

_____ DATE _____

DR. PAUL SPRUELL, GRADUATE STUDY COMMITTEE

_____ DATE _____

DR. JENIFER B. WALKE, GRADUATE STUDY COMMITTEE

_____ DATE _____

DR. MIMI MARINUCCIE, GRADUATE STUDY COMMITTEE

_____ DATE _____

DR. JAMES C. LENDEMER, GRADUATE STUDY COMMITTEE

"One thing I have learned in the woods is that nothing is random.
Everything is steeped in meaning,
colored by relationship, one thing with another."

-Robin Wall Kimmerer, *Braiding Sweetgrass*

"Gaia is a tough bitch –
a system that has worked for over three billion years without people.
This planet's surface and its atmosphere and environment
will continue to evolve long after people and prejudice are gone."

-Lynn Margulis, *The Third Culture: Beyond the Scientific Revolution*,
Chapter 7 "Gaia is a tough Bitch"

"but solitude is only a human presumption.
Every quiet step is thunder to beetle life underfoot.
Every choice is a world made new for the chosen; all secrets are witnessed."

-Barbara Kingsolver, *Prodigal Summer*

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Chapter 1: Comparative genomics of *Lepraria* and other Lecanoromycete lichens to develop a working framework of lichen reproduction genomics

Introduction

The Dual Cost of Sex

Sexual recombination through meiosis is a key distinguishing characteristic of Eukaryotic organisms (Hörandl & Hadacek, 2020; Skejo & Franjević, 2020). The Last Eukaryotic Common ancestor was likely a unisexual, with sexes evolving later in Eukaryotic history (Heitman, 2015). Unisexuality is a mating system whereby a species is characterized by a single self-fertile mating type, which is capable of mating with an identical clone of themselves and with every other individual in the species. Sexual recombination represents a confounding paradox for evolutionary theory that has puzzled generations of biologists. Theoretically, a population of asexual females will produce twice the progeny of a sexual one (Gibson et al., 2017; Smith, 1978). Obligately sexual species with diecious mating systems must maintain the cost of males, which consume resources and cannot produce progeny. Furthermore, obligately sexual females effectively surrender almost half of their genetic contribution to the next generation to the males. This is the dual cost of sex. Furthermore, sex requires expending the energy to find a suitable mate, and sexual recombination is not necessarily selectively advantageous, as recombination can segregate favorable gene combinations, and high rates of speciation can lower the abundances of the resulting species (Melián

et al., 2012; Otto, 2009). Despite the enormous costs associated with sexual reproduction, sex is an extremely widespread mode of reproduction across domain Eukarya, and most described Eukaryotes are at least facultatively sexual (Hofstatter et al., 2018; Sato et al., 2011; Weedall & Hall, 2015). If sex is so costly, then why is it so ubiquitous among Eukaryotes?

Additionally, the evolutionary function of genetically distinct sexes or mating types represents a difficult-to-reconcile quandary. In most extant sexual species, individuals cannot mate with any individual of the same species but are restricted to a range of potential partners controlled by categories called 'sexes' or 'mating types' (Perrin, 2012). Here, 'sexes' refer to species with an observable degree of anisogamy and/or phenotypic asymmetry among sexes, whereas 'mating types' refer to species with isogamy and/or phenotypically similar mating types. Anisogamous egg-and-sperm gametic sexes have independently evolved in many lineages, resulting from disruptive selection for females and males to increase egg cell size and sperm encounter rate, respectively, to maximize their fitness to next-generation zygotes (Johnson et al., 2013; Nishimura & Hoshino, 2011; Randerson & Hurst, 2001). But why would isogamous organisms maintain mating types? And why do some lineages evolve exceedingly complex genetic machinery to circumvent self-infertility while maintaining mating types?

Though many hypotheses have been proposed to explain the fitness benefit of sex that must overcome the dual cost of sex, and by far the most widely accepted today is the oxidative damage hypothesis for meiosis (Hörandl &

Hadacek, 2013). The ODHM proposes that DNA repair mechanisms associated with early parasexual forms of meiosis provided the short-term fitness benefits necessary for sex to become fixed in the last Eukaryotic common ancestors (Hörandl & Hadacek, 2020; Thomson et al., 2019). Thus, sexual reproduction is often assumed to be essential for long-term survival of lineages, because finite asexual populations that forfeit purifying selection through recombination will be overpowered by genetic drift and succumb to mutational meltdown (Muller, 1932; Wahl & Tanaka, 2022; Wright, 1931). Without recombination and allele segregation, natural selection is weakened, and deleterious mutations will accumulate leading to a mutational meltdown. For instance, asexual whiptail lizards in the genus *Aspidoscelis* have higher synonymous mutation rates and lower nucleotide diversity of mitochondrial DNA than their sexual relatives (Maldonado et al., 2022). In the facultatively sexual plant species complex *Ranunculus auricomus,* one study measured a recombination rate of about 6.05% per generation, which was determined to be sufficient for purifying deleterious mutations out of the population (Hodač et al., 2019).

Fungal Reproduction

The true fungi are a taxon of Eukaryotic Opisthokonts, sister to the Microsporidia and Cryptomycota – together forming the monophyletic clade Holomycota (Torruella et al., 2015). Fungi are ectoheterotrophs that commonly form a hyphal cell structure that maximizes surface area: volume ratio for

enhanced interfacing with their environment (Kendrick, 2017). Some ancient lineages of fungi such as Chytridiomycota have uniflagellate gametes, while more derived lineages such as the supergroup Dikarya have no apparent gametes. The two Dikaryan phyla, Ascomycota and Basidiomycota, fuse the cytoplasm of compatible cells through a process called plasmogamy, initiating a dikaryotic phase with two independent nuclei in each cell. This dikaryotic phase tends to be short-lived in the Ascomycota and is frequently long-lived in the Basidiomycota. Finally, specialized dikaryotic cells undergo karyogamy, each fusing their nuclei to produce a single diploid cell, called the ascus and basidium, respectively. These fertile cells undergo meiosis to produce sexually recombined, haploid spores.

While the genetics governing sexual reproduction in basal lineages of fungi and their relatives remains poorly understood, derived taxa like the Ascomycota have been extensively studied. Ascomycete sexual reproduction involves the expression of mating-type genes found at one locus: *MAT1*, which consists of two highly conserved genes (*SLA2* and *APN2*) that flank the mating-type genes (Fig 2). The *MAT1* locus in a given species or individual may include either or both of the non-allelic *MAT1-2* and *MAT1-1* idiomorphs (Wilken et al., 2017). The primary *MAT1* genes, *MAT1-1-1* and *MAT1-2-1*, are much more conserved among species than the much more variable secondary *MAT1* genes – e.g. *MAT1-1-2, MAT1-2-14*, etc. (Billiard et al., 2012). The presence or absence of each of these *MAT* idiomorphs in single individuals or in different individuals of the same species varies throughout Ascomycota and dictates the mating system of the species.

Currently defined Ascomycete mating systems include heterothallism, primary homothallism, secondary homothallism, and unisexuality. Heterothallism involves the mating of genetically distinct individuals, each expressing one of these complementary idiomorphs (Dyer, 2008). Homothallism represents a large and diverse artificial category of genetically unrelated mechanisms which all allow for self-fertility. Primary homothallism involves the expression of both *MAT1-1* and *MAT1-2* idiomorphs in the *MAT1* locus of a single individual. (Billiard et al., 2012). Secondary homothallism is another artificial category which includes pseudohomothallism and mating-type switching. Pseudohomothallism involves the production of dikaryotic or multinucleate ascospores which can express compatible mating types and eventually mate from a single spore (Andrea M. Wilson et al., 2015). Mating type switching occurs in species which have a second, mating-type-like (*MTL*) locus, which express a complementary mating type. The *MTL* locus is typically kept inactive through tightly wound heterochromatin, but can be expressed under certain stimuli (Nasmyth & Tatchell, 1980; Rusche & Kirchmaier, 2003). Lastly, unisexual reproduction is a unique and rare case of homothallism which has only been observed in four species of Ascomycetes to date: *Neurospora africana, Huntiella moniliformis, Thermoascus aurantiacus,* and *Thermoascus crustaceus* (Wilson et al., 2021). Unisexuality frequently occurs through multiple pathways in any given taxon and involves sexual reproduction with the expression of only a single *MAT1* idiomorph. Sexual modes of reproduction are diverse, complex, and frequently cryptic.

Filamentous Ascomycete fungi often express characteristics of sexes and mating types (Perrin, 2012). For instance, the adult haploids of heterothallic filamentous Ascomycetes are hermaphroditic, each mating type being capable of producing conidia (asexual spores which can function similarly to male gametes) and ascogonia (receptive hyphae which function like female gametes), but fusion will only occur between complementary mating types (Coppin et al., 1997; Perrin, 2012). The literature often refers to conidia and ascogonia as male and female gametes, respectively (Fukumori et al., 2004; Lassagne et al., 2022) but that term is misleading here in part because haploid Ascomycete "gametophytes" do not fuse to produce a diploid zygote as in plants and animals. The complementary hyphae fuse cytoplasm (plasmogamy) to form a dikaryotic generation which grows, forms a fruiting body, and then undergoes karyogamy, meiosis, and sporulation. Additionally, conidia are nonessential for fertilization, as any spore or hypha of a complementary mating type is capable of plasmogamy (Dodge, 1932, 1936). Conidia cannot be considered spermatia/male gametes because they are capable of growing into adult mycelium, although microconidial germination is suppressed in some species and strains (Dodge, 1932; Maheshwari, 1999; Wesche & Weber, 2023). Despite all this, many researchers insist on applying a strict male/female orthodoxy to the nuanced and varied copulating hyphae of Ascomycetes.

Asexual diversity is similarly diverse and widespread throughout the Fungi. Asexual reproduction currently accounts for the entire phyla Deuteromycota and

Glomeromycota, and ~11.3% of described species in the Ascomycota (Dyer & Kück, 2017; Senanayake et al., 2022). The overwhelming asexual diversity of fungi seems to contradict the prevailing interpretations from the ODHM that asexuality is an evolutionary dead end. In addition to oxidative damage, asexual lineages must manage the accumulation of transposable elements (TE's), which may have proliferated in their sexual ancestors (Arkhipova & Meselson, 2005). Research has confirmed that transposable elements are prone to proliferating in asexual lineages (Bast et al., 2019; Chen & Zhang, 2021). These facts, combined with the discovery of asexual "scandals" has led to speculation of sexuality in other putatively asexual taxa (Schurko & Logsdon, 2008). Further, the apparently ubiquitous observation of conserved meiosis and *MAT* genes in fungi has lent support for the perennial hypothesis of cryptic sexuality in asexual lineages (Halary et al., 2011; Pfeffer et al., 2023). But the inheritance of these genes only confirms sexual ancestry, while the apparently universal conservation of *MAT* and meiosis-associated genes implies that they are essential for lineage survival.

