Sex in Fungi

Min Ni, Marianna Feretzaki, Sheng Sun, Xuying Wang, and Joseph Heitman*

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710; email: heitm001@duke.edu

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*Corresponding author.

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Abstract

Sexual reproduction enables genetic exchange in eukaryotic organisms as diverse as fungi, animals, plants, and ciliates. Given its ubiquity, sex is thought to have evolved once, possibly concomitant with or shortly after the origin of eukaryotic organisms themselves. The basic principles of sex are conserved, including ploidy changes, the formation of gametes via meiosis, mate recognition, and cell-cell fusion leading to the production of a zygote. Although the basic tenants are shared, sex determination and sexual reproduction occur in myriad forms throughout nature, including outbreeding systems with more than two mating types or sexes, unisexual selfing, and even examples in which organisms switch mating type. As robust and diverse genetic models, fungi provide insights into the molecular nature of sex, sexual specification, and evolution to advance our understanding of sexual reproduction and its impact throughout the eukaryotic tree of life.

INTRODUCTION

Homothallism: fungi can undergo sexual reproduction and form sexual structures and progeny during solo culture

Heterothallism: fungi with two compatible mating types are required for a complete sexual cycle

***MAT* locus:** mating-type locus

Sexual reproduction drives genetic recombination throughout eukaryotic organisms and serves to purge deleterious mutations, producing better-adapted progeny. The processes of sex involve mate recognition, cell-cell fusion yielding a zygote, generation of gametes via meiosis, and ploidy changes. Although these basic tenants are shared, a diversity of sexual reproduction strategies is encountered throughout nature. The fungal kingdom includes outbreeding systems with up to thousands of mating types at one end of the spectrum and self-fertile, inbreeding systems at the other, and provides a window to investigate the molecular nature of sex, sexual specification, and evolution. There are three central dichotomies to consider regarding sex in fungi: (*a*) inbreeding/selfing (homothallism) versus outbreeding (heterothallism) modes of reproduction; (*b*) the sex determinants encoded by the mating-type (*MAT*) locus; (*c*) sex systems with a single biallelic locus (bipolar) versus systems with two unlinked, multiallelic sex loci (tetrapolar). Our aim in this review is to investigate these specific aspects of sex in fungi. In addition, we discuss the connections between sexual development and other aspects of the life cycle, including evolution of sex chromosomes, mitochondrial inheritance, and genome defense mechanisms.

SEX AND MATE RECOGNITION

An early key step in sexual reproduction is mate recognition. Both yeast and filamentous fungi have evolved systems to detect mating partners via mating type–specific peptide pheromones and receptors. In *Saccharomyces cerevisiae*, α cells produce the α factor peptide pheromone to signal **a** cells, and **a** cells signal α cells via **a** factor, a lipid-modified peptide pheromone. Mating type-specific pheromone receptors decorate the cell surface and sense these reciprocal signals to activate the pheromone-sensing mitogen-activated protein (MAP) kinase signal transduction pathway common to both mating types. Mate recognition through pheromone sensing is widespread throughout the fungal kingdom. In the filamentous ascomycete *Neurospora crassa*, a pheromone-like protein was recently identified that participates in mating, ascospore formation, and vegetative growth (73).

Interestingly, pheromone-receptor interactions exhibit considerable plasticity between species. In the filamentous ascomycete *Aspergillus nidulans*, pheromone receptors are required for self-fertilization, whereas the outcrossing of pheromone receptor mutants results in the production of fertile ascospores but reduced levels of cleistothecia, the sexual structures bearing the spores (130). Surprisingly, pheromones have functions beyond their role in initiating **a**-α mating in the human pathogen *Candida albicans*. Alby et al. (2, 3) found that both intraspecies and interspecies pheromones initiate an autocrine signaling pathway leading to same-sex mating in *C. albicans* **a** opaque cells. The same set of pheromones promotes biofilm formation in α white cells, which may reflect an important role of the pheromones in pathogenesis (31). Basidiomycetes have only **a** factor–like pheromones and pheromone receptors, which are encoded by genes in the *MAT* locus. In the human pathogenic basidiomycete *Cryptococcus neoformans*, pheromones and their receptors contribute to both opposite- and same-sex mating (85, 131, 137). We can therefore conclude that pheromone-receptor systems are integral for both heterothallism and homothallism. Indeed, the presence of pheromones and their receptors in a given genome suggests an ability to mate, even in the absence of a known extant sexual cycle. As the pheromone system is a common element of sexual differentiation in both basidiomycetes and ascomycetes, it is likely that their last common ancestor possessed a pheromone-receptor system that governed the sexual cycle in either a homothallic or heterothallic fashion.

