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SYMPOSIUM

Sex Without Sexes: Can the Cost of Finding a Mate Explain Diversity in Fungal Mating Systems?

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Synopsis Eukaryotes have evolved myriad ways of uniting gametes during sexual reproduction. A repeated pattern is the convergent evolution of a mating system with the fusion of larger gametes with smaller gametes (anisogamy) from that of fusion between morphologically identical gametes (isogamy). In anisogamous species, sexes are defined as individuals that produce only one gamete type. Although sexes abound throughout Eukarya, in fungi there are no biological sexes, because even in anisogamous species, individuals are hermaphroditic and produce both gamete types. For this reason, the term mating types is preferred over sexes, and, thus defined, only individuals of differing mating types can mate (homoallelic incompatibility). In anisogamous fungal species, there is scant evidence that there are more than two mating types, and this may be linked to genetic constraints, such as the use of mating types to determine the inheritance of cytoplasmic genomes. However, the mushroom fungi (Agaricomycetes) stand out as having both large numbers of mating types within a species, which will allow nearly all individuals to be compatible with each other, and reciprocal exchange of nuclei during mating, which will avoid cytoplasmic mixing and cyto-nuclear conflicts. Although the limitation of mating types to two in most fungi is consistent with the cyto-nuclear conflicts model, there are many facets of the Agaricomycete life cycle that also suggest they will demand a high outbreeding efficiency. Specifically, they are mostly obligately sexual and outcrossing, inhabit complex competitive niches, and display broadcast spore dispersal. Subsequently, the Agaricomycete individual pays a high cost to being choosy when encountering a mate. Here, I discuss the costs of mate finding and choice and demonstrate how most fungi have multiple ways of reducing these costs, which can explain why mating types are mostly limited to two per species. Nevertheless, it is perplexing that fungi have not evolved multiple mating types on more occasions nor evolved sexes. The few exceptions to these rules suggest that it is dictated by both molecular and evolutionary constraints.

Introduction

Sex is a near-ubiquitous phenomenon among eukaryotic species, but sexes are not. Sex supplies numerous functions in the evolutionary stability of species, such as removal of deleterious mutations, facilitation of adaptation via natural selection, repair of DNA lesions, and in generating diversity to counteract pathogens (Agrawal 2006; Otto 2009; Roze 2012). Sexes, however, only exist when a species has individuals who specialize in the production of different-sized gametes (Billiard et al. 2011; Beukeboom and Perrin 2014). The basic premise of sex as the formation of a diploid via fusion of haploid gametes followed by reversion to haploidy via meiosis is essentially unchanged across all eukaryotes and has apparently a single origin (Lenormand et al. 2016). On the other hand, sexes have evolved numerous times (Umen and Coelho 2019), and there is extreme variation among organisms in the process of sexuality, including how gametes find each other, the genetic mechanisms that control which gametes can fuse, and morphological and behavioral elaborations due to sexual selection. These variations provide a fascinating window into the importance of sex in fueling diversification, but they also create a conundrum. Why is sex so common across eukaryotic lineages that have evolved myriad means of facilitating it when it demands cost relative to **apomixis**

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(Box 1) or parthenogenesis (Lehtonen et al. 2012; Otto 2009)? And why do many lineages evolve sexes, but they mostly come in twos (Hurst 1996)?

The fungal kingdom provides an excellent framework within which to understand much about the evolution of sex (Billiard et al. 2011; Lee et al. 2010). Fungi display a wide range of mating systems, are well-characterized genetically and physiologically, and can be easily manipulated in the lab. Because of the extensive information on fungal mating systems, the comprehensive investigation of sex in fungi should provide insights into the major question of the nature and number of sexes. While sexes predominate in animals and may be found in plants and other protist lineages, they are mostly absent in fungi (Kondrashov 1997; Billiard et al. 2012). Fungi instead are either **isogamous** or when **anisogamous** are hermaphroditic, with individuals producing both sizes of gametes. That is not to say that fungi are free-wheelers with no barriers to outcrossing. Rather, genetic control of fungal mating exists in the form of **mating types**, and individuals of the same mating type are unable to mate. Mating types are the manifestation of genes at **mating-type loci (*MAT*)**, and typically in fungi one or two loci each comprised of a couple or few genes is enough to direct a cell to produce a particular mating type (Heitman et al. 2013).

Just as for the case of sexes, mating types, when present, usually come in pairs. Even in isogamous organisms such as the alga *Chlamydomonas* and baker's yeast *Saccharomyces*, there are only two mating types. The fact that this is so should be unacceptable, because in the process of finding a mate, the rarity of a sex provides a strong advantage via negative frequency selection (Iwasa and Sasaki 1987; May et al. 1999). For example, if a new mating type were to arise via mutation, then it would immediately have access to matings with many more individuals than the pre-existing mating types. Why then are two mating types so common? This topic has received considerable attention, with explanations ranging from genomic constraints on creating new alleles, dynamics of mate finding and choice, and mutation accumulation at sex determining loci (Billiard et al. 2011). Some of the best insights come from observations of the few organisms that actually have more than two mating types, most prominently, the mushroom fungi (Agaricomycetes), the ciliates, and plasmodial slime molds. One leading hypothesis is that mating types themselves may have evolved to modulate the conflict between inheritance of nuclear and cytoplasmic genomes due to their different modes of transmission, the **organelle inheritance model** (Hurst and Hamilton 1992; Hutson and Law 1997). The theory postulates that competition between cytoplasmically inherited genomes can be minimized by the evolution of a nuclear type that both suppresses transmission of its own cell's organelles and can preferentially mate with cells that are not suppressors. In such donor-recipient models of cytoplasmic transmission, more than two mating types are unlikely to be maintained because of an unstable hierarchy of inheritance (Hurst 1996). The best, albeit correlative, data to support this hypothesis is the fact that in both organismal groups with large numbers of mating types (Agaricomycetes and ciliates) mating occurs via nuclear exchange between gametes, thus avoiding mixing of cytoplasmic genomes, which are urged by natural selection to compete to the detriment of the zygote. Compelling as this argument is, it is not supported by the many species with biparental organellar inheritance in single celled eukaryotes, such as *Saccharomyces*, and it does not explain why there are not more than two mating types in hermaphroditic species.

As an alternative or additional explanation, it has been investigated if mating type number is controlled by the dynamics of mate finding and union of gametes (Iwasa and Sasaki 1987). In their models, under most conditions mating type numbers advance to infinity, which seems appropriate given the effects of natural selection. However, when the cost of finding a mate is low, such as when it is relatively easy to find a conspecific and to determine their compatibility, mating type numbers collapse to two. It is important to understand what the "cost of mating" means in such models. According to their model, the cost is paid by waiting around for a mate or in the energy put into finding a mate, such that at some point, an individual may never find a mate if it is too choosy or unlucky. This model would predict that species that could signal mate preference at long distance, have high motility, or have some degree of self-compatibility would be those with the lowest cost for finding a mate. Because this topic has been poorly covered in the literature, in this paper, I explore the idea that the cost of mate finding may explain some of the trends in mating systems in fungi.