Population Genomics

Molecular genetic analyses typically detect some level of recombination in fungal populations, regardless of presumed sexuality (Henk et al., 2012). A genomics study of *Cryptococcus gattii* in the Pacific Northwest detected three highly clonal subpopulations (Billmyre et al., 2014). They found evidence that the VGIIa and VGIIb strains arose from mitotic clonality, while the VGIIc strain, which

is endemic to the PNW, is likely the result of a recent sexual recombination event. Recombination is highly correlated with the *MAT1* locus, and *Saccharomyces paradoxus,* which possesses asexual, heterothallic, and two modes of unisexual reproduction exhibits high levels of recombination only on the chromosome which contains the *MAT1* locus (Tsai et al., 2008). This pattern is in contrast with the more general pattern of recombination suppression around the *MAT1* locus (Hartmann et al., 2021; Tsui et al., 2013; Vittorelli et al., 2023). In the lichen *Lobaria pulmonaria* which can reproduce asexually through isidia or sexually in a heterothallic mating system, researchers correlated populations which reproduce asexually with a skewed ratio of *MAT1-1* and *MAT1-2* idiomorphs (Singh et al., 2012). It was later discovered that this *MAT* locus skewing of populations was correlated with wildfire disturbance (Singh et al., 2015). The *MAT* locus is a key component in the population structure of clonal and sexual fungi alike.

Lichens are the symbiotic unions formed through community among fungi, algae, and a diversity of other microbes to form a stable thallus with emergent properties (Allen & Lendemer, 2022; Spribille et al., 2022). Lichens are named for the primary mycobiont partners, which constitute the majority of lichen biomass and form the taxonomically pertinent reproductive structures. Sexual reproduction in lichens involves the production of ascospores which, after germination, must encounter complementary photobionts to resynthesize the symbiosis. Many species also produce conidia which can grow into a new thallus or enmesh into a preexisting thallus, likely for sexual fertilization. Asexual

propagation is extremely common through specialized diaspores, which transmit a sample of the entire lichen holobiome. Lichens are frequently observed in "species pairs," in which two closely related species exhibit divergent reproductive preferences (Degtjarenko et al., 2020; Ohmura, 2020; Poelt, 1970). One species will reproduce entirely via sexual ascospores, where the other will reproduce entirely or predominantly through asexual propagules. Many species of predominantly vegetatively asexual lichens occasionally produce fertile apothecia with meiospores, but a study of the ascospores from *Physconia grisea* (vegetative asexual) and *Physconia distorta* (sexual) demonstrate that the ascospores of *P. grisea* have a low rate of viability (0.43%) and only on one tested medium (Molina et al., 2013).

Lichen population genomics is still in its infancy, and consequently few lichen genomes have been characterized at the population level. Some lichens like the Appalachian endemic *Cetradonia linearis* demonstrate strong spatial structure, with geographically and genetically isolated populations (Allen et al., 2018). Conversely, other species like the Mediterranean *Parmelina carporrhizans* demonstrate high rates of gene flow and outcrossing even among geographically distant populations. A study of microsatellite markers in *Xanthoria parietina* in Switzerland demonstrated that most of the genetic variation occurred within, not between populations, suggesting that within the country all individuals potentially form one large panmictic population (Itten & Honegger, 2010). On a global scale, researchers investigated Arctic and Antarctic populations paired with nearby

temperate populations of the widespread lichen *Cetraria aculeata* (Fernández-Mendoza et al., 2011). They confirmed that the Arctic and Antarctic populations were genetically distinct from each other. Furthermore, the populations in Svalbard and South America had similar levels of genetic distance from their respective paired polar location, suggesting similar levels of gene flow in two distinct regions.

Lepraria spp.

Lepraria s. str. represents an excellent study system for the investigation of asexual evolution. *Lepraria* is a genus of lichenized fungi in the family Stereocaulaceae colloquially referred to as the 'dust' or 'powder' lichens. They have a leprose growth form, the thallus consisting entirely of ecorticate granules, which function simultaneously as thallus and diaspore (Brodo et al., 2001). *Lepraria* s.l. is widely understood to be polyphyletic, and a monophyletic *Lepraria* s.str. has emerged (Lendemer and Hodkinson 2013). Delimiting the species that belong to *Lepraria* s.str. remains an active area of research. For instance, *Lepraria stephaniana* was recently discovered to belong to the distantly related family Ramalinaceae, and the new genus *Pseudolepraria* was described to accommodate it (Kukwa et al., 2023). Many species in the *Lepraria* s.str. are widely distributed. For instance, *Lepraria finkii* and *Lepraria neglecta* are found in North America, Europe, and Asia. In stark contrast, *Lepraria lanata* is endemic to the high elevations of southern North America on non-calcareous rock. *Lepraria sensu lato*

(s.l.) comprises approximately 60 species, all of which have never been observed to produce sexual reproductive structures, and are therefore assumed to be exclusively asexual (Lendemer, 2013). Despite this, previous research demonstrated that *Lepraria neglecta* possesses an intact and conserved mating type *(MAT)* locus (Pfeffer et al., 2023). This observation raises questions about the reproduction of *Lepraria* and about the function of *MAT* genes in fungi generally. Is the interspecific structure of the *MAT* locus largely consistent across the genus, or do species diverge in the expression of their *MAT* locus?

In this present study, we sought to develop a working foundation of reproduction genomics in *Lepraria* s. str. To this end, we sought to 1) sequence, assemble, and annotate reference genomes for four *Lepraria* spp. and map landscape-level population data to reference genomes for *Lepraria lanata* and *Lepraria finkii,* 2) characterize and analyze the mating-type gene sequences in the reference genomes, and 3) Analyze substitution ratios of genes relating to reproduction in *Lepraria* and closely related lichens.

We sequenced, assembled, and annotated the whole genomes of four *Lepraria* species: *Lepraria finkii, Lepraria lanata, Lepraria normandinoides,* and *Lepraria oxybapha* from long-read nanopore data. We analyzed these genome assemblies along with previously published Lecanoromycete lichens and six others we sequenced, assembled, and annotated in our lab: *Punctelia appalachiensis, Punctelia rudecta, Sticta Deyana, Sulcaria isiidifera, Usnea strigosa,* and *Usnea*

subfusca. These data represent a remarkable new synthesis of genomic data at high taxonomic resolution and scale.

Methods

Sample collection, DNA extraction, sequencing, and genome assembly (plus lichen repository)

Specimens were collected with nitrile gloves and alcohol-sterilized forceps or wood chisels then placed into a paper collection bag with a unique identification label. *Lepraria lanata* was collected from a NW-facing overhang on Cattail Peak, North Carolina (35.7964 N, -82.2569 W; *Allen s.n.*). *Lepraria finkii* was collected from Lumber Ridge in Great Smoky National Park, North Carolina (35.6531 N, -83.6711 W; *Lendemer XXX*). *Lepraria normandinoides* was collected from a vertical shale outcrop at the pigeon River Whitewater Boat Launch, Tennessee (35.7756N, -83.101 W; *Hollinger 27,155*). *Lepraria oxybapha* was collected from a quartzite outcrop in Great Smoky National Park, on a roadbank off Old Cataloochee Turnpike, North Carolina (35.6465 N, -83.0633 W; *Hollinger 27,129*). After returning from the field, specimens were placed in a -20°C freezer until ready for DNA extraction.

Six other reference genomes were also sequenced, assembled, annotated, and are presented here. *Punctelia appalachensis* and *Punctelia rudecta* were collected from Cataloochee divide, Great Smoky Mountain National Park, North Carolina (35.585 N, -83.074 W; *Allen 6097 and Allen 6098*). *Sticta Deyana* was collected from Sipsey wilderness area, Alabama (34.3241 N, -87.4513 W; *Tripp 6,599*). *Sulcaria isidiifera* was collected from Elfin Forest, California (35.3325 N, -120.8238 W; *Balderas 70*). *Usnea strigosa* was collected from Cataloochee divide,

Great Smoky Mountain National Park, North Carolina (35.582012, N, -83.07037; *Allen 6099*) and *Usnea subfusca* from the West slopes of Jack Mountain, Highlands Wildlife Management Area, Virginia (38.3746, -79.5034; *Allen 6100*). Each of these species was loaded in 16 disrupter tubes. All genome voucher specimens are retained at EWU and accessioned in the herbarium.

The *L. lanata* sample was loaded into six Qiagen tissue disruptor tubes, the *L. finkii* sample was loaded into 16 Qiagen tissue disruptor tubes, *L. oxybapha* into 8 disruptor tubes, and *Lepraria normandinoides* into 16 disruptor tubes. Cell walls were disrupted using the TissueLyser II, DNA was extracted using the Qiagen DNeasy™ Plant Pro Kit, then the resulting DNA extracts were pooled into one sample for each species for quantification and sequencing. DNA content was quantified using the Qubit high-sensitivity double-stranded DNA assay (Van Dyke, 2017). DNA was sent to Cold Spring Harbor National Laboratory for sequencing on the Oxford Nanopore PromethION platform. Base-calling nucleotides from raw data was also completed by Cold Spring Harbor Laboratory using Guppy v5 in sup mode (https://github.com/nanoporetech/pyguppyclient).

Reference genomes were assembled and annotated using a suite of bioinformatics tools. Reads were assembled into contigs using flye v2.9 in --meta mode (Kolmogoroov et al., 2020). The assembly was polished using Medaka v1.6.1 (https://github.com/nanoporetech/medaka). A Diamond v0.9.32 blastx search against the NCBI nonredundant protein database was performed with the custom BITAT python script to assign contigs to taxa and build a GC depth plot (McKenzie

et al., 2020). Contigs ascribable to Ascomycota with an average coverage greater than a threshold value were then extracted and used for all following assessments and analyses. Reads were then aligned to the filtered Ascomycete assembly using minimap2, and only the reads which aligned to the filtered assembly were used during reassembly with flye v2.9, then polished with Medaka v1.6.1 and filtered as described above. Specific details of each final assembly are listed in table 1.

BUSCO predictions using the Ascomycota odb10 database were used to assess genome completeness (Allen et al., 2021; Simão et al., 2015). Assembly contiguity and quality was then assessed with QUAST in --fungus mode (Mikheenko et al. 2018). The assembly was then annotated with funannotate v1.8.9 (https://github.com/nextgenusfs/funannotate). In this workflow, repeat regions are masked, then *ab initio* predictors are compiled with EVidenceModeler (Haas et al., 2008). Predictors were trained using a lab-standard pezizomycotina dataset, which includes *Cladonia grayi, Lobaria pulmonaria, Usnea florida, Xanthoria parietina,* and *Lepraria neglecta* (Allen et al., 2021; Pfeffer et al., 2023).

Twenty-three Lecanoromycete lichen genome assemblies were also collected from a Comparative Genomics research project (Gerasimova et al., 2022). We downloaded the contigs.fsa files, evaluated the assemblies with BUSCO and QUAST, and annotated them using the same funannotate workflow as above.

Species tree

Orthofinder v2.5.4 was used to generate a maximum likelihood species tree from whole-genome protein data for all available lichen species (Emms & Kelly, 2019). Orthofinder first identifies orthologs and assigns them to orthogroups. We used the -M msa option to generate multiple sequence alignments for each orthogroup with fasttree. Orthofinder then generated gene trees for all orthogroups and identifies all gene duplication events in the gene trees. From thousands of gene trees, Orthofinder then infers several rooted species trees. Knowing that *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* represent the outgroup for the Lecanoromycete tree, the correct rooted species tree was then selected as the input to rerun the final Orthofinder Analysis steps from pre-computed trees. The final species tree was then visualized in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Inkscape (https://inkscape.org) to label species and families.