However, the molecular nature of the pheromone system is not universal in the fungal kingdom. In basal fungi such as the zygomycetes, trisporic acid derivatives serve as mating pheromones. These are small, organic molecules completely unrelated structurally to

the **a**-factor and α-factor peptide pheromones of the ascomycetes and basidiomycetes (146). In chytridiomycetes, another basal fungal lineage, the pheromone system involves chemical compounds with structures completely unrelated to those of the zygomycete, ascomycete, or basidiomycete pheromones. In the aquatic chytrid *Allomyces macrogynus*, the pheromone produced by the female is named sirenin, and the male produces parisin. It is possible that the initiation of the sexual cycle in aquatic, ancestral fungi was achieved through chemical compounds similar to the sirenin/parisin system in *A. macrogynus*, or the trisporic acid compounds observed in zygomycetes, whereas the **a**-factor and α-factor peptide pheromones probably evolved later in the Dikarya as an adaptation to terrestrial ecosystems.

Following cell recognition via pheromone sensing, mating cells undergo cell-cell fusion, resulting in a dikaryotic state that prepares the cells for nuclear fusion and meiosis. In *S. cerevisiae*, cells of opposite mating type form projections (shmoos) toward a pheromone source and then fuse at the tips of these projections. Pheromone sensing causes a microtubule-associated nuclear migration and then nuclear fusion occurs to form an α/**a** diploid cell. Under certain environmental conditions (e.g., low nitrogen, presence of acetate), α/**a** diploid cells undergo meiosis to produce four haploid recombinant spores (progeny) enclosed in an ascus.

The *S. cerevisiae* paradigm serves as a basic model to understand the process of mating when there is no structural difference between the gametes of each mating type (termed isogamy). This system is common in the closely related *Candida* genus and in the rather distantly related archiascomycete *Schizosaccharomyces pombe*, where both haploid and diploid states of the sexual cycle are characterized by yeast cells. However, some ascomycetes and the majority of basidiomycetes are filamentous, and sexual reproduction occurs during the hyphal state. In filamentous fungi, cell fusion can occur between a hyphal and a specialized cell, or between two hyphal partners. In filamentous ascomycetes such as *N. crassa*, the mating type–specific pheromones from the male microconidium attract the reproductive hyphae that emanate from the female reproductive structures, and the female hypha fuses with the male cell following physical contact (72). Following cell-hypha fusion, the nucleus from the male cell migrates through the hypha to the female reproductive structure. The nuclei of both mating types proliferate, pair, and migrate to the dikaryotic ascus where they fuse and undergo meiosis. Mitosis then produces four pairs of recombinant ascospores (117).

A similar mating process is observed in the Aspergilli and filamentous basidiomycetes, where the mating partners are two hyphae of the same or opposite mating type. An interesting example is the model mushroom *Coprinopsis cinerea*, where monokaryotic hyphae fuse through hyphal anastomosis (22). If monokaryons have different mating types, they establish a fertile dikaryotic mycelium in which nuclear exchange and reciprocal migration of the nuclei occur within the hyphae. At the tip of the hyphae, the opposite mating-type nuclei pair, proliferate, and migrate in the hyphae. Under certain environmental conditions, the dikaryon produces the fruiting body that contains the basidia, where nuclear fusion occurs and subsequent meiosis and sporulation produce basidiospores (22). An interesting feature of basidiomycetes is the long delay between cell fusion and nuclear fusion. In addition, the absence of the pheromone-receptor interplay in mating partner recognition in mushrooms is striking, as fusion can occur between any type of hyphae. However, genetic compatibility of mating partners is required for sexual development to continue.

Hypha-hypha fusion is common in basidiomycetes considering that the majority are filamentous at some point during their life cycle. However, some exist as haploid yeasts in nature and fuse upon stimulation by pheromones, at which point the dikaryon undergoes a dimorphic transition from yeast to hypha. An interesting example is *C. neoformans*, where yeast cells respond to pheromones

by forming conjugation tubes toward the pheromone source (the mating partner) (69, 93). The cells fuse and establish a dikaryotic state that initiates hyphal formation. The basidium is formed at the tip of the hypha, where nuclear fusion, meiosis, and sporulation occur (69). The three morphological states of mating—yeast cells, hyphae, and the dimorphic transition from yeast to hyphae—are common in both phyla of the Dikarya; therefore, it is challenging to determine the ancestral morphological condition as there is evidence to support all three. However, species of the distantly related, basal zygomycete lineage mate through hyphal fusion in a manner similar to that described above. *Phycomyces blakesleeanus* mycelia of opposite mating types produce trisporic acid chemical signals that induce the formation of fruiting bodies with thicker hyphae at the tip, which are known as zygophores (23). Following physical contact, zygophores twist to form a circle where at the top, two cells of opposite mating types fuse to form a zygospore. Nuclear fusion then occurs and gives rise to a sporangium filled with spores (23). Based on these observations and previous phylogenetic analyses that support a filamentous species as a possible precursor of the Dikarya, it is conceivable that the ancestral mating process involved the presence of hyphae. Alternatively, the sexual cycle of *A. macrogynus* involves fusion of two motile gametes, and thus might more closely approximate the cellular nature of mating of the unicellular yeasts. Which of these is the ancestral state, or the possibility of both being ancestral states, invites future investigation.