Background on fungal mating systems

The fungi are a diverse kingdom of an estimated 3–5 million species and 13 phyla (Blackwell 2011; Hawksworth and Lücking 2017). A majority of the species diversity lies in the subkingdom Dikarya comprised of the phyla Ascomycota and Basidiomycota, and this group includes the lichens, many molds, mushrooms, and yeasts (James et al. 2020). The name Dikarya derives from a special heterokaryotic cell type, the dikaryon, that forms from the mating of compatible mating types. In these mated cells, the two nuclei of the mating partners divide synchronously, and the cells are functionally diploid, though the two nuclei remain

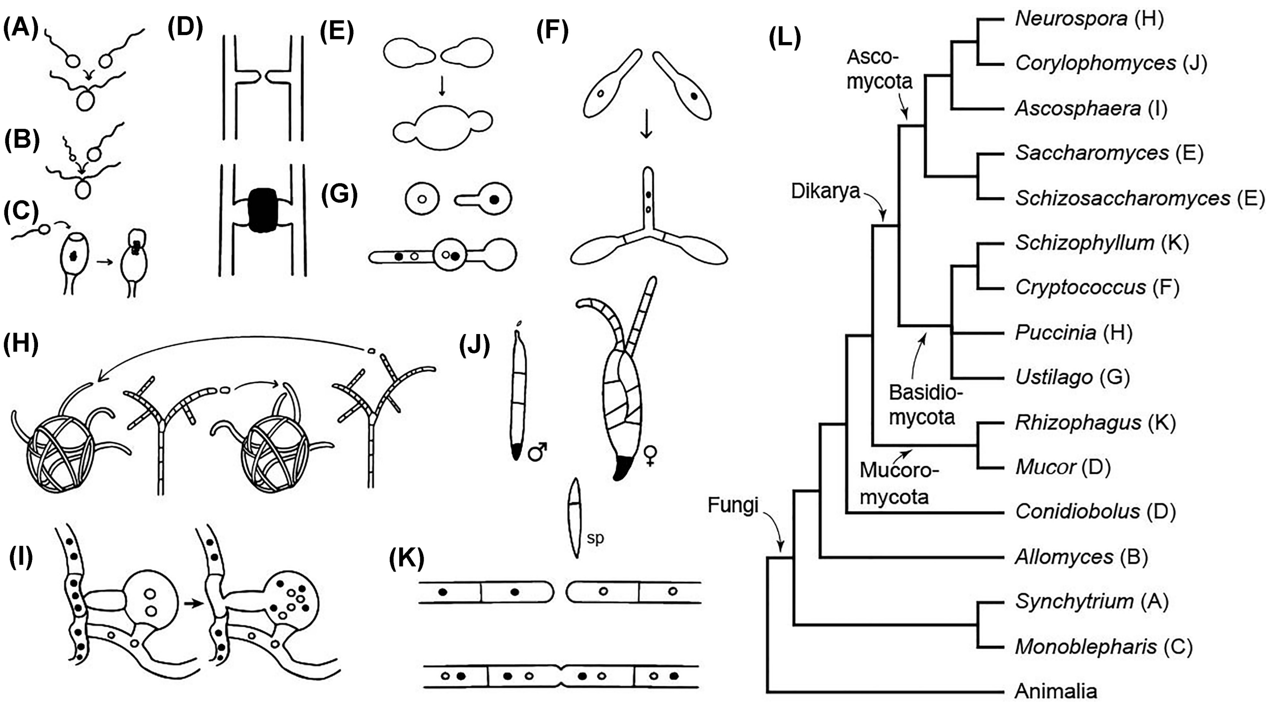

Fig. 1 The diversity of gamete fusion in fungi. Illustrated are the many modes in which gametes undergo sex. Fusion with motile gametes can be (A) isogamous (e.g., *Synchytrium*), (B) anisogamous (*Allomyces*), or (C) oogamous (*Monoblepharis*). (D) Hyphal fusions leading to single-cell zygotes are common in pin molds (*Mucor*) and entomopathogens (*Conidiobolus*). (E) Isogamous fusion of yeasts through the formation of mating projections followed by budding (*Saccharomyces* and *Schizosaccharomyces*). (F) Cytoplasmic fusion of yeasts can also occur solely in conjugation tubes (*Ustilago*), or (G) may be through the mating-type-specific production of a conjugation tube followed by nuclear migration (*Cryptococcus*). (H) Many Ascomycota and rusts are hermaphroditic and have fertilization of a trichogyne through an aerially dispersed spermatium or spore (*Neurospora* and *Puccinia*). (I) Some hyphal fusions can be through movement of nuclei from barely distinctive trichogynes and antheridia (*Ascosphaera*). (J) Laboulbeniales are unusual among fungi in showing dioecy with male and female thalli. Male thalli produce spermatia that fertilize trichogynes produced by females. Spores (sp) are often asymmetrically septate (*Corylophomyces*). (K) Hyphal fusion may result in the formation of a heterokaryon, such as in a typical mushroom mating, isogamous hyphae exchange nuclei after fusion and form a dikaryon (*Schizophyllum*), or in AMF fungi where a highly multinucleate heterokaryon forms (*Rhizophagus*). (L) Phylogeny of representative genera showcasing the distribution of the various mating patterns across the fungal tree of life. Phylogeny is approximated based on James et al. (2020).

separated. These heterokaryotic cell types are very different between Ascomycota and Basidiomycota, however. The dikaryotic stage in Ascomycota is very limited in duration and space, generally forming on the surface of a supporting haploid mycelium. In most Basidiomycota, on the other hand, the dikaryotic cell type is extensive and has a vegetative role separate from sexual reproduction; it may be the dominant stage of the life cycle.

Fungal gametes range across a diversity of morphologies. Isogamous gametes can be pairs of motile cells fusing, pairs of non-distinctive hyphae fusing, or fusion of single celled yeasts. Fertilization may also be anisogamous: specialized receptive hyphae fertilized by aerially dispersed cells, a motile cell fertilizing an immotile cell, or unequally sized motile gametes (Fig. 1). In the case of anisogamy, a number of terms are used to describe the different roles that connotate sexes, based on stereotypical male–female roles associated with sexes, but not necessarily prescriptive of any dichotomy. In this paper, I will use these terms as needed, but I will also discuss how these terms are antiquated and could lead to a bias in how we view these systems. Male–female terms may refer to the size, wherein the male gamete is smaller than the female, as in the swimming gametes of *Allomyces* (Fig. 1B), or it may refer to dispersal, such as **spermatia**, which are small cells dispersed by wind or insects that fertilize immotile **trichogynes** or female-hairs (Nieuwenhuis and Aanen 2012). Following cell fusion, nuclear migration is often observed. For nuclei that enter into a cell, the migrating nucleus is ascribed a male function, whereas the cell receiving of the nucleus the female function. Despite these asymmetries, in nearly all of the cases where mating is anisogamous in fungi, there are no sexes to speak of; instead, the fungi show a clear pattern of hermaphroditism, where both male and female roles are conducted by the same individual.

Mating types, therefore, control only the compatibility of gametes, and not the type of gametes an individual produces. For example, the model mold species *Neurospora crassa* has two mating types **A** and **a**, and mating occurs when micro or macroconidia of one mating type land on a trichogyne of the other mating type (Fig. 1H). Two mating types per species predominates in the Ascomycota, and all species have only one *MAT* locus, which encodes either high mobility group box or homeodomain motif transcription factors (Lee et al. 2010). In the Basidiomycota, many species have two *MAT* loci, one of which encodes homeodomain transcription factors and another that encodes G protein-coupled pheromone receptors and pheromones (Kües et al. 2011). Basidiomycota groups, including the smuts (Ustilaginomycotina) and jelly fungi (Tremellomycetes: Agaricomycotina), have a pattern wherein the homeodomain locus has many alleles and the pheromone receptor locus has only two, the so-called modified tetrapolar system (Coelho et al. 2017). In contrast, for the mushroom-forming fungi (Agaricomycetes: Agaricomycotina), there has been a wide proliferation in mating types at both loci, and this provides a very high outcrossing efficiency, the probability that two random haploid individuals will be mating compatible (Kües et al. 2011; James 2015). The two-locus system provides no benefit to outcrossing efficiency, and though it reduces inbreeding among full sibs by 50%, the full advantages of such a system are unclear.

Outside the Dikarya, there are at least 11 phyla of less speciose, mostly microscopic fungi, and there is less information on mating systems and mating types. Where known, the pin molds (Mucoromycotina) have a single locus with two mating types, and for the majority of the other phyla, there is little information on mating types outside the insect parasite *Coelomomyces* (Idnurm et al. 2007).

Anisogamy and mating types as options for control of organellar inheritance

Anisogamy creates a scenario where one gamete contributes more of the cytoplasm and is also expected to be the donor of the organellar genomes. Overall, there is not extensive data on mitochondrial inheritance throughout the fungi, though some systems have been well studied (Xu and Li 2015; Mendoza et al. 2020). In general, mitochondrial inheritance is largely found to be uniparental in fungi as in other eukaryotes (Wilson and Xu 2012). This is true for most Ascomycota with anisogamy, such as the pink bread mold *Neurospora*, as well as the isogamous yeasts *Cryptococcus* and *Ustilago*. Biparental inheritance is observed in some isogamous species, such as the yeasts *Saccharomyces* and *Schizosaccharomyces*. The Agaricomycetes show the unusual pattern of nuclear exchange without cytoplasmic fusion, making mtDNA inheritance biparental as a whole but spatially uniparental (Wilson and Xu 2012).