MAT locus characterization and visualization

The full set of annotated genes for each species generated from funannotate v1.8.9 was used to create a BLAST database. The *APN2* and *SLA2* genes previously recovered from *Lepraria neglecta* (Pfeffer et al. 2023) were used as query against the protein database for each species. Several species from Gerasimova et al. (2022) did not produce good BLAST hits to *APN2* or *SLA2*, or the peripheral *MAT* genes were not close together on the assembly. These species were not included in further *MAT1* analysis, leaving seven species from

Gerasimova et al.: *Cladonia macilenta, Cladonia metacorallifera, Cladonia rangiferina, Evernia prunastri Letharia columbiana, Letharia lupina,* and *Usnea hakonensis*. For species with good BLAST hits, the resulting annotations, and all annotations lying between *APN2* and *SLA2* were extracted from the .gff3 annotation file and the cds-transcripts exon file. A visualization of the *MAT1* locus alignment for each idiomorph was generated using Clinker v0.0.27 (Gilchrist & Chooi 2020). The final alignment visualization was edited in Microsoft Powerpoint.

Substitution analysis

To investigate the evolution of genes relating to reproduction, we analyzed the rate of nonsynymous mutations *(d_N)* to synonymous mutations *(d_S)*: $\omega = d_N/d_S$. For these analyses we searched for gene homologs from *Candida spp.* involved in the yeast-to-hypha dimorphic switching pathway, which is regulated by the highly conserved cAMP/PKA and MAPK pathways (Borges-Walmsley & Walmsley, 2000; Tagirdzhanova et al., 2021). We also searched for meiosis-specific gene homologs (Schurko & Logsdon, 2008). Query genes were taken from the NCBI database and queried against *Lepraria* neglecta. The best result from *L. neglecta* was queried against the NCBI protein database to confirm its identity. That protein annotation was queried against the other genome assemblies belonging to *Lepraria* (Stereocaulaceae) and *Cladonia* (Cladoniaceae). The homologous protein-coding exons were extracted with Samtools v1.15.1 (Li et al., 2009)and aligned with Muscle v3.8.1551 (Edgar, 2004). Substitution analyses require perfect alignments,

so they were manually trimmed to represent the true reading frame. Raw and final coding-sequence alignments are presented here (supplementary data 1)

BayesCode v1.1.2 (https://github.com/ThibaultLatrille/bayescode, Rodrigue et al., 2021) was used on the resulting alignments to estimate ω *and* ω_0. We first estimated ω_0, a nearly-neutral model that assumed all sites exists at mutation-selection equilibrium. The equilibrium is balanced by nucleotide-level mutation parameters and codon-level selection parameters, and dN/dS is an emergent property of the model rather than an explicit parameter (Latrille et al., 2023). Each Monte Carlo Markov Chain (MCMC) was run for 2,000 generations with a sliding selection window of 20 codons. The results were read with a burn-in of 1,000 generations to obtain the posterior mean and 95% confidence intervals of ω_0 for each site. We then used the classical model to estimate a classical model of ω which explicitly parameterizes dN/dS, again running each MCMC for 2,000 generations and reading with a 1,000 generation burnin (Rodrigue et al., 2021). For all sites in which the lower credibility bound for ω (α=0.05) is above the upper credibility bound for ω_0 (α=0.05), the null model is rejected and the site is assumed to be under positive selection. It is worth noting that these are unilateral tests where $\omega > \omega_0$, and the credible intervals are independent, so each test has a $(\alpha/2)^2$ = 0.000625 risk (an estimated rate of 0.625 false positives per 1,000 rejections of the null model).

Results

Comparative genomics

The genome assemblies presented here are highly complete and contiguous, all recovering >92% single-copy BUSCO genes (Table 1). Further, they are highly pure, each returning <1.0% duplicate BUSCO genes except for *Lepraria normandinoides* (1.2%), *Lepraria oxybapha* (1.1%), and *Sticta deyana* (2.4%). Funannotate predicted between 9,579 and 11,857 genes for each genome assembly (Table 2). Orthofinder assigned a total of 254,608 genes to 15,668 orthogroups. The consensus species tree has 100% support for all nodes, and all species were placed in their expected families (Fig. 1).

MAT locus characterization and visualization

All five species of *Lepraria* possess an intact and apparently conserved mating type locus, complete with peripheral *APN2* and *SLA2* genes, a primary *MAT* gene, and a secondary *MAT* gene (Fig. 2). The *Lepraria finkii, Lepraria normandinoides,* and *Lepraria oxybapha* reference genomes possess a *MAT1-1* idiomorphic mating-type locus (Fig. 2a), whereas the *Lepraria lanata* and *Lepraria neglecta* reference genomes possess *MAT1-2* idiomorphs of the mating-type locus (Fig. 2b).

Substitution analysis

We searched for eight meiosis-specific genes and eight genes key in the yeast-hypha dimorphic switching pathway (Table 3). All genes recovered homologs, and their identity was confirmed with reciprocal BLAST searches against the entire NCBI protein database. Further, the genes demonstrated similar – though not identical – patterns in Cladoniaceae and in the substitution model.

According to the model, sites for which $\omega = \omega_0$ are inferred to be under a nearly neutral selection regime, sites for which $\omega < \omega_0$ are inferred to be under negative (purifying) selection, and sites for which $\omega > \omega_0$ are inferred to be under positive selection. Most sites in each gene are inferred to be evolving under a neutral or negative (purifying) selection Regime (Fig. 3, Fig. 4). Most genes also possess a few sites that are likely under positive selection. However, likely due to a small sample size, these results are not statistically significant, and so inferences must be taken lightly.

Discussion

Here we demonstrate that the presence of a mating-type locus in four additional species of *Lepraria*, showing that previously reported mating-type locus in *L. neglecta* is not an anomaly in the genus *Lepraria* (Pfeffer et al. 2023). Further, the architecture of that mating type locus is not consistent species to species. Three species have a MAT1-2 idiomorph in the reference genome generated (*L. finkii, L. oxybapha, and L. normandinoides*) and two species have a MAT1-1 idiomorph (*L. lanata and L. neglecta*) The presence, conservation, and diversity of the mating type locus in a genus of presumed asexual raises questions about the function of the MAT1 locus.

The precise functions of *MAT* genes have historically been a major point of contention. Since Billiard et al. (2011) reviewed *MAT* several hypotheses to explain the origin of the *MAT* locus, researchers have consistently tacitly or explicitly assumed that the primary function of the *MAT* locus is to determine sexual compatibility and incompatibility (Böhm et al., 2013; W. Li et al., 2013; Pizarro et al., 2019; Wilken et al., 2017). Contrastingly, Perrin (2011) suggests that "the first and main function of mating types is indeed that of controlling key developmental switches along the haploid-diploid life cycle." Billiard et al (2011) were aware of this hypothesis but disregarded the basis that mating types are not universal. But genomic research in the fungi has observed consistent and apparently universal conservation of a *MAT* locus in all studied fungi. Perhaps it is worthwhile to revisit

the question of *MAT* gene functions, especially in the context of putatively asexual lineages.

Because of the centrality of the *MAT1* locus in determining a species' mating system, it is often assumed in the literature that the *MAT1* locus is the center of control for sexual reproduction in Ascomycetes. This may be so, but it is also clear that the genetic control of reproduction extends far beyond the *MAT1* locus. *MAT1* genes code for transcription factors which regulate the expression of potentially hundreds of pheromone and pheromone receptor genes (Jones & Bennett, 2011). *MAT*-derived pheromones in *Fusarium oxysporum* have been shown to be important for quorum sensing functions, including density-dependent regulation of asexual sporulation (Martin, 2019; Vitale et al., 2019). Evidently, the *MAT* locus is tightly connected to many regulatory pathways, and it can not be accurately assumed that its primary function is sex determination. Rather, as Perrin (2011) suggested, evidence strongly indicates that the *MAT* locus is primarily responsible for managing key developmental switches in an organism's life cycle, regardless of sexuality.

Previous research has uncovered the *MAT* locus in the genus *Lepraria* (Pfeffer et al., 2023),and found the same genes relating to dimorphic switching in other Lecanoromycete lichens (Tagirdzhanova et al., 2021).Our results confirm and expand on these findings. The analyses of substitution ratios revealed similar patterns in Cladoniaceae and Stereocaulaceae. However, these results lack statistical significance, and therefore cannot be relied on. The presence and

putative functionality of these genes may indicate the presence of a sexual lifecycle, possibly involving a yeast-like phase. However, it may also be that the genes are vital to lineage survival for other reasons.

Much work remains to characterize the evolution of reproductive strategies in the context of lichen symbiosis. Pairs of sexual and vegetatively asexual lichen species are frequently observed, and are often very genetically similar to one another, indicating recent common ancestry (Degtjarenko et al., 2020; Ohmura, 2020). Clonal lichenized fungi have an increased coevolutionary potential with their photobionts, which may increase their fitness. These results demonstrate that *Lepraria spp.* possess the genetic machinery for sexual reproduction and dimorphic switching. It is possible that *Lepraria* may maintain a clonal morphology while simultaneously reaping the benefits of mixis and meiosis. Further research on the population structure of *Lepraria spp.* will be essential to determining whether this genus is cryptically sexual.

Literature Cited

Allen, J. L., Jones, S. J. M., & McMullin, R. T. (2021). Draft Genome Sequence of the Lichenized Fungus Bacidia gigantensis. *Microbiology Resource Announcements*, *10*(44), 1–3. https://doi.org/10.1128/mra.00686-21

Allen, J. L., & Lendemer, J. C. (2022). A call to reconceptualize lichen symbioses. *Trends in Ecology & Evolution*, 1–8. https://doi.org/10.1016/j.tree.2022.03.004

Allen, J. L., McKenzie, S. K., Sleith, R. S., & Alter, S. E. (2018). First genome-wide analysis of the endangered, endemic lichen Cetradonia linearis reveals isolation by distance and strong population structure. *American Journal of Botany*, *105*(9), 1556–1567. https://doi.org/10.1002/ajb2.1150

Arkhipova, I., & Meselson, M. (2005). Deleterious transposable elements and the extinction of asexuals. *BioEssays*, *27*(1), 76–85. https://doi.org/10.1002/bies.20159

Bast, J., Jaron, K. S., Schuseil, D., Roze, D., & Schwander, T. (2019). Asexual reproduction reduces transposable element load in experimental yeast populations. *ELife*, *8*, 1–10. https://doi.org/10.7554/eLife.48548

Billiard, S., López-Villavicencio, M., Hood, M. E., & Giraud, T. (2012). Sex, outcrossing and mating types: Unsolved questions in fungi and beyond. *Journal of Evolutionary Biology*, *25*(6), 1020–1038. https://doi.org/10.1111/j.1420-9101.2012.02495.x

Billmyre, B. R., Croll, D., Li, W., Mieczkowski, P., Carter, D. A., Cuomo, C. A., Kronstad, J. W., & Heitman, J. (2014). Highly recombinant VGII Cryptococcus gattii population develops clonal outbreak clusters through both sexual macroevolution and asexual microevolution. *MBio*, *5*(4), 1–16. https://doi.org/10.1128/mBio.01494-14

Böhm, J., Hoff, B., O'Gorman, C. M., Wolfers, S., Klix, V., Binger, D., Zadra, I., Kürnsteiner, H., Pöggeler, S., Dyer, P. S., & Kück, U. (2013). Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus Penicillium chrysogenum. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(4), 1476–1481. https://doi.org/10.1073/pnas.1217943110

Borges-Walmsley, M. I., & Walmsley, A. R. (2000). *cAMP signalling in pathogenic fungi: control of dimorphic switching and pathogenicity*.