HOMOTHALLISM VERSUS HETEROTHALLISM

Sexual reproduction is central to eukaryotic evolution via its ability to increase genetic diversity and eliminate deleterious mutations. Fungi have evolved two paradigmatic sexual systems: heterothallism and homothallism (**Figure 1**). Heterothallic fungi require two compatible partners for mating to occur, whereas homothallic fungi are self fertile with a single individual capable of sexual reproduction even in solo culture. Both modes of sexual reproduction share key features (e.g., ploidy changes, meiosis, production of recombinant progeny) but differ in other key features involving aspects of cell or hyphal fusion. Transitions between heterothallic and homothallic patterns of sexuality are common throughout the fungal kingdom, and both modes can be observed concomitantly in different species of the same genus and sometimes even within the same species (reviewed in 55, 84).

Both heterothallic and homothallic sexual reproduction modes are dynamic and have evolved to fulfill the mating requirements of each fungal species. Heterothallic fungi require two partners of opposite mating types with compatible *MAT* idiomorphs, which contain genes controlling cell identity, cell fusion, and the formation of the dikaryotic zygote state that leads to nuclear fusion, meiosis, and sporulation. In homothallic fungi, the same genes are often required for successful mating; however, there is no mating partner, the partners are of the same mating type, both mating type idiomorphs are present or fused or one partner switches mating types. The canonical model of homothallism is the *MAT* switching paradigm in ascomycetes, in which a *MAT* cassette system enables mother cells to switch mating type to mate with a daughter cell. Studies of mating-type switching in *S. cerevisiae*, *S. pombe*, and *Kluyveromyces lactis* reveal three elaborate mechanisms that have been acquired through both shared and distinct evolutionary paths (**Figure 2**). Mating-type switching may also have independently arisen in basidiomycetes, based on a report on *Agrocybe aegerita*, indicating that this form of homothallism has evolved repeatedly and independently (78, 84).

In several types of homothallism, the two *MAT* idiomorphs required for mating coexist in the same genome and are either fused into one *MAT* locus, as observed in *Cochliobolus* spp., or they are unlinked and lie at different positions in the genome, as in *A. nidulans* and *Neosartorya fischeri* (**Figure 1*b***; for details, see 84, 123). Homothallic *Cochliobolus* spp. carry both *MAT*