For isogamous eukaryotes, such as *Chlamydomonas*, *Physarum*, and some yeasts, it is observed that mating types control the uniparental inheritance of organellar genomes as predicted by the organellar inheritance hypothesis for the origination of sexes (Hurst and Hamilton 1992). In these isogamous species, uniparental mtDNA is typically ensured by degradation of mtDNA or mitochondria from one parent (Basse 2010; Xu and Li 2015). Over time, mating types could readily turn into sexes by incorporating genes into the *MAT* loci that influence the size of gametes, which could reinforce organellar inheritance and improve assortative mating (Billiard et al. 2011). Despite this possibility, there is little evidence that organellar inheritance drives fungal mating types to diverge genetically into different sexes.

Understanding the cost of mating in fungi

We find ourselves now poised to address the question of why aren't more fungal species mating type diverse, given that the system is rife for invasion by rare alleles? Specifically, why is it that so few groups have evolved numerous mating types? Although the reciprocal migration of nuclei in Agaricomycetes is consistent with organellar inheritance model, the idea that mating kinetics could explain patterns of fungal mating systems needs better exploration. Mating kinetics dictates the process of locating and choosing a mate. Iwasa and Sasaki (1987) found that multiple modes of mating kinetics create scenarios where mating types can proliferate to infinity. In one model, the cost of mating is high; specifically, the model dynamics work such that each individual gets one shot at mating, and only if they encounter a compatible individual they can mate. At first blush, this seems to be similar to the mating dynamics of mushrooms and many other highly dispersed (broadcast) fungi where dispersal distances are wide. At the other extreme is a model with mating kinetics where the cost of mating is low such that all individuals encounter each other multiple times and will eventually find a mate unless there are no more remaining compatible mates in the population. This seems appropriate perhaps for humans but may have little analogy in fungi. The general impression, therefore, is that the cost of mating skews high for fungi, and the kinetics of mate finding implies that many individuals will go unmated. In the following sections, we will explore how many fungi circumvent these costs, whether the Agaricomycetes are unusual among fungi in the cost they bear, and what mating system anomalies teach us about the genetic constraints.

Anisogamy can reduce the cost of finding a mate

Anisogamy allows the production of cheap gametes, which can foster a greater chance at being dispersed over long distances and a better chance at outcompeting other males (Parker 1982; Lehtonen and Kokko 2011). A prerequisite for anisogamy may be the evolution of multicellularity and a high energetic cost of producing gametes. While a decrease in the size of one gamete and copious production may lead to wasted gametes, it may increase the overall proportion of individuals that mate in a population, thus reducing the cost of mate finding. Anisogamy is only observed in multicellular fungi, and in many cases, small male gametes that may be cheaply produced have evolved. Classic examples of cheap male gametes are spermatia produced by rust fungi and Pezizomycotina, e.g., *Neurospora*. Asexual conidia of molds, such as *Neurospora*, appear to have a dual function as both asexual propagules and male gametes (Backus 1939; Zimmerman et al. 2016). Production of cheap gametes is therefore a major way of reducing the cost of mate finding in fungi, but it is not one that Agaricomycetes often employ.

Locating a mate is costly but signaling can help

There are numerous modes by which fungi find mates, each with different opportunities for selection (Fig. 1). In the case of motile gametes, motility provides a mechanism wherein gametes can actively search for mates. In the model water mold *Allomyces*, powerful pheromones are used to attract the male and female gametes to each other (Olson 1984). Such strong signaling reduces the overall cost of mate finding as less time needs to be spent swimming to find compatible gametes. Lack of species-specificity, however, may relate to tendency for *Allomyces* to form interspecific hybrids (Emerson and Wilson 1954). A second dominant mode involves the aforementioned trapping of spermatia or spores on a receptive mycelium. This mode is common in Dikarya, and after the deposition of the male gamete, signaling between male and female gametes occurs via diffusible mating-type-specific pheromones as evidenced by chemotropism between spermatia and trichogynes (Bistis 1981; Bobrowicz et al. 2002). It is likely that pheromone receptors and pheromones are constitutively expressed by both spermatia and trichogynes, and upregulation of male signal via secreted pheromones occurs once a compatible mate is encountered (Seibel et al. 2012). These pheromones and their cognate seven-pass transmembrane G-protein-coupled receptors are produced by essentially all Dikarya, and they are key in reducing costs of mate finding.

The final mode of locating a mate is one of fusion of hyphae or yeast cells. In yeast fungi, the secretion and use of mating pheromones for attraction are well documented (Merlini et al. 2013), and extracellular pheromones are apparently used for mate finding in pin molds (Mucoromycotina). However, in hyphal Dikarya, as useful as it might appear for chemical signaling via secretion to distinguish partners, it is unclear whether pheromones are used for mate finding. For example, in the mushroom fungi, pheromones involved in mate compatibility recognition are also believed to play a role primarily after cell fusion between potential mates, and secretion of pheromones is debated (Kües 2015). It is possible that other non-mating-type chemical signals are involved. If extracellular signals are not commonly used to find mates in fungi as they grow through their habitats, it may be because the signals are easy to fake and cheating and parasitism occurs via false signals. Or it is possible that there is not enough diversity in sequence in the system to support multiple species signals and chemotaxis. Simplicity of signal diversity has its costs. As an example, the trisporic acid signal used by Mucoromycotina leads to homing and mating between highly unrelated species (Blakeslee and Cartledge 1927). Moreover, there are sexual parasites (*Parasitella*) that use trisporic acid signals to trick hosts into cell fusion (Schultze et al. 2005).

Locating a high-quality mate is useful for any sexual encounter. Signaling by chemical cues can not only help in finding a mate could also foster sexual selection. Could signaling work to advertise individual fitness much like it does in other organisms, e.g., bird song? It is conceivable that signaling strength via lipopeptide pheromones could indicate cellular vigor and a high-quality mate, but such a signal could lead to cheaters that falsely advertise their fitness by over-investing in pheromone production (Rogers and Greig 2008). Sexual selection is readily detected in fungi, particularly when genotypes are allowed to be choosy, and while the traits being selected are still unknown, pheromone signaling is a leading candidate (Nieuwenhuis and Aanen 2012).

Apomixis as the simplest fix to costly mate finding

Apomixis eliminates the cost of finding a mate. Many fungi display apomixis, but obligate apomixis is not observed as a long-term strategy for fungi (Nieuwenhuis and James 2016), despite some fungi showing no morphological evidence of any amphimixis (i.e., sex). Instead, a dominant mode of reproduction across fungi is one of facultative apomixis or mixed reproductive strategies. Apomixis may occur via asexual spores (primarily conidia) formed by individual mycelia, which can then be dispersed and clonally propagate individuals into other locales. A mixed strategy with asexual sporulation effectively allows a genotype to explore multiple habitats before needing to find a suitable mate. However, apomixis has a severe cost in the longer term due to clonal organisms having an inability to adapt quickly and accumulating deleterious mutations that cannot be removed by sexual recombination (Judson and Normark 1996). Another major mode of mixed mating is called homothally. In this system, a genetic individual arising from a single spore is able to reproduce sexually in the absence of another partner. Homothallism does not prevent outcrossing from happening, but instead outcrossing can happen without barriers of mating types within a species (Attanayake et al. 2014). Multiple modes of homothallism are possible, including mating type switching, haploid fertility, and presence of compatible mating type alleles in the same genome (Wilson et al. 2015). Homothallism can be shown to be beneficial in reducing costs of mate finding in nature. One scenario in which finding a mate might be costly is when colonizing a new territory. Such expectation defines Baker's Law as the idea that invasive species are selected to be more selfing (Cheptou 2012). A recent example of this Baker's Law in fungi comes from a study showing the North American invasion of the death cap mushroom *Amanita phalloides* is characterized by a form of homothallism called haploid fruiting that is only observed in the invasive but not the native range (Wang et al. 2023).