Brodo, I. M., Sharnoff, S. D., & Sharnoff, S. (2001). *Lichens of North America*. Yale University Press.

Chen, P., & Zhang, J. (2021). Asexual Experimental Evolution of Yeast Does Not Curtail Transposable Elements. *Molecular Biology and Evolution*, *38*(7), 2831–2842. https://doi.org/10.1093/molbev/msab073

Coppin, E., Debuchy, R., Arnaise, S., & Picard, M. (1997). Mating types and sexual development in filamentous ascomycetes. *Microbiology and Molecular Biology Reviews*, *61*(4), 411–428. https://doi.org/10.1128/mmbr.61.4.411-428.1997

Degtjarenko, P., Mark, K., Moisejevs, R., & Himelbrant, D. (2020). Low genetic differentiation between apotheciate Usnea fl orida and sorediate Usnea sub fl oridana (Parmeliaceae , Ascomycota) based on microsatellite data. *Fungal Biology*, *124*(10), 892–902. https://doi.org/10.1016/j.funbio.2020.07.007

Dodge, B. O. (1932). The Non-Sexual and the Sexual Functions of Microconidia of Neurospora. *Torrey Botanical Society*, *59*(6), 347–360.

Dodge, B. O. (1936). Spermatia and Nuclear Migrations in Pleurage anserina A. *Mycologia*, *28*(3), 284–291.

Dyer, P. S. (2008). Evolutionary Biology: Genomic clues to original sex in fungi. *Current Biology*, *18*(5), 207–209. https://doi.org/10.1016/j.cub.2008.01.004

Dyer, P. S., & Kück, U. (2017). Sex and the Imperfect Fungi. *Microbiology Spectrum*, *5*(3). https://doi.org/10.1128/microbiolspec.funk-0043-2017

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*(1), 1–14. https://doi.org/10.1186/s13059-019-1832-y

Fernández-Mendoza, F., Domaschke, S., Garcia, M. A., Jordan, P., Martin, M. P., & Printzen, C. (2011). Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. *Molecular Ecology*, *20*, 1208–1232. https://doi.org/10.1111/j.1365-294X.2010.04993.x

Fukumori, Y., Nakajima, M., & Akutsu, K. (2004). Microconidia act the role as spermatia in the sexual reproduction of Botrytis cinerea. *Journal of General Plant Pathology*, *70*(5), 256–260. https://doi.org/10.1007/s10327-004-0124-9

Gerasimova, J. V., Beck, A., Werth, S., & Resl, P. (2022). High Diversity of Type I Polyketide Genes in Bacidia rubella as Revealed by the Comparative Analysis of 23 Lichen Genomes. *Journal of Fungi*, *8*(5). https://doi.org/10.3390/jof8050449

Gibson, A. K., Delph, L. F., & Lively, C. M. (2017). The two-fold cost of sex: Experimental evidence from a natural system. *Evolution Letters*, *1*(1), 6–15.

Haas, B. J., Salzberg, S. L., Zhu, W., Pertea, M., Allen, J. E., Orvis, J., White, O., Robin, C. R., & Wortman, J. R. (2008). Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. *Genome Biology*, *9*(1). https://doi.org/10.1186/gb-2008-9-1-r7

Halary, S., Malik, S. B., Lildhar, L., Slamovits, C. H., Hijri, M., & Corradi, N. (2011). Conserved meiotic machinery in Glomus spp., a putatively ancient asexual fungal lineage. *Genome Biology and Evolution*, *3*(1), 950–958. https://doi.org/10.1093/gbe/evr089

Hartmann, F. E., Ament-Velasquez, S. L., Vogan, A. A., Gautier, V., Le Prieur, S., Berramdane, M., Snirc, A., Johannesson, H., Grognet, P., Malagnac, F., Silar, P., & Giraud, T. (2021). Size variation of the nonrecombining region on the mating-type chromosomes in the fungal Podospora anserina species complex. *Molecular Biology and Evolution*, *38*(6), 2475–2492. https://doi.org/10.1093/molbev/msab040

Heitman, J. (2015). Evolution of sexual reproduction: A view from the fungal kingdom supports an evolutionary epoch with sex before sexes. *Fungal Biology Reviews*, *29*(3–4), 108–117. https://doi.org/10.1016/j.fbr.2015.08.002

Henk, D. A., Shahar-Golan, R., Devi, K. R., Boyce, K. J., Zhan, N., Fedorova, N. D., Nierman, W. C., Hsueh, P. R., Yuen, K. Y., Sieu, T. P. M., Van Kinh, N., Wertheim, H., Baker, S. G., Day, J. N., Vanittanakom, N., Bignell, E. M., Andrianopoulos, A., & Fisher, M. C. (2012). Clonality Despite Sex: The Evolution of Host-Associated Sexual Neighborhoods in the Pathogenic Fungus Penicillium marneffei. *PLoS Pathogens*, *8*(10). https://doi.org/10.1371/journal.ppat.1002851

Hodač, L., Klatt, S., Hojsgaard, D., Sharbel, T. F., & Hörandl, E. (2019). A little bit of sex prevents mutation accumulation even in apomictic polyploid plants. *BMC Evolutionary Biology*, *19*(1), 1–11. https://doi.org/10.1186/s12862-019-1495-z

Hofstatter, P. G., Brown, M. W., & Lahr, D. J. G. (2018). Comparative genomics supports sex and meiosis in diverse amoebozoa. *Genome Biology and Evolution*, *10*(11), 3118–3128. https://doi.org/10.1093/gbe/evy241

Hörandl, E., & Hadacek, F. (2013). The oxidative damage initiation hypothesis for meiosis. *Plant Reproduction*, *26*(4), 351–367. https://doi.org/10.1007/s00497-013-0234-7

Hörandl, E., & Hadacek, F. (2020). Oxygen, life forms, and the evolution of sexes in multicellular eukaryotes. *Heredity*, *125*(1–2). https://doi.org/10.1038/s41437-020-0317-9

Itten, B., & Honegger, R. (2010). Population genetics in the homothallic lichen-forming ascomycete Xanthoria parietina. *Lichenologist*, *42*(6), 751–761. https://doi.org/10.1017/S0024282910000411

Johnson, D. W., Monro, K., & Marshall, D. J. (2013). The maintenance of sperm variability: Context-dependent selection on sperm morphology in a broadcast spawning invertebrate. *Evolution*, *67*(5), 1383–1395. https://doi.org/10.1111/evo.12022

Jones, S. K., & Bennett, R. J. (2011). Fungal mating pheromones: Choreographing the dating game. *Fungal Genetics and Biology*, *48*(7), 668–676. https://doi.org/10.1016/j.fgb.2011.04.001

Kendrick, B. (2017). *The fifth kingdom: an introduction to mycology* (4th editio). Hacket Publishing Company.

Kolmogoroov, M., Bickhart, D. M., Behsaz, B., Gurevich, A., Rayko, M., Shin, S. B., Kuhn, K., & Et, Al. (2020). metaFlye: scalable long-read metagenome assembly using repeat graphs. *Nature Methods*, *17*(11), 1103–1110.

Kukwa, M., Kosecka, M., Jabłońska, A., Flakus, A., Rodriguez-flakus, P., & Guzow-krzemińska, B. (2023). *Pseudolepraria , a new leprose genus revealed in Ramalinaceae (Ascomycota , Lecanoromycetes , Lecanorales) to accommodate Lepraria stephaniana*. *112*, 97–112. https://doi.org/10.3897/mycokeys.96.98029

Lassagne, A., Brun, S., Malagnac, F., Adreit, H., Milazzo, J., Fournier, E., & Tharreau, D. (2022). Male fertility in Pyricularia oryzae: Microconidia are spermatia. *Environmental Microbiology*, *24*(12), 6365–6375. https://doi.org/10.1111/1462-2920.16226

Latrille, T., Rodrigue, N., & Lartillot, N. (2023). Genes and sites under adaptation at the phylogenetic scale also exhibit adaptation at the population-genetic scale. *EASTERN WASHINGTON UNIVERSITY JFK LIBRARY*, 100. https://doi.org/10.1073/pnas

Lendemer, J. C. (2013). A monograph of the crustose members of the genus Lepraria Ach. s. str. (Stereocaulaceae, Lichenized Ascomycetes) in North America north of Mexico. *Opuscula Philolichenum*, *12*(1), 27–141.

Li, W., Sullivan, T. D., Walton, E., Averette, F., Sakthikumar, S., Cuomo, C. A., & Klein, B. S. (2013). Identification of the Mating-Type (MAT) Locus That Controls Sexual Reproduction of Blastomyces dermatitidis. *Eukaryotic Cell*, *12*(1). https://doi.org/10.1128/EC.00249-12

Maheshwari, R. (1999). Microconidia of Neurospora crassa. *Fungal Genetics and Biology*, *26*(1), 1–18. https://doi.org/10.1006/fgbi.1998.1103

Maldonado, J. A., Firneno Jr., T. J., Hall, A. S., & Fujita, M. K. (2022). Parthenogenesis doubles the rate of amino acid substitution in whiptail mitochondria. *Evolution*, *76*(7), 1434–1442. https://doi.org/10.1111/evo.14509

Martin, S. G. (2019). Quorum sensing with pheromones. *Nature Microbiology*, *4*(September), 1430–1431. https://doi.org/10.1038/s41564-019-0538-y

McKenzie, S. K., Walston, R. F., & Allen, J. L. (2020). Complete, high-quality genomes from long-read metagenomic sequencing of two wolf lichen thalli reveals enigmatic genome architecture. *Genomics*, *112*(5), 3150–3156. https://doi.org/10.1016/j.ygeno.2020.06.006

Melián, C., Alonso, D., Allesina, S., Condit, R. S., & Etienne, R. S. (2012). Does sex speed up evolutionary rate and increase biodiversity? *PLoS Computational Biology*, *8*(3), 1–9.

Molina, M. C., Divakar, P. K., Zhang, N., González, N., & Struwe, L. (2013). Non-developing ascospores in apothecia of asexually reproducing lichen-forming fungi. *International Microbiology*, *16*(3), 145–155. https://doi.org/10.2436/20.1501.01.189

Muller, H. J. (1932). Some genetic Aspects of Sex. *American Society of Naturalists*, *703*, 118–138.

Nasmyth, K. A., & Tatchell, K. (1980). The structure of transposable yeast mating type loci. *Cell*, *19*(3), 753–764. https://doi.org/10.1016/S0092-8674(80)80051-1

Nishimura, K., & Hoshino, N. (2011). Adaptive significance of egg size variation of aquatic organisms in relation to mesoscale features of aquatic environments. In *The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection* (pp. 131–167). Cambridge University Press. https://doi.org/10.1017/CBO9780511975943.006

Ohmura, Y. (2020). *presuming morphological , chemical and molecular phylogenetic data*. *65*(2), 265–271.