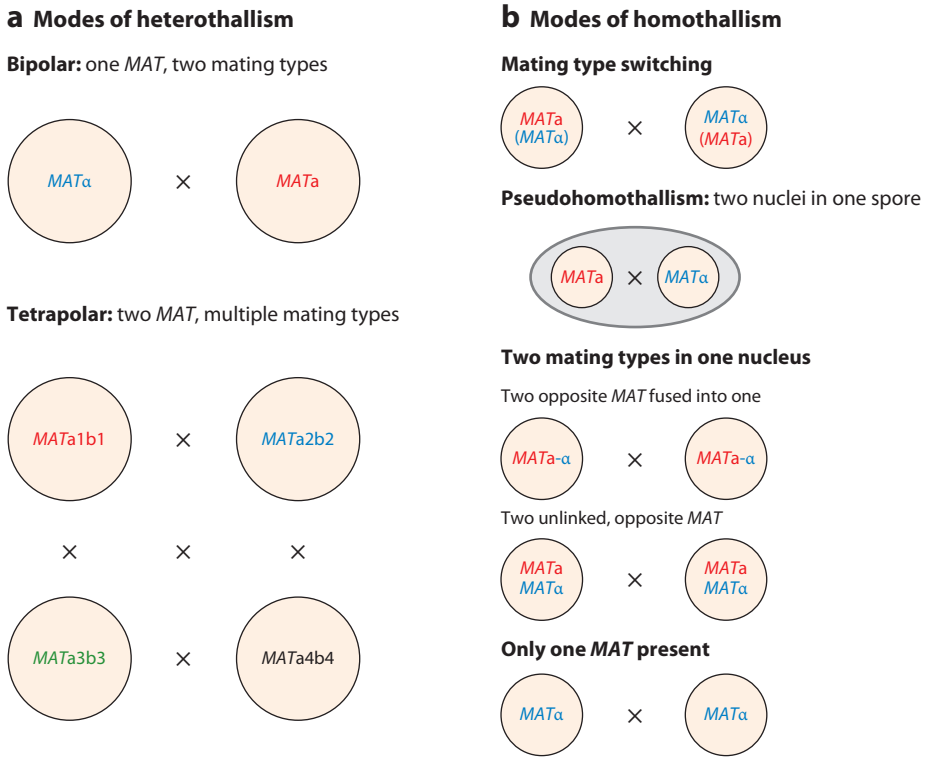


Figure 1

Modes of sexual reproduction in fungi. (*a*) Modes of heterothallism. Bipolar: one mating-type (*MAT*) locus regulates sexual development, and two isolates need to possess opposite *MAT* alleles to mate. Tetrapolar: two *MAT* loci regulate sexual development and are often multiallelic, and two isolates need to possess opposite alleles at both loci for sexual reproduction. (*b*) Modes of homothallism: mating-type switching in which an α daughter cell mates with an **a** mother cell; pseudohomothallism in which two nuclei of opposite mating types are packaged into one spore; two *MAT*s in one nucleus in which the two opposite *MAT* loci are either fused in one locus or reside at different loci; and finally, there is only one *MAT* idiomorph present and cells reproduce via same sex mating.

idiomorphs, fused or closely linked on the same chromosome, whereas heterothallic species carry one or the other in each isolate (150). The pattern of sexual reproduction can be readily altered by genetically manipulating the *MAT* locus, and thus the two patterns of sexuality may share a common evolutionary origin (150). The structural organization of *MAT* in *Cochliobolus* spp. supports the hypothesis that heterothallism is the ancestral form of sexual reproduction in these species and that homothallism is a derived state. Similarly, the homothallic species *A. nidulans* contains two different unlinked *MAT* idiomorphs, whereas isolates of the heterothallic species *Aspergillus fumigatus* and *Aspergillus oryzae* carry only one or the other *MAT* idiomorph (49). The recent discovery of an extant heterothallic *A. fumigatus* sexual cycle strengthens the argument that homothallism arose later in the evolution of *Aspergillus* spp. and that they descend from a heterothallic last common ancestor (105). Remarkably, some homothallic fungal species contain only one *MAT* idiomorph and yet exhibit a complete sexual cycle.

Some heterothallic fungi exhibit homothallism under specific environmental conditions. The heterothallic basidiomycete