Habitat matters: niches and dispersal mechanisms modulate costs

Agaricomycetes are dominant components of microbial communities of many habitats, but perhaps notoriously: plant roots, soil, and in decaying wood. All of these habitats have in common a complexity in microbial diversity that would predict challenging mate finding, which may have selected for outcrossing efficiency. Habitat clearly matters, and fungal groups with fewer mating types might have ecological parameters that would reduce the cost of mate finding. Some examples will show that this might be the case. Consider *Neurospora*, the bread mold, whose true habitat appears to be as an early succession post-fire mold on vegetation or trees (Jacobson et al. 2004). Other fungi, such as the whiskey fungus *Baudoinia compniacensis*, halophiles such as *Wallemia*, fungi occurring in utility poles, fruit juice, gut fungi, or indoor fungi might all experience reduced competition from other species by adaptions to specific niches and would therefore be expected to have less trouble finding mates after colonization. Among the Agaricomycetes, some of the "extremophilic" species, such as the dry rot fungus known to inhabit basements (*Serpula lacrymans*) are known to have reduced mating types (Skrede et al. 2013). Highly specific biotrophic species should also not bear the cost in finding a mate. This logic that habitat specificity reduces habitat complexity and narrows the physical space over which mating partners must find each other predicts the observed lower numbers of *MAT* alleles in the rusts and smuts, species that otherwise have the same mating type locus arrangements as the Agaricomycetes with many alleles (Coelho et al. 2017).

The flip side of this coin is the cost of dispersal. In order to take advantage of the reduced costs of finding a mate, dispersal into the more specific niches must be more efficient. Animal dispersal of fungi is common and can replace more passive methods such as wind dispersal. Numerous examples of mechanisms to facilitate adaptive dispersal of fungi into their hosts exist. Production of scents, as in the stink horn fungi, or production of spores inside nectar, as in some rust spermagonia, are both means of attracting insects for dispersing spores or gametes. Coprophilous fungi may have specific adaptations to be deposited into dung. For example, bird's nest fungi have spores dispersed as a packet with a sticky cord to reduce drag and wrap around vegetation that will be consumed by an herbivore and ultimately pass through them into their habitat of choice (Brodie 1975). Despite these obvious adaptations, the overall impression of fungal dispersal might appear more superfluous and even maladaptive, with Agaricomycetes apparently the most wasteful. Mushroom individuals can produce unfathomably large numbers of spores (Hassett et al. 2015), the majority of which have nothing to do with anything, except perhaps to serve as food for amoebae and arthropods. Accuracy has lost the war to a broadcast strategy. Yet, spores are effectively cheap, and there is likely intense pressure on colonization as priority effects clearly can dictate fungal communities (Hiscox et al. 2015).

The dikaryon of Agaricomycetes reduces the cost of finding a perfect mate

I have established that the high number of mating types in Agaricomycetes is associated with reciprocal nuclear migration, consistent with the organellar inheritance model for the origins of mating types. In the previous sections, I outlined ways in that costs of mate finding in fungi are reduced, most of which do not apply to Agaricomycetes. Instead, it is the long-lived dikaryon phase of the Agaricomycetes that reduces some of this cost and facilitates, or even demands, the high outcrossing efficiency. Agaricomycetes maintain the competency for mating throughout their entire lifecycle because they lack specific gamete structures, and instead, the nucleus functions as the gamete. For this reason, the cost of producing gametes can be very low, and importantly, gametogenesis is not a programmed development. Instead, all nuclei in the vegetative mycelium are capable of mating.

Because the nuclei within a dikaryon have not yet committed to karyogamy and meiosis, they may switch partners in a type of mating called dikaryon-monokaryon (di-mon), heterokaryon-homokaryon (he-ho), also known as the **Buller phenomenon** (Anderson and Kohn 2007). When dikaryotic mycelia of Agaricomycetes encounter monokaryotic mycelia, the nuclei of the dikaryon can fertilize by donating nuclei into the monokaryotic mycelium, thus creating new associations. These di-mon matings are typically subject to sexual selection and may foster competition among nuclei (Nieuwenhuis and Aanen 2012; Auxier et al. 2022), and usually one nucleus is favored as being the one to enter into the monokaryon. These di-mon matings, therefore, reduce the cost of mate finding by allowing mating to be a reversible process. Fluidity in mating partners would suggest that species could function more of as collectives of nuclear genotypes with a large degree of fluidity in associations over space (namely mycelia with many nuclei all recognizing each other as distinct, but cooperating), and this would be only possible in a system with multiple mating types. Also limited to species with multiple mating types is mating between more than two partners. In Agariomycetes species with heterokaryons comprised of cells of more than two nuclei, it has been shown that even three or four nuclei of different mating types can reside in a single cell (Johannesson and Stenlid 2004; James et al. 2009; Carlson et al. 2017). Despite the potential for dynamism of individuals, evidence for mycelial collectives is actually scant, and indeed in many species the dikaryon is a highly stable association, as evidenced by fairy rings or logs with the same dikaryotic genotype isolated from extreme ends (Kay and Vilgalys 1992; Hiltunen et al. 2019).

Remating is also facilitated in Agaricomycetes by the production of asexual conidia by heterokaryons. This phenomenon is not seen in all species, and indeed conidiation is far less common and abundant than in the Ascomycota (Kües et al. 2016). However, when produced, conidia of heterokaryons are shown to often break down the association of mated nuclei by packaging only one or one type of nucleus into the spore, and therefore the mycelium germinating from such conidia may return to homokaryotic (James et al., 2008, 2011). In this way, the cost of mating may be reduced, better partners may be had, and mating may not necessarily culminate in meiosis.

Rule breakers challenge our understanding of fungal mating

Up to this point, I have often stated that fungi do not actually have sexes, and instead have mating types. I also discussed Agaricomycetes as if they are the only group with more than two mating types in a species. On these two points, there are exceptions, and these exceptions provide clues to the genomic, physiological constraints, and evolutionary constraints that force the general paradigm.

Most of the models of number of sexes predict that sexes can increase to infinity or decrease to two, with intermediate numbers unlikely (Iwasa and Sasaki 1987; Hurst 1996). If population genetic constraints are the cause for only two mating types, one might predict that intermediate numbers of mating types between two and many as might be occasionally detected as mating types are created but ultimately fated to be lost by selection or drift. In fact, there is mounting evidence of long-term stability of three mating types in many smut fungi (Ustilaginomycotina: Basidiomycota). *Sporisorium reilianum* is a smut fungus known to have 3 alleles at the *P/R MAT* locus and at least 5 alleles at the *HD MAT* locus, providing an outbreeding efficiency of at least 53% in a randomly mating population (Schirawski et al. 2005). Phylogenetic data show that the three mating types have been around for millions of years suggesting they are at a stable equilibrium (Kellner et al. 2011; Coelho et al. 2017). *Sporisorium*, other smuts, jelly fungi, and basidiomycetous yeasts are haploid in the yeast phase and convert into a filamentous mycelium after mating and cytoplasmic fusion (Fig. 1F). As mentioned, use of extracellular pheromones for signaling may pose challenges for creating diversity and maintaining species specificity. Indeed, in interspecific mating tests with the Ustilaginaceae smuts, pairings between isolates of different mating type led to either attempted or successful hyphal fusion and the formation of hybrids, most likely which would be of low pathogenicity or reproductively isolated, given the up to 100 mya divergence of some species pairs (Kellner et al. 2011). These data hint that the origin of new alleles may be limited by genomic architecture rather than population processes, since new receptor and pheromone alleles have to emerge on the same haplotype.

The arbuscular mycorrhizal fungi (Glomeromycotina) have been a fascinating sexual conundrum as they have been described as heterokaryotic (Kuhn et al. 2001; Ropars and Corradi 2015), yet they had also been considered "ancient asexual scandals" having no evidence for morphological sex in any portion of their life cycles (Judson and Normark 1996). However, recent evidence provides a strong indication that Glomeromycotina may have a mating system with at least six mating types (Ropars et al. 2016). The nuclear composition of heterokaryotic isolates is similar to some Agaricomycetes, wherein the mycelium is comprised of two nuclear types, but these types may not be equally represented in the mycelium (Kokkoris et al. 2021). Whether these fungi undergo cytoplasmic mixing during mating versus nuclear migration is important for resolving the organellar inheritance model for the maintenance of two sexes. Some insights are already available. Pairing of germinating spores in the lab, suggests genetically distinct isolates can fuse and apparently undergo nuclear and mitochondrial exchange or at least transient mixing (Croll et al. 2009; de la Providencia et al. 2013; Purin and Morton 2013). Heteroplasmy of mitochondrial markers would suggest cytoplasmic mixing, contradicting predictions of the organellar inheritance model, but crosses need to be conducted with consideration of the new *MAT* locus model.