Otto, S. P. (2009). The Evolutionary Enigma of Sex. *The American Naturalist*, *174*(S1), 1–14. https://doi.org/10.1086/599084

Perrin, N. (2012). What uses are mating types? The “developmental switch” model. *Evolution*, *66*(4), 947–956. https://doi.org/10.1111/j.1558-5646.2011.01562.x

Pfeffer, B., Lymbery, C., Booth, B., & Allen, J. L. (2023). Chromosomal genome sequence assembly and mating-type (MAT) locus characterization of the leprose asexual lichenized fungus Lepraria neglecta (Nyl.) Erichsen . *The Lichenologist*, *55*(1), 41–50. https://doi.org/10.1017/s002428292200041x

Pizarro, D., Dal Grande, F., Leavitt, S. D., Dyer, P. S., Schmitt, I., Crespo, A., Thorsten Lumbsch, H., Divakar, P. K., & Barluenga, M. (2019). Whole-Genome Sequence Data Uncover Widespread Heterothallism in the Largest Group of Lichen-Forming Fungi. *Genome Biology and Evolution*, *11*(3), 721–730. https://doi.org/10.1093/gbe/evz027

Poelt, J. (1970). Das Konzept der Artenpaare bei den Flechten. *Vorträge Aus Dem Gesamtgebiet Der Botanik, Neue Folge*, *4*, 187–198.

Randerson, J. P., & Hurst, L. D. (2001). A comparative test of a theory for the evolution of anisogamy. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1469), 879–884. https://doi.org/10.1098/rspb.2000.1581

Rodrigue, N., Latrille, T., & Lartillot, N. (2021). A Bayesian Mutation-Selection Framework for Detecting Site-Specific Adaptive Evolution in Protein-Coding Genes. *Molecular Biology and Evolution*, *38*(3), 1199–1208. https://doi.org/10.1093/molbev/msaa265

Rusche, L. N., & Kirchmaier, A. L. (2003). The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. *Annual Review of Biochemistry*, *72*(1), 481–516. https://doi.org/10.1146/annurev.biochem.

Sato, S., Beakes, G., Idei, M., Nagumo, T., & Mann, D. G. (2011). Novel sex cells and evidence for sex pheromones in diatoms. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0026923

Schurko, A. M., & Logsdon, J. M. (2008). Using a meiosis detection toolkit to investigate ancient asexual “scandals” and the evolution of sex. *BioEssays*, *30*(6), 579–589. https://doi.org/10.1002/bies.20764

Senanayake, I. C., Pem, D., Rathnayaka, A. R., Wijesinghe, S. N., Tibpromma, S., Wanasinghe, D. N., Phookamsak, R., Kularathnage, N. D., Gomdola, D., Harishchandra, D., Dissanayake, L. S., Xiang, M., Ekanayaka, A. H., Mckenzie, E. H. C., Hyde, K. D., Zhang, H., & Xie, N. (2022). Predicting global

numbers of teleomorphic ascomycetes. In *Fungal Diversity* (Issue February). Springer Netherlands. https://doi.org/10.1007/s13225-022-00498-w

Simão, F. A., Waterhouse, R. M., Ioannidis, P., V., K. E., & M., Z. E. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212.

Singh, G., Dal Grande, F., Cornejo, C., Schmitt, I., & Scheidegger, C. (2012). Genetic Basis of Self-Incompatibility in the Lichen-Forming Fungus Lobaria pulmonaria and Skewed Frequency Distribution of Mating-Type Idiomorphs: Implications for Conservation. *PLoS ONE*, *7*(12). https://doi.org/10.1371/journal.pone.0051402

Singh, G., Grande, F. D., Werth, S., & Scheidegger, C. (2015). Long-term consequences of disturbances on reproductive strategies of the rare epiphytic lichen Lobaria pulmonaria : clonality a gift and a curse. *FEMS Microbiology Ecology*, *91*(November 2014), 1–11. https://doi.org/10.1093/femsec/fiu009

Skejo, J., & Franjević, D. (2020). Eukaryotes Are a Holophyletic Group of Polyphyletic Origin. *Frontiers in Microbiology*, *11*(July), 1–6. https://doi.org/10.3389/fmicb.2020.01380

Smith, J. M. (1978). Cambridge University Press. *The Journal of British Studies*, *32*(1), b1–b9. https://doi.org/10.1017/S0021937100024837

Spribille, T., Resl, P., Stanton, D. E., & Tagirdzhanova, G. (2022). Evolutionary biology of lichen symbioses. *New Phytologist*, *234*, 1566–1582. https://doi.org/10.1111/nph.18048

Tagirdzhanova, G., Saary, P., Tingley, J. P., Díaz-Escandón, D., Abbott, D. W., Finn, R. D., & Spribille, T. (2021). Predicted Input of Uncultured Fungal Symbionts to a Lichen Symbiosis from Metagenome-Assembled Genomes. *Genome Biology and Evolution*, *13*(4). https://doi.org/10.1093/gbe/evab047

Thomson, G. J., Hernon, C., Austriaco, N., Shapiro, R. S., Belenky, P., & Bennett, R. J. (2019). Metabolism-induced oxidative stress and DNA damage selectively trigger genome instability in polyploid fungal cells. *The EMBO Journal*, *38*(19), 1–17. https://doi.org/10.15252/embj.2019101597

Torruella, G., De Mendoza, A., Grau-Bové, X., Antó, M., Chaplin, M. A., Del Campo, J., Eme, L., Pérez-Cordón, G., Whipps, C. M., Nichols, K. M., Paley, R., Roger, A. J., Sitjà-Bobadilla, A., Donachie, S., & Ruiz-Trillo, I. (2015). Phylogenomics Reveals Convergent Evolution of Lifestyles in Close Relatives of Animals and Fungi. *Current Biology*, *25*(18), 2404–2410. https://doi.org/10.1016/j.cub.2015.07.053

Tsai, I. J., Bensasson, D., Burt, A., & Koufopanou, V. (2008). Population genomics of the wild yeast Saccharomyces paradoxus: Quantifying the life cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(12), 4957–4962. https://doi.org/10.1073/pnas.0707314105

Tsui, C. K. M., DiGuistini, S., Ye, W., Feau, N., Dhillon, B., Bohlmann, J., & Hamelin, R. C. (2013). Unequal Recombination and Evolution of the Mating-Type (MAT) Loci in the Pathogenic Fungus Grosmannia clavigera and Relatives. *G3*, *3*(March), 465–480. https://doi.org/10.1534/g3.112.004986

Van Dyke, M. (2017). Direct Double-Stranded DNA Quantitation from PCR Reactions V.2 Michael Van Dyke 1 1. *Protocols.Io*, 5–7. rotocols.io/view/direct-double-stranded-dna-quantitation-from-pcr-r-k5pcy5n

Vitale, S., Di Pietro, A., & Turrà, D. (2019). Autocrine pheromone signalling regulates community behaviour in the fungal pathogen Fusarium oxysporum. *Nature Microbiology*, *4*(9), 1443–1449. https://doi.org/10.1038/s41564-019-0456-z

Vittorelli, N., Rodríguez de la Vega, R. C., Snirc, A., Levert, E., Gautier, V., Lalanne, C., De Filippo, E., Gladieux, P., Guillou, S., Zhang, Y., Tejomurthula, S., Grigoriev, I. V., Debuchy, R., Silar, P., Giraud, T., & Hartmann, F. E. (2023). Stepwise recombination suppression around the mating-type locus in an ascomycete fungus with self-fertile spores. *PLoS Genetics*, *19*(2). https://doi.org/10.1371/journal.pgen.1010347

Wahl, L. M., & Tanaka, M. M. (2022). Hazardous loss of genetic diversity through selective sweeps in asexual populations. *The American Naturalist*, *199*(3).

Weedall, G. D., & Hall, N. (2015). Sexual reproduction and genetic exchange in parasitic protists. *Parasitology*, *142*, S120–S127. https://doi.org/10.1017/S0031182014001693

Wesche, J., & Weber, R. W. S. (2023). Are microconidia infectious principles in Neonectria ditissima? *Journal of Plant Diseases and Protection*, *130*(1), 157–162. https://doi.org/10.1007/s41348-022-00669-6

Wilken, P. M., Steenkamp, E. T., Wingfield, M. J., de Beer, Z. W., & Wingfield, B. D. (2017). Which MAT gene? Pezizomycotina (Ascomycota) mating-type gene nomenclature reconsidered. *Fungal Biology Reviews*, *31*(4), 199–211. https://doi.org/10.1016/j.fbr.2017.05.003

Wilson, A. M., Gabriel, R., Singer, S. W., Schuerg, T., Wilken, P. M., van der Nest, M. A., Wingfield, M. J., & Wingfield, B. D. (2021). Doing it alone: Unisexual reproduction in filamentous ascomycete fungi. *Fungal Biology Reviews*, *35*, 1–13. https://doi.org/10.1016/j.fbr.2020.12.003

Wilson, A. M., Wilken, P. M., van der Nest, M. A., Steenkamp, E. T., Wingfield, M. J., & Wingfield, B. D. (2015). *Homothallism : an umbrella term for describing diverse sexual behaviours*. *6*(1), 207–214.

Wright, S. (1931). Evolution in mendelian populations. *Genetics*, *16*(2), 18–159. https://doi.org/10.1007/BF02459575

Table 1.- Assembly metrics of all genome sequence assemblies presented here. Read statistics represent the file used in the final assembly, after first filtering short reads and mapping initial reads to the first Ascomycete assembly. BUSCO scores show the number of genes and percentage recovered in parentheses. N50 and L50 are measures of assembly contiguity. L50 is the number of the longest contigs which together represent >= 50% of the total assembly Length. Within that of longest contigs which together represent 50% of the genome, N50 is the length of the shortest contig.

Species	Number of reads	Minimum read length (bp)	Mean read length (bp)	Maximum read length (bp)	Minimum Ascomycete contig depth	BUSCO complete, single-copy genes (S)	BUSCO duplicate genes (D)	Total Length (Mb)	Number of Contigs	N50 (Mb)	L50
Lepraria lanata	500,114	5,000	9,407	138,419	70x	1660 (97.3%)	7 (0.4%)	47.2	39	2.16	6
Lepraria finkii	775,811	5,000	8,659	484,830	70x	1644 (96.4%)	10 (0.6%)	40.9	34	4.15	4
Lepraria normandinoides	747,255	2,000	6,371	365,263	35x	1592 (93.3%)	21 (1.2%)	34.9	234	0.37	29
Lepraria oxybapha	1,343,229	2,000	5,722	612,472	35x	1636 (95.9%)	19 (1.1%)	42.3	110	1.24	12
Punctelia appalachiensis	847,937	4,000	8,269	458,243	50x	1639 (96.1%)	7 (0.4%)	45.69	25	2.21	9
Punctelia rudecta	1,313,391	4,000	7,854	212,403	80x	1654 (96.9%)	7 (0.4%)	42.75	31	1.87	10
Usnea strigosa	768,997	8,000	11,398	390,710	50x	1585 (92.9%)	3 (0.2%)	43.17	32	2.17	9
Usnea subfusca	1,702,241	4,000	7,493	268,685	80x	1649 (96.7%)	8 (0.5%)	49.09	30	2.17	9
Sticta deyana	1,775,800	1,000	5,093	79,651	40x	1531 (91.7%)	41 (2.4%)	43.51	127	0.89	18
Sulcaria isiidifera	1,175,087	112	4,676	65,986	50x	1659 (97.0%)	4 (0.2%)	71.39	23	3.46	9

Table 2.- Annotation results for each species presented here, produced with funannotate v1.8.7. Between 9,579 and 11,857 genes are predicted for each assembly. Gene categories listed here include transfer RNA (tRNA) and Carbohydrate-Activate Enzymes (CAZymes).