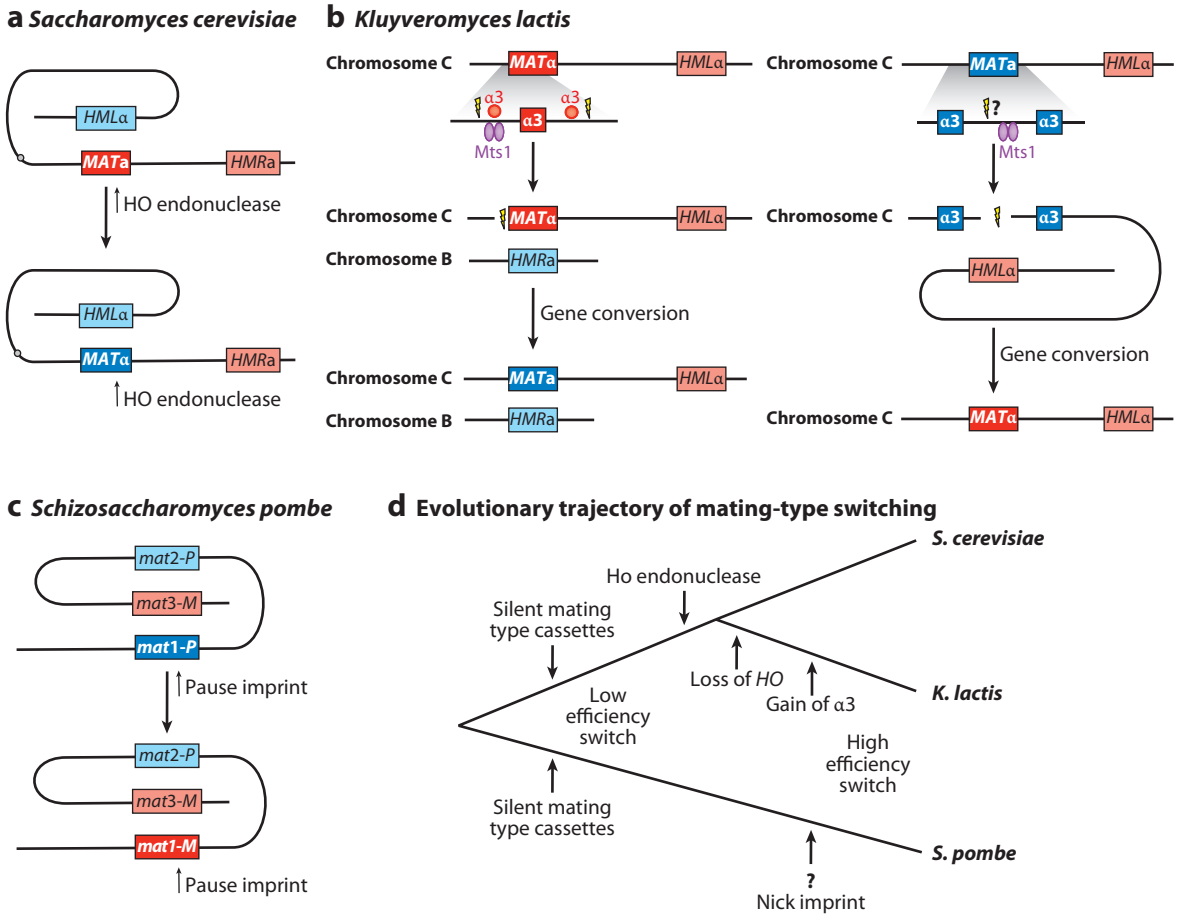


Figure 2

Mating-type switching in *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, and *Schizosaccharomyces pombe*. (*a*) In *S. cerevisiae*, the Ho endonuclease creates a double-strand break (DSB) in *MAT* that is repaired via homologous recombination with one of the silent cassettes as a donor, leading to gene conversion. (*b*) *K. lactis* utilizes the native α3 transposase or an unknown nuclease to induce a DSB in *MAT* directed by the binding of Mts1. Homologous recombination repairs the break via gene conversion. (*c*) In *S. pombe*, an unknown nuclease promotes a lesion at the imprint, which is repaired through homologous recombination. (*d*) Evolution of mating-type switching in *S. cerevisiae*, *S. pombe*, and *K. lactis*. Initially, the silent cassettes were independently acquired in *S. cerevisiae* and *K. lactis*, resulting in inefficient mating-type switching via mitotic recombination. The Saccharomycotina gained the *HO* gene, which increased switching efficiency. However, in the diverged lineage of *K. lactis*, the *HO* gene degenerated and the α3 transposase or an unknown nuclease was conscripted to promote mating-type switching.

C. neoformans can undergo a transition from yeast to hyphae and complete opposite-sex mating or, in the absence of a compatible mating partner, undergo same-sex mating (**Figure 1*b***; 85). The heterothallic ascomycete *C. albicans*, in the absence of the Bar1 protease or the presence of α pheromone produced by α cells in ménage à trois matings, can also undergo autocrine or paracrine pheromone signaling and same-sex mating (3). Homothallism has also been observed in the basal fungal zygomycete and chytridiomycete lineages. In the homothallic species *A. macrogynus*, a single haploid meiospore can produce both female and male gametangia to complete the sexual cycle (98).

The transition from heterothallism to homothallism appears to be a choice between outcrossing and inbreeding in a population, and the frequency with which they occur is under the control of specific environmental and genetic conditions that favor one or the other breeding strategy. Conversions between the two sexual modes are common in the same genus and even in isolates of the same species. Whether homothallism evolved from heterothallism or vice versa is not known, as there is evidence to support both hypotheses. The pervasiveness of heterothallism may favor this as the ancestral mode of reproduction, and examples of heterothallic species with recently derived closely aligned homothallic species provides further support for heterothallism as ancestral. However, the finding that some homothallic species involve just one mating type (same-sex mating) suggests that homothallism could represent an original sexual state of fungi with only one mating type. One way to envision this is as a primitive homothallic fungus with a self-fertile unisexual mode of reproduction, possibly similar to that of *C. neoformans*. Heterothallism could have been derived later, with two distinct idiomorphs evolving to govern the sexual cycle and identity. If this is the case, the pervasiveness of heterothallism throughout the fungal kingdom may be due to its advantages in response to distinct evolutionary pressures. What then could have been an ancestral homothallic state? It is clearly not mating-type switching or the fused *MAT* alleles observed in *Cochliobolus* spp., as these are more recently derived. However, an argument can be advanced for either unisexual reproduction (observed in *Cryptococcus* and *Candida*) or two unlinked *MAT* locus alleles (observed in *A. nidulans* and *N. fischeri*) as candidate ancestral homothallic states.