The rule of no sexes in fungi is actually broken by a fascinating group called the Laboulbeniales (Pezizomycotina: Ascomycota). These fungi are ectoparasites of arthropods, particularly mites, and insects. Development is limited to production of small thalli of few to dozens of cells and no mycelial growth. Many species are hermaphroditic, producing both trichogynes and spermatia. However, some species are dioecious, where individual thalli are either female and produce only a trichogyne or male and produce only spermatia (Benjamin and Shanor 1950; Benjamin 1986). Laboulbeniales are notorious for showing high specificity to hosts, including in some cases showing specificity to the species, sex of host, and even body site (Benjamin and Shanor 1952; Sundberg et al. 2018; Haelewaters et al. 2022). Such specificity could greatly reduce the cost of mate finding. Contrary to expectations, a well-known dioecious species, *Laboulbenia formicarum*, is found on multiple genera of ants and even is associated with invasive ant species (Espadaler et al. 2011), breaking with Baker's Law. Understanding the mating genetics of Laboulbeniales is a critical gap due to their uncultivability, and it may be that they have plastic or environmentally determined sex development. Interestingly, many Laboulbeniales spores are two celled, and the location of the septum (rarely symmetric) (Fig. 1J) could influence sexual development. Another possibility is that individuals are constitutively female and produce a pheromone that converts immature thalli into the compatible male sex. This mode of development would be similar to that observed in the Oomycete *Achlya ambisexualis* in that secreted hormones stimulate the development of other individual's gametes and drive sexual differences (Raper 1940). A final possibility would be that ascospores segregating haploid males and females are always co-dispersed as a spore pair, which would reduce any issues in mate finding.

The agent of chalkbrood disease of bees, *Ascosphaera apis* (Eurotiomycetes), is considered dioecious because strains of different mating type are reported to grow at different rates and differ in production of female (ascogonia) or male (nucleus donating) gametes (Spiltoir 1955; Mráz et al. 2021). This species produces "spore balls" in which the ascospores are produced inside a packet and probably codispersed as intercompatible progeny, reducing the cost of mate finding (Aronstein and Murray 2010). The genetics behind this dioecy has not been explored, though the mating-type genes are typical for Ascomycota (Aronstein et al. 2007). Codispersal of both mating types may select for differences to evolve that facilitate mate finding and specialization of mating types to improve mate finding among progeny, a type of functional homothallism at the diploid level.

Cryptococcus neoformans sensu lato (Tremellomycetes; Basidiomycota) is a special fungus that has received a great deal of attention due to its role as a human pathogen and a model system for understanding pathogenicity, signaling, and mating. Its life cycle is dimorphic with mating of yeast leading to development of dikaryotic hyphae that undergo meiosis. This clinically relevant species presents multiple phenomena that buck the rules for mating systems in fungi. There are two mating types in *C. neoformans s. l.*, and these are programmed by a complex ∼100 kb mating type locus with ∼20 genes that mostly differ between the **a** and α mating types (Fraser et al. 2004). This species also shows a nascent form of dioecy. Mating between **a** and α cells involves the secretion of corresponding pheromones; however, this is followed by the development of a conjugation tube towards the **a** type by the α type (but not vice versa; Fig. 1G), and the migration of the α nucleus into the **a** cell post-fusion (McClelland et al. 2004). The dikaryotic hyphae then emerge from the **a** cell, and this typically leads to inheritance of cytoplasm from the **a** cell, a phenomenon that is well established (Yan et al. 2004). The presence of the large mating-type locus (unusual for fungi) is coincident with this mating behavior and provides ample room for the development of morphological and behavioral differences between mating types. Dioecy or mating-type-specific roles may yet be rule rather than the exception, even in isogamous mating. Recently, it was shown that chemical differences in the pheromones secreted between the **a** and *α* mating types of *Saccharomyces cerevisiae* lead to partitioning of mate finding roles (Anders et al. 2021).

Mating types promote outcrossing by ensuring heteroallelism at mating. But what if it did not? *Cryptococcus neoformans s. l.* has two more surprising findings, same-mating-type sex and pseudosex. Evidence both in the lab and in nature now shows that fusion between cells of the same mating type can occur, albeit at much lower rates than that of heteroallelic pairs, and that same-mating-type fusion is increased ∼1000 in the presence of a compatible mating types, which presumably provides a pheromone signal (Lin et al., 2005, 2007, 2009). Same-mating-type sex also leads to filamentation and apparently normal meiosis, albeit at a slightly reduced recombination rate (Roth et al. 2018). The recently discovered pseudosexual process in *C. neoformans* found in some cases mating could trigger the development of sexual structures, yet only one of the two partner's genetic information was incorporated into the offspring (Yadav et al. 2021). Although these two unusual phenomena are only known from one genus of fungi, when phenomena are found in 1 of the 10 or so major model genera of fungi, it begs the question as to how widespread they might be. It also forces a reconsideration of how important sexual identity is for governing the mating process—there are no hard and fast rules.

The use of gender and sex terms in mycology

The concept of sexes explains much about behavior associated with the sexual process in eukaryotes, particularly animals. As discussed in the Symposium Sex, Science, and Society: Sex Diversity and Variation hosted at the 2023 Society for Integrative and Comparative Biology, scientists may develop preconceived ideas about the importance of sexual differences and focus on binary divisions within species to the detriment of understanding sexual variation within species, such as intersex individuals, sex switching, and hermaphroditism. In mycology, terms associated with the sexes are used widely. They are associated with ideas that both are grounded in the ultimate definition of sex, such as gamete size, but also on roles that we as humans often assign to sexes, such as choosiness, reception of gametes, and nurturing roles, which are easily shown to be malleable even in animals, for example, internal fertilization and paternal care in seahorses (Stölting and Wilson 2007). For this reason, it is advisable to review and restrict the use of sex terms appropriately. Trichogynes and spermatia (fungal terms of female and male gametangia) of hermaphroditic rusts and ascomycetes pass the muster as they are associated with gamete size. Male and female roles during mating of isogamous basidiomycetes would not. It is not the author's intention to provide any rubric for the treatment of these terms, but to ask fungal biologists to seriously consider whether the use of stereotypical sex roles of animals when discussing fungal biology is an important clarification, an over-simplification, or a misappropriation. Failing to consider this may miss some of the aspects of fungal biology that make this kingdom unique and have led to their massive success in terms of diversification and ecosystem prevalence. Even in a clear-cut case of smaller male and larger female gametes of the water mold *Allomyces*, these terms should be appropriate, but they provide little information as the mitochondrion appears to be inherited from the male gamete (Borkhardt and Olson 1983). What more will we miss if we make assumptions about the male and female roles of gametes and individuals because we have "assigned" them based on equivalency in other kingdoms?

Conclusions and future directions

In this paper, I highlighted how the cost of finding a mate predicts the diversity of mating-type genes by showcasing the numerous ways fungi have evolved to reduce this cost. In a kingdom of some 5 million species, virtually anything goes. Yet, by and large, fungi go without sexes and instead have mating types that cannot be distinguished morphologically. And while anisogamy and hermaphroditism are common, they nearly always co-occur. Both isogamous and anisogamous fungal species have, generally, two mating types, and in many cases, their mating-type-determining loci control cytoplasmic inheritance. The Agaricomycetes stand as the great exception. Members of this group have a huge diversity of mating types, affording a high outbreeding efficiency. That the group is the only one that has the high diversity and also has evolved reciprocal nuclear exchange conforms with the organellar inheritance model for the maintenance of only two mating types. So mating in fungi has much in common with mating in other eukaryote groups, and the study of fungi has much to teach us about why sexes exist and why both sexes and mating types mostly come in twos. Fungi also show numerous ways in which binary sexes are not the norm for many species, via homothallism, hermaphroditism, matings between multiple partners, to pseudosex, and same mating type mating.