Species	total genes	tRNA	CAZymes
Lepraria finkii	10091	98	192
Lepraria lanata	10107	62	205
Lepraria normandinoides	10762	71	200
Lepraria oxybapha	9795	68	215
Punctelia appalachiensis	9633	65	231
Punctelia rudecta	11857	58	256
Sticta deyana	13368	60	229
Sulcaria isidiifera	9579	91	195
Usnea Strigosa	10523	89	256
Usnea subfusca	10852	64	274

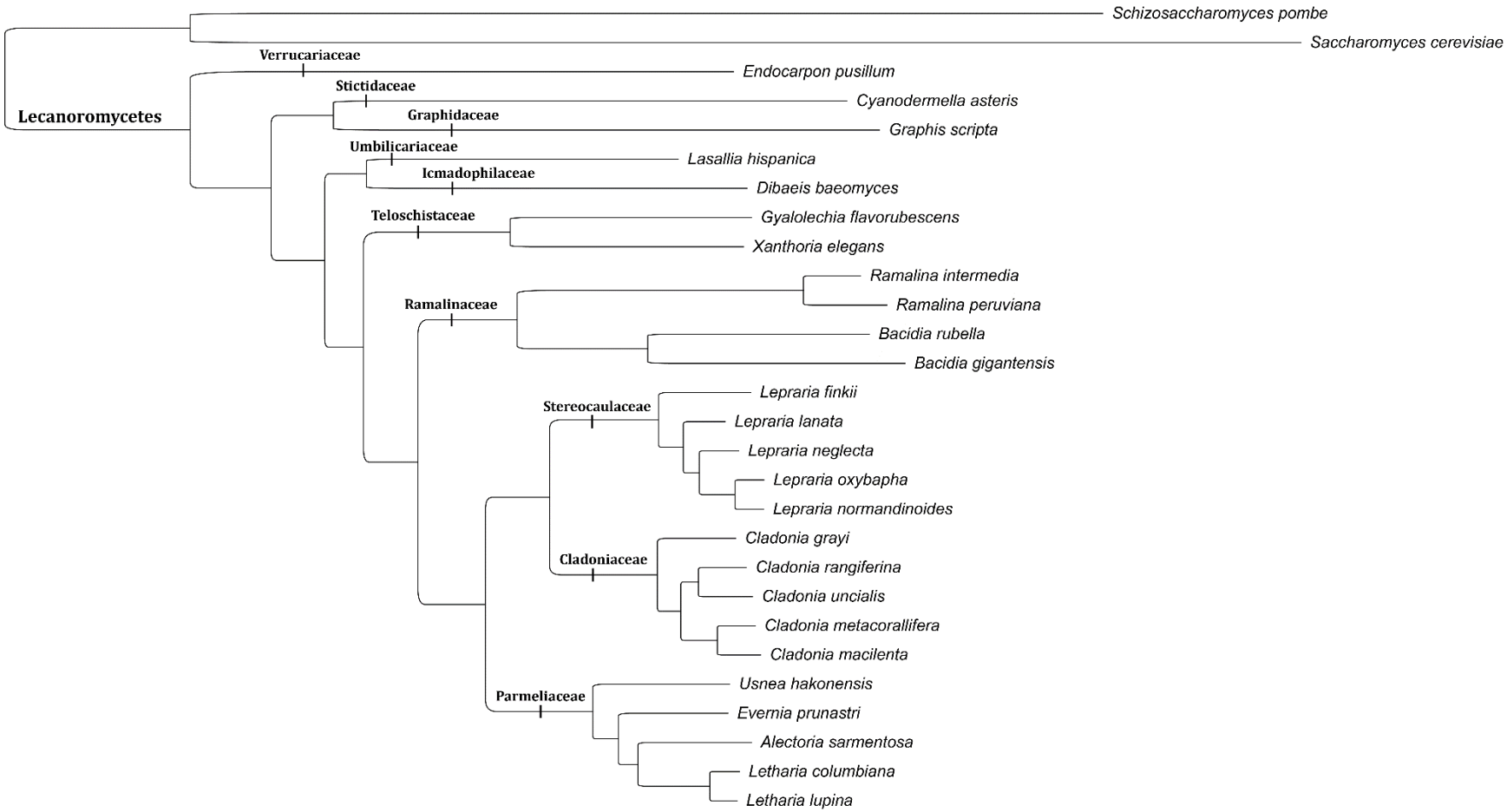

Fig. 1.- Species tree produced with Orthofinder v2.5.4 through multiple sequence alignments of all annotated protein-coding genes. All nodes have 100% support.

A.	B.

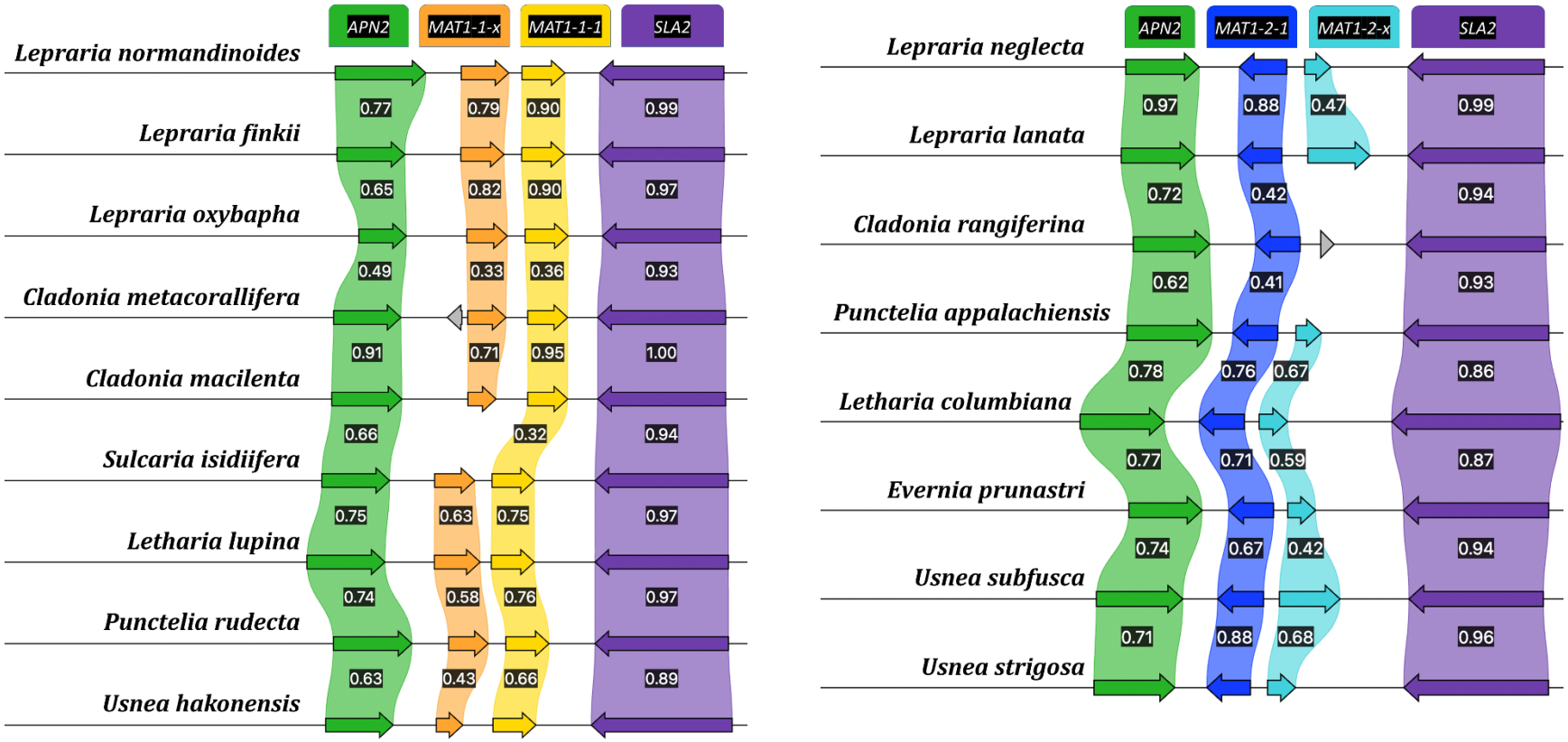

Fig. 2- Mating-type locus recovered from various Lecanoromycete lichens. Genes are colored by their homology, and the two idiomorphic loci: A. the MAT1-1 locus and B. *MAT1-2* locus, are visualized with links to homologous genes. Numbers indicate the proportion of identical nucleotides.

Table 3.- BLAST results against *Lepraria neglecta* of genes associated with Dimorphic switching and Meiosis. Query proteins were retrieved from the NCBI protein database. Bit score is a metric of alignment quality with higher numbers indicating better quality. E-value refers to the number of identical results that are expected to be recovered by random chance from a database of equal size. Percent identity is the proportion of identical nucleotides shared by query and subject.

function of interest	gene	query protein ID	query species	best annotation result	bit score	e-value	% identity
Dimorphic switching	CDC42	KAG8204675.1	Candida africana	OEA41_003131	313	6.00E-111	79%
	CYR1	BAA93553.1	Candida albicans	OEA41_002316	931	0.0	36%
	GPA2	KAG8203374.1	Candida africana	OEA41_006220	359	1.00E-121	51%
	RAS2P	AOW28466.1	Candida albicans	OEA41_002194	65.1	1.00E-12	26%
	STE2	XP_051673087.1	Candida margitis	OEA41_007595	837	0.0	37%
	STE11	XP_051669659.1	Candida margitis	OEA41_008723	298	1.00E-87	62%
	STE7	XP_051673087.1	Candida margitis	OEA41_006556	287	2.00E-90	49%
	STE20	KAG8203151.1	Candida africana	OEA41_007595	837	0.0	37%
Meiosis	SPO11	XP_051670339.1	Candida margitis	OEA41_003607	45.8	8.00E-06	21%
	HOP1	XP_003868223.1	Candida orthopsilosis	OEA41_010075	31.2	0.87	27%
	HOP2	XP_003870138.1	Candida orthopsilosis	OEA41_006892	37.4	0.001	26%
	MND1	XP_051671904.1	Candida margitis	OEA41_000341	52	1.00E-08	37%
	REC8	XP_049260990.1	Candida subhashii	OEA41_006358	31.2	0.96	32%
	DMC1	KAG0690054.1	Candida californica	OEA41_007057	315	3.00E-107	53%
	MSH4	XP_051669776.1	Candida margitis	OEA41_004289	385	7.00E-122	31%
	MSH5	KAG0691138.1	Candida californica	OEA41_007791	202	9.00E-56	25%

A.