MATING-TYPE SWITCHING

Sexual identity in fungi is controlled by the *MAT* locus, which encodes key regulators of mating. In haploid cells, *MAT* is defined by two alleles: *MAT*α and *MAT***a**. Two cells of opposite mating type are able to fuse and mate in response to pheromone and environmental cues. Some fungal species, specifically the ascomycetes *S. cerevisiae*, *S. pombe*, and *K. lactis*, exhibit the unusual property of being able to undergo mating-type switching, a process in which a haploid cell gives rise to a cell of opposite mating type (**a**→α or α→**a**). These systems involve a cassette mechanism with one active expression locus, two silent *MAT* allele copies, and machinery for DNA lesion-promoted recombination (**Figure 2**). Although the mechanisms differ between species, full genome sequences and molecular genetic studies have revealed conserved features and evidence of independent origins of the cassettes and switching mechanisms.

In *S. cerevisiae* and the majority of the Saccharomycotina, two additional *MAT* cassettes of each opposite mating type are present in the genome in transcriptionally inactive, heterochromatin-like structures (19). The opposite *MAT* silent cassettes are an essential part of the system, functioning as templates for mating-type switching. The presence of the cassettes in the genome underlies the ability of the organism to switch mating types; the species that feature them switch mating types at various frequencies. Interestingly, the cassettes are not present in closely related *Candida* spp. or other ascomycetes, but they are present in the distantly related archiascomycete *S. pombe* (101). These observations support an independent acquisition of the silent cassettes in the genomes of *S. cerevisiae* and *S. pombe* subsequent to their divergence from a last common ancestor.

The cassette *MAT* switching model proposed by Herskowitz and colleagues has been extensively studied in *S. cerevisiae* (**Figure 2*a***). In this model, a haploid cell switches mating type through a gene conversion event driven by a DNA double-strand break (DSB) in the active *MAT* locus (58). The Ho endonuclease causes the DNA DSB at the boundary between the Y**a** or Yα sequence and the common flanking Z sequence (100). *MAT* switching occurs through a DSB repair mechanism known as

Silent cassette: additional copies of the two opposite mating idiomorphs in the genome that are transcriptionally inactive because of modified chromatin structure

Gene conversion: nonreciprocal substitution of a gene with a donor sequence because of homologous recombination induced by a DNA double-strand break

DSB: double-strand break

synthesis-dependent strand annealing using the silent cassette of the opposite mating type as a template (100). The Ho endonuclease is cell cycle–controlled and only expressed in mother cells during late G1. Ash1 mRNA from the mother is localized to the daughter cell and inhibits *HO* transcription by suppressing its activator complex Swi4/Swi6 (52, 87). Thus, only mother cells (cells that have undergone at least one mitotic division) are licensed to switch.

The *HO* gene (or pseudogene) is present in all members of the Saccharomycotina that harbor silent cassettes, and all of these members have been reported to exhibit mating-type switching. Interestingly, *K. lactis* lacks a Ho site in any *MAT* allele (19). In addition, *K. lactis* contains a nonfunctional, highly degenerate *HO* pseudogene, which suggested that mating-type switching in this species might occur through spontaneous mitotic recombination (41). Herman & Roman (57) studied two natural, heterothallic isolates that were able to undergo mating-type switching and noticed that the low frequency of switching on rich media increased in a nutrient-limited medium (malt extract agar). Recent studies revealed a novel alternative Ho-independent mechanism of high efficiency switching in *K. lactis* (**Figure 2*b***). Barsoum et al. found that mating-type switching is induced by Mts1 (the *K. lactis RME1* ortholog, regulator of meiosis) binding at sequences present in both *MAT***a** and *MAT*α (11). Mts1 binds to the 5′ and 3′ regions of the *α3* gene in *MAT*α and acts as a transposase that creates a DSB and excises itself as a circular DNA molecule that is lost in subsequent cell cycles (11). In *MAT***a**, Mts1 binding induces the formation of a DSB through an unknown nuclease. The DSB is repaired through homologous recombination employing the silent cassettes as templates and results in mating-type switching via gene conversion (11). Moreover, Booth et al. (15) found that Mts1 (like its ScRme1 ortholog) is induced by phosphate starvation, which increases the frequency of mating-type switching, explaining the initial observation of Herman & Roman with respect to nutrient limitation.

These results suggest a common evolutionary path of mating-type switching in *S. cerevisiae* and *K. lactis* (**Figure 2*d***). A first step in the evolution of mating-type switching involved the acquisition of the silent cassettes. Initially, the switching efficiency was likely very low; however, the acquisition of the *HO* gene into the regulatory circuit improved the efficiency of mating-type switching. The *S. cerevisiae* lineage retained all of the elements necessary for successful mating-type switching, but the *HO* gene degenerated and lost its function in *K. lactis*. Nevertheless, *K. lactis* acquired a new mechanism with α3 transposases playing the role of the endonuclease driven by the newly acquired function of Kl*MTS1*.