Changes in our planetary ecosystem have coincided with the diversification of certain groups of fungi and the strategies they employ for dispersal (Berbee et al.

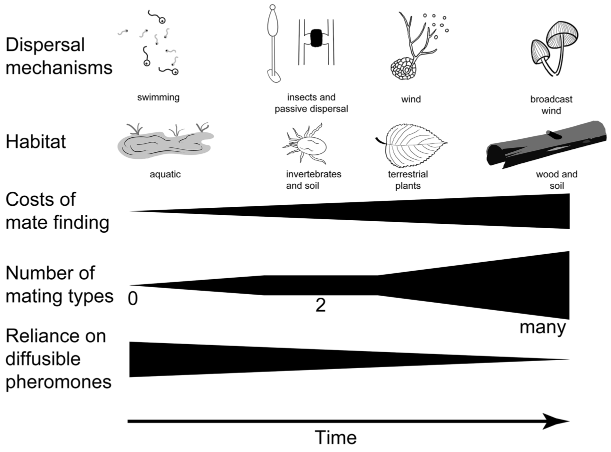

Fig. 2 The interactions of dispersal mechanisms and niche preferences over geologic time highlight how the costs of mating have shaped fungal mating type diversity. Shown are very broad patterns in terms of transitions in fungal habitat since their early diversification began approximately 1 billion years ago (Berbee et al. 2020). Early diverging lineages of fungi were primarily aquatic and dispersed gametes and spores actively via flagellated cells. To find each other, gametes likely required a strong pheromone signal. As terrestrial biota developed by the Silurian, fungi colonized land and terrestrial organisms, such as arthropods, and mating was between hyphae that found each other through pheromone signaling. As land plants diversified, so did fungi, and fungi used aerial dispersal to colonize plant tissues as endophytes, epiphytes, and pathogens. The importance of finding a mate chemically was reduced for most fungi, which was traded for habitat specificity. Modern terrestrial ecosystems have a large percentage of their biomass in Agaricomycetes, which are major components of wood and soil habitats. These habitats are extremely diverse, and these fungi find mates by producing copious numbers of spores in combination with a high outbreeding efficiency ensured by high numbers of mating-type alleles.

2020) (Fig. 2). Early diverging fungal lineages had motile gametes when life was more aquatic focused. As terrestrial life proliferated, fungi flourished on land, but met the challenge of finding mates and substrates; asexual sporulation with diverse adaptations for specific hosts and substrates and isogamy may have dominated. Then, as large plants began to dominate the landscape, both spores and gametes took to the wind, and in many cases propagules assumed dual roles as gametes and for spores clonal propagation. In doing so, the cost of finding a mate remained low in most of these species, and homothallism may have evolved when costs were too high. Agaricomycete diversification is coincident with the diversification of Angiosperms and woody debris and led to the evolution of diverse and prolific sporocarps for aerial dispersal (Varga et al. 2019). Agaricomycetes also largely avoid clonality in favor of obligate sexuality, a trait that makes them not only the most genetically diverse among the fungi but perhaps amongst all eukaryotes (Baranova et al. 2015).

Continued study of fungal mating systems in a broader phylogenetic and ecological context promises to provide fertile grounds for testing whether the scenario outlined in the previous paragraph holds merit. Research on fungi is not just for mycologists; questions regarding sexual identity, sexual selection, the relationship between ecology and mating systems, and much more are all research areas for which the diversity and simplicity of fungi have much to offer. I conclude with a few areas in immediate need of further research.

1. Until recently, it was nearly impossible to test the organelle inheritance model as there was presumably a single origin of multiple mating types in the fungi (Agaricomycetes), but it appears that the Glomeromycotina is also multiallelic (Ropars et al. 2016). The theory could be tested by determining whether reciprocal nuclear migration without cytoplasmic mixing also occurs in Glomeromycotina as it does in Agariomycetes.
2. With the emergence of single-cell genomic sequencing methods, it would now be possible to determine whether males of Laboulbeniales are genetically or epigenetically differentiated from females. These data are needed to understand whether sexes could be programmed using the genetic architecture used to create mating types in other fungi.
3. Agaricomycetes genomes encode a large number of "pheromone" receptors, but it is still unclear what role they actually play in the sexual cycle (Wirth et al. 2021). The dogma is that pheromones are not secreted, yet when pheromone and cognate receptor of Agaricomycetes are expressed in budding yeast, the proteins facilitate extracellular mate finding (Fowler et al. 1999). Understanding whether the pheromones are secreted can help understand how mates are found, whether true and false sexual signaling can occur, and why it is that the Agaricomycetes are the only species to produce a highly diverse set of pheromones alleles.
4. The early stages of mating among Agaricomycetes in the wild are poorly known. Agaricomycetes isolated from nature are almost always dikaryotic (e.g., Kay and Vilgalys 1992), but not always (Crockatt et al. 2008). How often are spores of mushrooms acting as spermatia to fertilize monokaryotic mycelia or to displace resident nuclei from mycelia? How frequently are Agaricomycete heterokaryons comprised of more than two nuclear types, and what effect does this have on genome plasticity and stability?
5. Can conidia in Agaricomycetes reduce the cost of finding a mate? Conidia are poorly studied as components of the Agaricomycete life cyle (Kües et al. 2016), and they may function both as spermatia or

as dispersal to new habitats. According to the mating kinetics model, species that produce conidia would have a lower cost of mate finding and would be expected to have reduced mating type diversity relative to those that lack conidia.

Box 1: Glossary of technical terms.

Anisogamy: sexual reproduction involving fusion of gametes of different sizes.
Apomixis: asexual reproduction without gamete fusion.
Buller phenomenon: mating between a dikaryon and a monokaryon in which a nucleus from the dikaryon migrates into the monokaryon, also known as di-mon mating.
Dikaryon: a cell type produced after mating of members of Dikarya (Ascomycota and Basidiomycota) in which the two nuclei of the mating partners do not fuse but undergo synchronous mitosis.
Heterothallism: mating system in which sexual reproduction involves outcrossing.
Homothallism: mating system in which sexual reproduction occurs by individuals that are self-fertile
Isogamy: sexual reproduction involving fusion of gametes of the same size.
Mating types: phenotypic groups of individuals that differ with respect to their ability to mate with other individuals. Normally, only individuals of differing mating types are able to mate.
Mating-type locus: a genomic region that determines the mating type of an individual.
Oogamy: sexual reproduction involving a motile gamete and a larger immotile gamete (egg).
Organelle inheritance model: model explaining the evolution of sexes or mating types to reduce cytoplasmic competition by enforcing uniparental inheritance.
Spermatium: a fungal gamete that is dispersed by wind or insects to fertilize a trichogyne.
Trichogyne: a receptive hypha that functions as a female gamete to receive the nucleus from a spermatium.

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Conflict of interest

The authors have no conflict of interest.

Data availability statement

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References

Agrawal AF. 2006. Similarity selection and the evolution of sex: revisiting the red queen. PLoS Biol 4:e265.

Anders A, Colin R, Banderas A, Sourjik V. 2021. Asymmetric mating behavior of isogamous budding yeast. Sci Adv 7:eabf8404.

Anderson JB, Kohn LM. 2007. Dikaryons, diploids, and evolution. In: Heitman J, Kronstad JW, Taylor J, Casselton L, editors. Sex in Fungi: Molecular Determination and Evolutionary Implications. Washington, DC: ASM Press. p.333–48.

Aronstein KA, Murray KD, de León JH, Qin X, Weinstock GM. 2007. High mobility group (HMG-box) genes in the honeybee fungal pathogen Ascosphaera apis. Mycologia 99:553–61.

Aronstein KA, Murray KD. 2010. Chalkbrood disease in honey bees. J Invertebr Pathol 103:S20–9.

Attanayake RN, Tennekoon V, Johnson DA, Porter LD, del Río-Mendoza L, Jiang D, Chen W. 2014. Inferring outcrossing in the homothallic fungus *Sclerotinia sclerotiorum* using linkage disequilibrium decay. Heredity 113:353–63.

Auxier B, Czárán TL, Aanen DK. 2022. Modeling the consequences of the dikaryotic life cycle of mushroom-forming fungi on genomic conflict. eLife 11:e75917.