B.

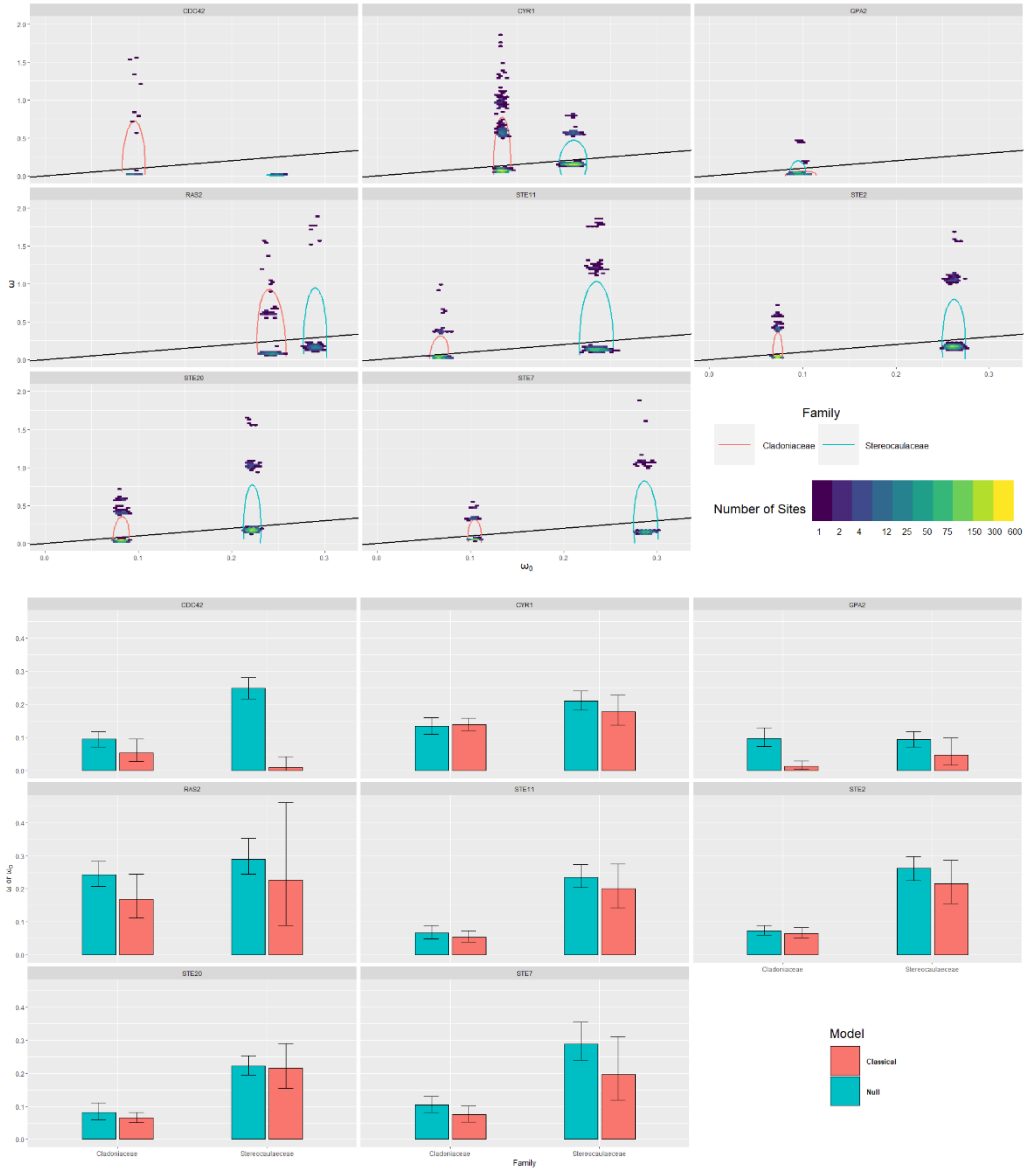

Fig. 3- Substitution analysis results for genes relating to dimorphic switching. Data were produced with Bayescode v1.1.2. A: The x-axis represents the mutation-selection (null) model and the y-axis represents the Classical ω model. The slanted line indicates the plane where $\omega = \omega_0$ B: Average mean and 95% confidence interval for the null and classical model for each species

A.

B.

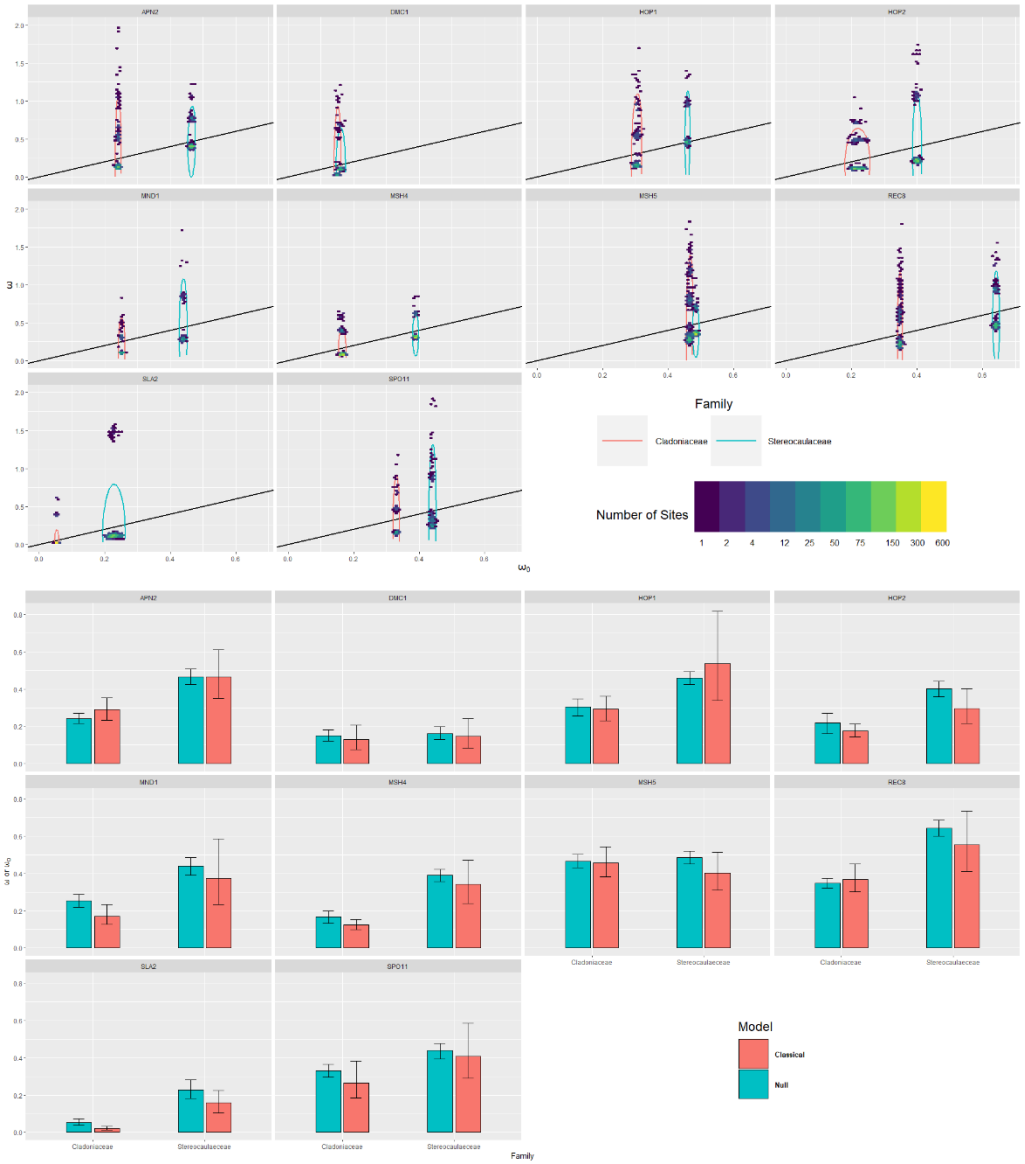

Fig. 4- Substitution analysis results for genes relating to meiosis. Data were produced with Bayescode v1.1.2. A: The x-axis represents the mutation-selection (null) model and the y-axis represents the Classical ω model. The slanted line indicates the plane where $\omega = \omega_0$ B: Average mean and 95% confidence interval for the null and classical model for each species.

Chapter 2: The Red Queen, Karl Popper, and Queer Theory

The Red Court

The Red Queen hypothesis posits that species must adapt, evolve, and overcome a constantly shifting landscape of antagonistic inter-specific interactions (Van Valen, 1973). Based on Lewis Carrol's titular who says "it takes all the running you can do, to keep in the same place" (Carroll, 1871),Red Queen dynamics have been used to explain some complex mating systems. In this understanding, sex offers a more rapid rate of evolution to maintain pace with antagonistic species (Bell, 1982; Hamilton, 1980; Jaenike, 1978). The Red Queen has since inspired a plethora of competing hypotheses. One such alternative hypothesis, coined the Court Jester, considers abiotic factors to be the primary driver of evolution (Barnosky, 1999, 2001). Court Jester dynamics may involve periods of punctuated equilibrium during stable climate and may work in concert with Red Queen Dynamics. The Vicar of Bray (the Fisher-Muller model) is yet another notable member of this court of evolutionary theory. Named for a caricaturized English clergy who "was first a Papist, then a protestant, then a Papist, then a protestant again" (Fuller, 1662), the Vicar of Bray hypothesis explains sex as a form of group selection to avoid clonal interference and allow for faster fixation of beneficial alleles (Fischer, 1930; Muller, 1932, 1964). Species capable of mixis are thus expected to evolve more rapidly to changing circumstances and survive out asexual species. And so, the Vicar changes reproductive modes to stay alive and keep up with a constantly changing fitness

landscape. The red court – including the Red Queen, the Court Jester, the Vicar of Bray, and other such evolutionary hypotheses – offers several distinct theories of the selective forces that drive character traits such as reproduction.

As their strict leprose growth form facilitates vertical and horizontal transmission of the entire lichen holobiont via clonal granules, *Lepraria* offers a unique case study for competing evolutionary hypotheses. The Red Queen suggests that without the ability to escape symbiotic relations through sporulation and resynthesis, *Lepraria spp.* should be particularly susceptible to lichen parasites. The Court Jester suggests that this leprose growth form offers superb fitness advantages that can outpace the Red Queen. But the Vicar of Brays insists that without the potential for mixis, clonal interference will bog down a species' ability to adapt quickly enough. A substantial body of research in Eukaryotic reproduction has contended that sex is universal while asexuality is an unstable and short-lived evolutionary dead end (Arkhipova & Meselson, 2005; Cooper et al., 2007; Gioti et al., 2013; Hillis, 2007; Hofstatter et al., 2018; Laine et al., 2022; Malik et al., 2008; Muller, 1932). Other researchers have suggested that asexual lineages manifest an enormous source of evolutionary innovation (Tripp, 2016). The Red Court cannot reach a consensus! The genus *Lepraria* represents an excellent opportunity to consider these pivotal theories on the fundamental processes of evolution.