Although *S. cerevisiae* and *K. lactis* share common evolutionary steps to acquire mating-type switching, *S. pombe* has a distinct evolutionary trajectory. In *S. pombe*, a distantly related archiascomycete, switching occurs during replication and is initiated by an imprinted signal on the leading strand at the *mat* (101). The nature of the imprint has been the subject of active debate, but recent evidence supports the hypothesis that a protected single-strand nick is introduced on the leading strand template, possibly during maturation of Okazaki fragments in the prior replication cycle, by an unknown nuclease (101, 142). During replication, the incoming replication fork is delayed at the imprint, and the newly synthesized leading strand invades the opposite *MAT* donor cassette, using it as a replication template for the *mat* region (101). This mechanism is unique among known homothallic species, indicating that the mating-type switching mechanism in *S. pombe* followed an independent evolutionary pathway from the Saccharomycotina (**Figure 2*c***). Initially, the acquisition of the silent *MAT* cassettes may have allowed mating-type switching with low efficiency in an ancestral species of *S. pombe*. However, a subsequent, unknown event, possibly the evolution or acquisition of a nuclease, increased the efficiency of mating-type switching, similar to *S. cerevisiae* and *K. lactis*.

MATING-TYPE LOCI IN FILAMENTOUS ASCOMYCETES: *NEUROSPORA*, *PODOSPORA*, AND *SORDARIA*

A previous review from our group covered mating-type systems in filamentous ascomycetes, using the genus *Aspergillus* as an example (82). Here, we overview *MAT* in three additional ascomycetes: *Sordaria macrospora*, *Podospora anserina*, and *N. crassa*, which develop through similar sexual cycles but represent three paradigmatic genetic breeding mechanisms: homothallism, pseudohomothallism, and heterothallism, respectively. Both *S. macrospora* and *P. anserina* belong to a group of coprophilous fungi that are found in the dung of herbivorous animals and are important in recycling nutrients from animal feces (30, 76). They are exemplary genetic models to study fungal sexual development and meiosis because their sexual spores are arranged linearly in fruiting bodies (76, 140). In addition, *P. anserina* serves as a model for aging because it has a limited, strain-specific life span (126), and the pink bread mold *N. crassa* is the model system in which the one gene–one enzyme hypothesis was elucidated, as well as the molecular mechanisms of circadian rhythm (reviewed in 32). All three genome sequences are available (40, 48, 103).

Sexual development of these fungal species (**Figure 3*a***) begins with the germination of ascospores, followed by the growth of vegetative mycelium with the formation of an ascogonium (female gametangia), which further develops into a perithecium (the fruiting body). Inside the perithecium, two nuclei fuse to generate a diploid nucleus, which undergoes meiosis followed by a postmeiotic mitosis, resulting in the formation of eight haploid, linearly arranged ascospores in *N. crassa* and *S. macrospora* and four binucleate ascospores in *P. anserina* (one percent of the asci contain three binucleate and two uninucleate ascospores) (32, 115, 151).