Backus MP. 1939. The mechanics of conidial fertilization in *Neurospora sitophila*. Bulletin of the Torrey Botanical Club 66:63–76.

Baranova MA, Logacheva MD, Penin AA, Seplyarskiy VB, Safonova Y, Naumenko SA, Klepikova AV, Gerasimov ES, Bazykin GA, James TY et al. 2015. Extraordinary genetic diversity in a wood decay mushroom. Mol Biol Evol 32:2775–83.

Basse CW. 2010. Mitochondrial inheritance in fungi. Curr Opin Microbiol 13:712–9.

Benjamin R. 1986. Laboulbeniales on semiaquatic Hemiptera V. Triceromyces. Aliso 11:245–78.

Benjamin RK, Shanor L. 1950. The development of male and female individuals in the dioecious species *Laboulbenia* formicarum thaxter. Am J Bot 37:471–6.

Benjamin RK, Shanor L. 1952. Sex of host specificity and position specificity of certain species of *Laboulbenia* on *Bembidion picipes*. Am J Bot 39:125–31.

Berbee ML, Strullu-Derrien C, Delaux P-M, Strother PK, Kenrick P, Selosse M-A, Taylor JW. 2020. Genomic and fossil windows into the secret lives of the most ancient fungi. Nat Rev Microbiol 18:717–30.

Beukeboom LW, Perrin N. 2014. What are sexes, and why are there sexes?In: Beukeboom LW, Perrin N, editors. The Evolution of Sex Determination. Oxford University Press. p. 1–17.

Billiard S, Lopez-Villavicencio M, Devier B, Hood ME, Fairhead C, Giraud T. 2011. Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. Biol Rev 86:421–42.

Billiard S, Lopez-Villavicencio M, Hood ME, Giraud T. 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J Evol Biol 25:1020–38.

Bistis GN. 1981. Chemotropic interactions between trichogynes and conidia of opposite mating-type in *Neurospora crassa*. Mycologia 73:959–75.

Blackwell M. 2011. The Fungi: 1, 2, 3 … 5.1 million species? Am J Bot 98:426–38.

Blakeslee AF, Cartledge JL. 1927. Sexual dimorphism in Mucorales. Botanical Gazette 84:51–7.

Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ. 2002. The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804.

Borkhardt B, Olson LW. 1983. Paternal inheritance of the mitochondrial dna in interspecific crosses of the aquatic fungus allomyces. Curr Genet 7:403–4.

Brodie HJ. 1975. The Bird's Nest Fungi. Toronto: University of Toronto Press.

Carlson AL, Ishak HD, Kurian J, Mikheyev AS, Gifford I, Mueller UG. 2017. Nuclear populations of the multinucleate fungus of leafcutter ants can be dekaryotized and recombined to manipulate growth of nutritive hyphal nodules harvested by the ants. Mycologia 109:832–46.

Cheptou P-O. 2012. Clarifying Baker's Law. Ann Bot 109:633–41.

Coelho MA, Bakkeren G, Sun S, Hood ME, Giraud T. 2017. Fungal sex: the Basidiomycota. Microbiol Spectr 5:5.3.12.

Crockatt ME, Pierce GI, Camden RA, Newell PM, Boddy L. 2008. Homokaryons are more combative than heterokaryons in *Hericium coralloides*. Fungal Biol 1: 40–8.

Croll D, Giovannetti M, Koch AM, Sbrana C, Ehinger M, Lammers PJ, Sanders IR. 2009. Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 181:924–37.

de la Providencia IE, Nadimi M, Beaudet D, Rodriguez Morales G, Hijri M. 2013. Detection of a transient mitochondrial DNA heteroplasmy in the progeny of crossed genetically divergent isolates of arbuscular mycorrhizal fungi. New Phytol 200:211–21.

Emerson R, Wilson CM. 1954. Interspecific hybrids and the cytogenetics and cytotaxonomy of Euallomyces. Mycologia 46:393–434.

Espadaler X, Lebas C, Wagenknecht Tragust S. 2011. *Laboulbenia formicarum* (Ascomycota, Laboulbeniales), an exotic parasitic fungus, on an exotic ant in France. Vie et Milieu 61:41–4.

Fowler TJ, DeSimone SM, Mitton MF, Kurjan J, Raper CA. 1999. Multiple sex pheromones and receptors of a mushroom-producing fungus elicit mating in yeast. MBoC 10: 2559–72.

Fraser JA, Diezmann S, Subaran RL, Allen A, Lengeler KB, Dietrich FS, Heitman J. 2004. Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. PLoS Biol 2:2243–55.

Haelewaters D, Lubbers M, De Kesel A. 2022. The haustorium as a driving force for speciation in thallus-forming Laboulbeniomycetes. IMA Fungus 13:1.

Hassett MO, Fischer MWF, Money NP. 2015. Mushrooms as rainmakers: how spores act as nuclei for raindrops. PLoS One 10:e0140407.

Hawksworth DL, Lücking R. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectr 5, https://doi.org/10.1128/microbiolspec.FUNK-0052-2016

Heitman J, Sun S, James TY. 2013. Evolution of fungal sexual reproduction. Mycologia 105:1–27.

Hiltunen M, Grudzinska-Sterno M, Wallerman O, Ryberg M, Johannesson H. 2019. Maintenance of high genome integrity over vegetative growth in the fairy-ring mushroom *Marasmius oreades*. Curr Biol 29:2758–2765.e6.

Hiscox J, Savoury M, Müller CT, Lindahl BD, Rogers HJ, Boddy L. 2015. Priority effects during fungal community establishment in beech wood. ISME J 9:2246–60.

Hurst LD, Hamilton WD. 1992. Cytoplasmic fusion and the nature of sexes. Proc Royal Soc B 247:189–94.

Hurst LD. 1996. Why are there only two sexes? Proc R Soc Lond Ser B-Biol Sci 263:415–22.

Hutson V, Law R. 1997. Four steps to two sexes. Proc Royal Soc B 253:43–51.

Idnurm A, James TY, Vilgalys R. 2007. Sex in the rest: mysterious mating in the Chytridiomycota and Zygomycota. In: Heitman J, Taylor JK JW, Casselton LA, editors. Sex in fungi: molecular determination and evolutionary implications. Washington, DC: ASM Press. p. 407–18.

Iwasa Y, Sasaki A. 1987. Evolution of the number of sexes. Evolution 41:49–65.

Jacobson DJ, Powell AJ, Dettman JR, Saenz GS, Barton MM, Hiltz MD, Dvorachek WH, Glass NL, Taylor JW, Natvig DO. 2004. Neurospora in temperate forests of western North America. Mycologia 96:66–74.

James TY, Johansson SBK, Johannesson H. 2009. Trikaryon formation and nuclear selection in pairings between heterokaryons and homokaryons of the root rot pathogen *Heterobasidion parviporum*. Mycol Res 113:583–90.

James TY, Lee M, van Diepen LTA. 2011. A single mating-type locus composed of homeodomain genes promotes nuclear migration and heterokaryosis in the white-rot fungus *Phanerochaete chrysosporium*. Eukaryot Cell 10:249–61.

James TY, Stajich JE, Hittinger CT, Rokas A. 2020. Toward a fully resolved fungal tree of life. Annu Rev Microbiol 74: 291–313.

James TY, Stenlid J, Olson Å, Johannesson H. 2008. Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the basidiomycete fungus *Heterobasidion parviporum*. Evolution 62:2279–96.

James TY. 2015. Why mushrooms have evolved to be so promiscuous: insights from evolutionary and ecological patterns. Fungal Biol Rev 29:167–78.

Johannesson H, Stenlid J. 2004. Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. Fungal Genet Biol 41:563–70.

Judson OP, Normark BB. 1996. Ancient asexual scandals. Trends Ecol Evol 11:41–6.

Kay E, Vilgalys R. 1992. Spatial distribution and genetic relationships among individuals in a natural population of the oyster mushroom *Pleurotus ostreatus*. Mycologia 84:173–82.

Kellner R, Vollmeister E, Feldbrügge M, Begerow D. 2011. Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system. PLoS Genet 7:e1002436.