The Red Court offers several distinct, overlapping, and sometimes contradictory theories of evolutionary selection. The Red Queen imagines species

that must constantly run just to stay in place, locked in a sort of perpetual arms race with antagonistic species. The Court Jester imagines species which may occasionally evolve to keep up with interspecific antagonism, but mostly evolve to survive the omnipresent threat of abiotic factors. The Court Jester must never stop dancing, or it will be off with their head! Meanwhile, the Vicar of Bray will change to become whatever is needed to avoid persecution (or extinction). Each of these anthropomorphized perspectives offers a distinct explanation for what primarily drives evolution. The explanations are competing, in tension with one another because as one grows in power the others diminish. But perhaps they are not entirely mutually exclusive. Could the Red Queen, the Court Jester, and the Vicar of Bray all be dancing to the same tune?

Philosophical frameworks in science

Queer theory is a growing philosophical framework that emerged out of Lesbian and Gay studies in the late 1990's. Queer theory focuses on the constructed nature of dichotomies, especially binaries. "Simply, queer theory does not dictate the eradication of existing categories of gender, sex, and sexuality, though many people assume it must" (Marinucci, 2016). Rather, queer theory insists that such categories are empirically underdetermined, meaning that "empirical evidence alone provide an insufficient basis for choosing one paradigm over another" (Marinucci, 2016). Most of queer theory's work in the academic literature has been limited to the humanities, although David Griffith's "Queer

Theory for Lichens" argues for a queer ecological perspective on the notions of species, individuals, and relationships (Griffiths, 2015). Here, I hope to present a scientific queer theory that is more grounded in interdisciplinary scholarship, while providing examples of its implementation.

Karl Popper (Popper, 1959, 1963) argues for a scientific method that centers testable and falsifiable hypotheses to address the problem of induction. Much of the existing literature on the Red Court has proposed evidence to support one Red Court hypothesis over another (Barnosky, 1999, 2001; Condamine et al., 2018; Salathé et al., 2008). The most notable of these studies developed a model of the Red Queen – which assume a steady state of coevolutionary change – and compared it to so-called "stationary models" which assume that evolutionary rates will diminish to zero in a constant environment (Stenseth & Maynard, 1984). The researchers therefore developed a binary system whereby any ecosystem can be determined to be governed by their interpretation of the Red Queen or the stationary model. This experimental design is woefully flawed, as a single reductive model attempts to explain the necessarily plural selective pressures on an entire ecosystem. Their study assumes that selective pressures can be simplified. Natural selection has been in action for billions of years and has developed bizarre and complex systems. Furthermore, Chapter 1 demonstrates that even within a single gene, selective pressures vary substantially (Fig. 3, Fig. 4). Site-based dN/dS models emerged because of the observation that measurable genetic selection most often occurs at the codon level (Yang & Swanson, 2002).

Thus, dN/dS averaged across an entire gene is of little use. The idea that a scientist can simply generalize all the selective pressures of an entire organism, species, or ecosystem is preposterous because selective pressures are necessarily plural. Any attempt to fit these systems to such a broad and all-encompassing model is futile; I suggest that Stenseth and Smith's (1984) Red Queen model perfectly represents the kind of unfalsifiable, all-explaining hypothesis that Karl Popper warned to avoid the temptation of.

Conclusions

The Red Court presents grand, anthropocentric, and unfalsifiable hypotheses. As such, the Red Queen, Court Jester, Vicar of Bray, and other members of the growing Red Court represent empirically underdetermined categories. I propose that the attempts to prove the grand hypotheses proposed by the Red Queen, Court Jester, Vicar of Bray, or any other member of the Red Court (Barnosky, 1999, 2001; Condamine et al., 2018; Salathé et al., 2008) are broadly an inefficient and inappropriate use of the scientific method. A useful model is limited in scope, painfully aware of its assumptions, and as simple as possible without being too simple. Models based off the Red Court hypotheses are far more indicative of researcher bias and experimental design than of any natural phenomenon.

But the critical issues facing the Red Court do not necessarily mean that these perspectives ought to be abandoned. These hypotheses can be useful

because they provide a simple and anthropomorphic view of macro-evolutionary change. Oftentimes for evolutionary biologists, the reality of nature is far too large and complex to truly comprehend. In these cases, rhetorical tools such as those provided by the Red Court can be extremely useful in crafting an experimental design, developing more specific and falsifiable hypotheses, and uncovering our own biases. The issues in the literature surrounding the Red Court are pervasive, and must be addressed. It seems that many researchers have forgotten that ideas such as the Red Queen, Vicar of Bray, and Court Jester are rhetorical tools, not natural phenomenon. Queer theory may provide a useful tool for using the Red Court responsibly. Here, I have attempted to briefly outline a queer approach to understanding the Red Court, specifically in the context of *Lepraria*.

Karl Popper described natural phenomena on a continuum between "clouds" – natural systems which are inherently chaotic and random – and "clocks" – natural systems which can be described as a sum of constituent parts (Popper, 1991). With the advent of the genomics revolution and the unprecedented growth of computational power, Scientists now regularly face the issue of handling datasets far too large and complex to study directly. Genomics relies on robust algorithms to dissect these enormous new datasets. These algorithms produce summary statistics which we may interpret. Allegory is therefore quite useful for interpreting summary statistics of incomprehensible data. Further, recognition of the limitations of these datasets is crucial; Genomic data exist as a black box which cannot accurately be described as the sum of

constituent parts, and therefore do not lend themselves to a reductionist statistical approach.

Species must maintain homeostasis within a constantly shifting landscape of biotic and abiotic factors. A species is not a single, unified character, but a mess of closely related lineages. Each cell, each granule, each population, and each ecosystem may be pulled in every direction by the members of the Red Court. Lineages are ripped apart, and most go extinct for following the wrong evolutionary imperatives. In the case of *Lepraria*, the Red Court represents an excellent allegory for understanding and communicating the evolutionary conundrums present in the genus. However, It must be considered that the scientific method might not equipped to 'solve' these hypotheses presented by the red court. Rather, the Red Court may serve scientists best by remaining in a perpetual state of tension among members who can never stop.

Literature Cited

Arkhipova, I., & Meselson, M. (2005). Deleterious transposable elements and the extinction of asexuals. *BioEssays*, *27*(1), 76–85. https://doi.org/10.1002/bies.20159

Barnosky, A. D. (1999). Does evolution dance to the Red Queen or the Court Jester? *Journal of Vertebrate Paleontology1*, *19*, 31A.

Barnosky, A. D. (2001). Distinguishing the effects of the red queen and court jester on miocene mammal evolution in the northern rocky mountains. *Journal of Vertebrate Paleontology*, *21*(1), 172–185. https://doi.org/10.1671/0272-4634(2001)021[0172:DTEOTR]2.0.CO;2

Bell, G. (1982). The masterpiece of nature: the evolution and genetics of sexuality. In *Routledge*.

Carroll, L. (1871). *Through the Looking Glass* (Millenium Fulcrum1.7). Project Gutenberg.

Condamine, F. L., Rolland, J., Höhna, S., Sperling, F. A. H., & Sanmartín, I. (2018). Testing the role of the Red Queen and Court Jester as drivers of the macroevolution of Apollo butterflies. *Systematic Biology*, *67*(6), 940–964. https://doi.org/10.1093/sysbio/syy009

Cooper, M. A., Adam, R. D., Worobey, M., & Sterling, C. R. (2007). Population Genetics Provides Evidence for Recombination in Giardia. *Current Biology*, *17*(22), 1984–1988. https://doi.org/10.1016/j.cub.2007.10.020

Fischer, R. A. (1930). *The Genetical Theory of Natural Selection*. The Clarendon Press.

Fuller, T. (1662). *The History of the Worthies of England endeavoured*.

Gioti, A., Stajich, J. E., & Johannesson, H. (2013). Neurospora and the dead-end hypothesis: Genomic consequences of selfing in the model genus. *Evolution*, *67*(12), 3600–3616. https://doi.org/10.1111/evo.12206

Griffiths, D. (2015). Queer Theory for Lichens. *Undercurrents*, *19*, 36–45.

Hamilton, W. D. (1980). Sex versus Non-Sex versus Parasite. *Oikos*, *35*(2), 282–290.

Hillis, D. M. (2007). Asexual Evolution: Can Species Exist without Sex? *Current Biology*, *17*(14), 543–544. https://doi.org/10.1016/j.cub.2007.05.015

Hofstatter, P. G., Brown, M. W., & Lahr, D. J. G. (2018). Comparative genomics supports sex and meiosis in diverse amoebozoa. *Genome Biology and Evolution*, *10*(11), 3118–3128. https://doi.org/10.1093/gbe/evy241

Jaenike, J. (1978). An hypothesis to account for the maintenance of sex within populations. *Evolutionary Theory*, *3*, 191–194.

Laine, V. N., Sackton, T. B., & Meselson, M. (2022). Genomic signature of sexual reproduction in the bdelloid rotifer Macrotrachella quadricornifera. *Genetics*, *220*(2). https://doi.org/10.1093/genetics/iyab221

Malik, S. B., Pightling, A. W., Stefaniak, L. M., Schurko, A. M., & Logsdon, J. M. (2008). An expanded inventory of conserved meiotic genes provides

evidence for sex in Trichomonas vaginalis. *PLoS ONE*, *3*(8). https://doi.org/10.1371/journal.pone.0002879

Marinucci, M. (2016). *Feminism is Queer: The intimate connection between queer and feminist theory* (second). Bloomsbury Publishing.

Muller, H. J. (1932). Some genetic Aspects of Sex. *American Society of Naturalists*, *703*, 118–138.

Muller, H. J. (1964). The relation of recombination to mutational advance. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *1*(1), 2–9. https://doi.org/10.1016/0027-5107(64)90047-8

Popper, K. R. (1959). *The logic of scientific discovery*. Routledge.

Popper, K. R. (1963). Science as falsification. *Conjectures and Refutations*, *1*, 33–39.

Popper, K. R. (1991). Of clouds and clocks: an approach to the problem of rationality and the freedom of man. In D. Cicchetti & W. M. Grove (Eds.), *Thinking clearly about psychology: Essays in honnor of Paul E. Meehl. Matters of public interest.* (Vol. 1, pp. 100–139). University of Washington Press.

Salathé, M., Kouyos, R. D., & Bonhoeffer, S. (2008). The state of affairs in the kingdom of the Red Queen. In *Trends in Ecology and Evolution* (Vol. 23, Issue 8, pp. 439–445). https://doi.org/10.1016/j.tree.2008.04.010

Stenseth, N. C., & Maynard, J. (1984). *Coevolution in Ecosystems: Red Queen Evolution or Stasis?* (Vol. 38, Issue 4).

Tripp, E. A. (2016). Is asexual reproduction an evolutionary dead end in lichens? *Lichenologist*, *48*(5), 559–580. https://doi.org/10.1017/S0024282916000335

Van Valen, L. (1973). A new evolutionary law. *Evolutionary Theory*, *1*, 1–30.

Yang, Z., & Swanson, W. J. (2002). Codon-substitution models to detect adaptive evolution that account for heterogeneous selective pressures among site classes. *Molecular Biology and Evolution*, *19*(1), 49–57. https://doi.org/10.1093/oxfordjournals.molbev.a003981