Kokkoris V, Chagnon P-L, Yildirir G, Clarke K, Goh D, MacLean AM, Dettman J, Stefani F, Corradi N. 2021. Host identity influences nuclear dynamics in arbuscular mycorrhizal fungi. Curr Biol. 31:1531–1538.e6.

Kondrashov AS. 1997. Evolutionary genetics of life cycles. Annu Rev Ecol Syst 28:391–435.

Kües U, Badalyan SM, Gießler A, Dörnte B. 2016. 12 Asexual sporulation in Agaricomycetes. In: Wendland J, editors. Growth, Differentiation and Sexuality. The Mycota. Cham: Springer. p. 269–328.

Kües U, James TY, Heitman J. 2011. Mating type in Basidiomycetes: unipolar, bipolar, and tetrapolar patterns of sexuality. In: Pöggeler S, Wöstemeyer J, editors. Evolution of fungi and fungal-like organisms, The Mycota XIV. Berlin: Springer. p. 97–160.

Kües U. 2015. From two to many: multiple mating types in Basidiomycetes. Fungal Biology Reviews 29:126–66.

Kuhn G, Hijri M, Sanders IR. 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. Nature 414:745–8.

Lee SC, Ni M, Li W, Shertz C, Heitman J. 2010. The evolution of sex: a perspective from the fungal kingdom. Microbiol Mol Biol Rev 74:298–340.

Lehtonen J, Jennions MD, Kokko H. 2012. The many costs of sex. Trends Ecol Evol 27:172–8.

Lehtonen J, Kokko H. 2011. Two roads to two sexes: unifying gamete competition and gamete limitation in a single model of anisogamy evolution. Behav Ecol Sociobiol 65:445–59.

Lenormand T, Engelstäder J, Johnston SE, Wijnker E, Haag CR. 2016. Evolutionary mysteries in meiosis. Phil Trans R Soc B 371: 20160001.

Lin X, Hull CM, Heitman J. 2005. Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature 434:1017–21.

Lin X, Litvintseva AP, Nielsen K, Patel S, Floyd A, Mitchell TG, Heitman J. 2007. αADα hybrids of *Cryptococcus neoformans*: evidence of same-sex mating in nature and hybrid fitness. PLoS Genet 3:e186.

Lin XR, Patel S, Litvintseva AP, Floyd A, Mitchell TG, Heitman J. 2009. Diploids in the *Cryptococcus neoformans* serotype a population homozygous for the alpha mating type originate via unisexual mating. PLoS Pathog 5:e1000283.

May G, Shaw F, Badrane H, Vekemans X. 1999. The signature of balancing selection: fungal mating compatibility gene evolution. Proc Natl Acad Sci USA 96:9172–7.

McClelland CM, Chang YC, Varma A, Kwon-Chung KJ. 2004. Uniqueness of the mating system in *Cryptococcus neoformans*. Trends Microbiol 12:208–12.

Mendoza H, Perlin MH, Schirawski J. 2020. Mitochondrial inheritance in phytopathogenic fungi—everything Is known, or Is It? IJMS 21:3883.

Merlini L, Dudin O, Martin SG. 2013. Mate and fuse: how yeast cells do it. Open Biol 3:130008.

Mráz P, Hýbl M, Kopecký M, Bohatá A, Konopická J, Hoštičková I, Konvalina P, Šipoš J, Rost M, Čurn V. 2021. The effect of artificial media and temperature on the growth and development of the honey bee brood pathogen *Ascosphaera apis*. Biology 10:431.

Nieuwenhuis BPS, Aanen DK. 2012. Sexual selection in fungi. J Evol Biol 25:2397–411.

Nieuwenhuis BPS, James TY. 2016. The frequency of sex in fungi. Phil Trans R Soc B 371:20150540.

Olson LW. 1984. Allomyces—a different fungus. Opera Botanica 73:5–96.

Otto SP. 2009. The evolutionary enigma of sex. Am Nat 174:S1–S14.

Parker GA. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. J Theor Biol 96:281–94.

Purin S, Morton JB. 2013. Anastomosis behavior differs between asymbiotic and symbiotic hyphae of *Rhizophagus clarus*. Mycologia 105:589–602.

Raper JR. 1940. Sexuality in *Achlya ambisexualis*. Mycologia 32:710–27.

Rogers DW, Greig D. 2009. Experimental evolution of a sexually selected display in yeast. Proc R Soc B 276:543–9.

Ropars J, Corradi N. 2015. Homokaryotic vs heterokaryotic mycelium in arbuscular mycorrhizal fungi: different techniques, different results? New Phytol 208:638–41.

Ropars J, Toro KS, Noel J, Pelin A, Charron P, Farinelli L, Marton T, Krüger M, Fuchs J, Brachmann A, et al. 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. Nat Microbiol 1, 16033.

Roth C, Sun S, Billmyre RB, Heitman J, Magwene PM. 2018. A high-resolution map of meiotic recombination in *Cryptococcus deneoformans* demonstrates decreased recombination in unisexual reproduction. Genetics 209:567–78.

Roze D. 2012. Disentangling the benefits of sex. PLoS Biol 10:e1001321.

Schirawski J, Heinze B, Wagenknecht M, Kahmann R. 2005. Mating type loci of *Sporisorium reilianum*: novel pattern with three a and multiple b specificities. Eukaryot Cell 4: 1317–27.

Schultze K, Schimek C, Wostemeyer J, Burmester A. 2005. Sexuality and parasitism share common regulatory pathways in the fungus Parasitella parasitica. Gene 348:33–44.

Seibel C, Tisch D, Kubicek CP, Schmoll M. 2012. The role of pheromone receptors for communication and mating in *Hypocrea jecorina* (Trichoderma reesei). Fungal Genet Biol 49:814–24.

Skrede I, Maurice S, Kauserud H. 2013. Molecular characterization of sexual diversity in a population of *Serpula lacrymans*, a tetrapolar Basidiomycete. G3-Genes Genomes Genet 3:145–52.

Spiltoir CF. 1955. Life cycle of *Ascosphaera apis* (Pericystis apis). Am J Bot 42:501–8.

Stölting KN, Wilson AB. 2007. Male pregnancy in seahorses and pipefish: beyond the mammalian model. Bioessays 29:884–96.

Sundberg H, Kruys Å, Bergsten J, Ekman S. 2018. Position specificity in the genus *Coreomyces* (Laboulbeniomycetes, Ascomycota). Fungal Syst Evol 1:217–28.

Umen J, Coelho S. 2019. Algal sex determination and the evolution of anisogamy. Annu Rev Microbiol 73: 267–91.

Varga T, Krizsán K, Földi C, Dima B, Sánchez-García M, Sánchez-Ramírez S, Szöllősi GJ, Szarkándi JG, Papp V, Albert L, et al. 2019. Megaphylogeny resolves global patterns of mushroom evolution. Nat Ecol Evol 3:668–78.

Wang Y-W, McKeon MC, Elmore H, Hess J, Golan J, Gage H, Mao W, Harrow L, Gon ßalves SC, Hull CM, Pringle A. 2023.

Invasive Californian death caps develop mushrooms unisexually and bisexually. bioRxiv 2023.01.30.525609.

Wilson AJ, Xu J. 2012. Mitochondrial inheritance: diverse patterns and mechanisms with an emphasis on fungi. Mycology 3:158–66.

Wilson AM, Wilken PM, van der Nest MA, Steenkamp ET, Wingfield MJ, Wingfield BD. 2015. Homothallism: an umbrella term for describing diverse sexual behaviours. IMA Fungus 6:207–14.

Wirth S, Freihorst D, Krause K, Kothe E. 2021. What role might non-mating receptors play in Schizophyllum commune? JoF 7:399.

Xu J, Li H. 2015. Current perspectives on mitochondrial inheritance in fungi. CHC 7:143–54.

Yadav V., Sun S, Heitman J. 2021. Uniparental nuclear inheritance following bisexual mating in fungi. Elife 10: e66234.

Yan Z, Hull CM, Heitman J, Sun S, Xu J. 2004. SXI1α controls uniparental mitochondrial inheritance in the fungus *Cryptococcus neoformans*. Curr Biol 14: R743–4.

Zimmerman KCK, Levitis DA, Pringle A. 2016. Beyond animals and plants: dynamic maternal effects in the fungus *Neurospora crassa*. J Evol Biol 29:1379–93.