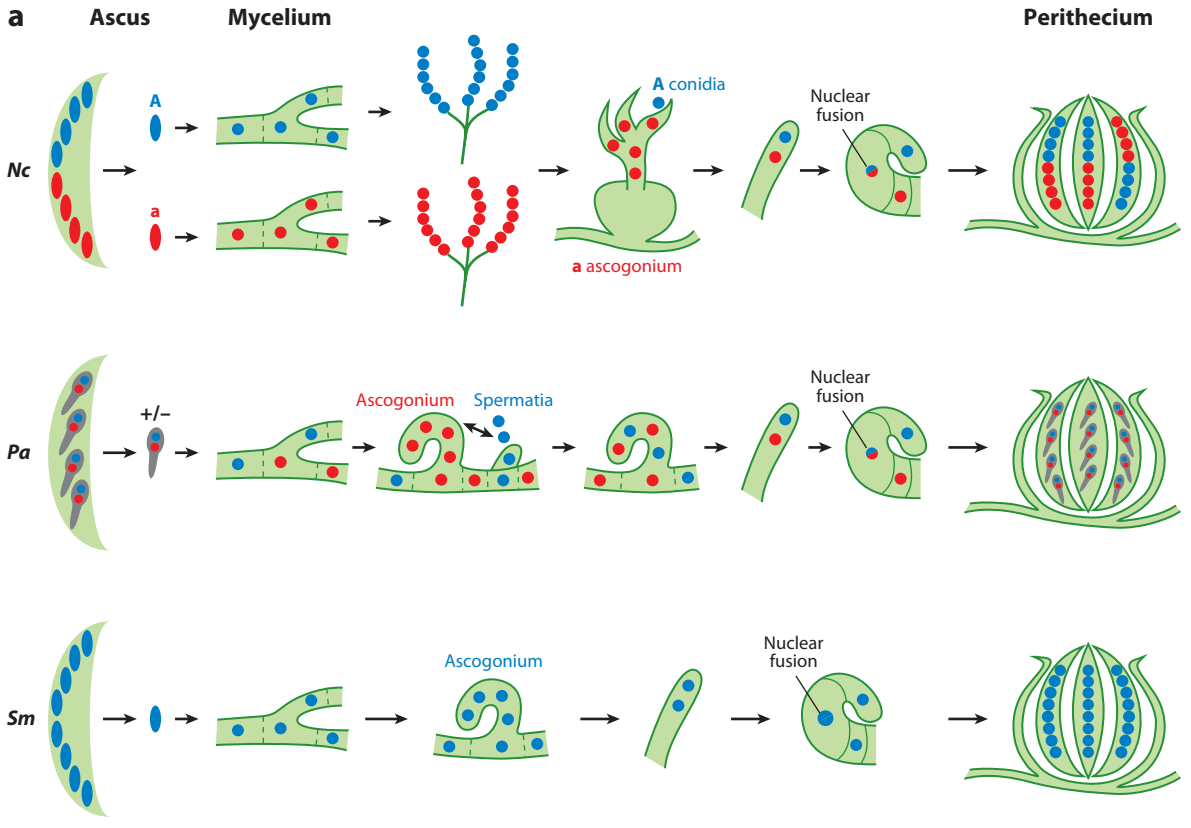
S. macrospora is homothallic with mycelia that grow from the germination of uninucleate ascospores, and it does not produce asexual spores during mycelial growth (115). *P. anserina* is pseudohomothallic, and its ascospores contain two nuclei, one of each mating type (*MAT1-1* or *MAT-* and *MAT1-2* or *MAT+*) (111). The binucleate ascospores germinate to form self-fertile, heterokaryotic mycelia carrying nuclei of both mating types that can complete the sexual cycle in solo culture (pseudohomothallic inbreeding) or segregate hyphae of opposite mating type to enable outcrossing. The mycelia of each mating type develop into spermatia (male) or ascogonia (female), and fertilization occurs between a spermatium and an ascogonium of opposite mating type (151). Unlike many other ascomycetes, spermatia (asexual spores) of *P. anserina* cannot germinate, and their function is purely sexual (37).

Pseudohomothallism: fungi that package two opposite mating-type nuclei in the same spore

N. crassa is heterothallic, and sexual reproduction in this species occurs only between partners of opposite mating types (*MAT1-1* or *MATA* and *MAT1-2* or *MATa*) (97). In contrast to the two species discussed above, *N. crassa* can undergo asexual development and produces two types of asexual spores: orange multinucleate macroconidia (6–8 mm in diameter) and smaller uninucleate microconidia (2.5–3.5 mm in diameter) (reviewed in 90). In contrast to *P. anserina*, *N. crassa* macroconidia and microconidia can both germinate to produce mycelial hyphae, whereas only microconidia can function as spermatia (male gametes) (90). The fertilization process is similar to that of *P. anserina* discussed above; however, similar to *S. macrospora*, the ascospores of *N. crassa* are uninucleate. As shown in **Figure 3*a***, all three fungi produce perithecia with linearly arranged ascospores, thus providing excellent model systems in which meiotic drive elements can be studied.

These three fungi share a similar genetic organization at the *MAT* locus (**Figure 3*b***). In *N. crassa*, ascospores carry either the *MAT1-1* or the *MAT1-2* idiomorph (97). The *MAT1-1* idiomorph comprises the α domain gene, the PPF domain (which contains conserved proline, proline, and phenylalanine residues) gene, and an HMG (high-mobility group) domain gene (35, 44, 51). The α domain protein is critical for 1-1 *MAT* identity and sexual development (14, 44, 51, 124). Deletion of the

PPF or HMG domain gene does not confer any apparent phenotype, whereas the deletion of both genes dramatically decreases fertility (43). The *MAT1-2* idiomorph includes two genes: an HMG domain gene and a small open reading frame (ORF) of unknown function (114, 136). The HMG domain gene is necessary to establish the 1-2 *MAT* identity (25).



S. macrospora is homothallic, and its *MAT* locus does not have distinguished idiomorphs. Instead, four genes are located within the *S. macrospora MAT* locus, and they are orthologs of the genes present in both *MAT1-1* and *MAT1-2* of *N. crassa* (**Figure 2*b***). These genes are all transcribed during the sexual life cycle in both *S. macrospora* and *N. crassa*, and each pair of orthologs shares high sequence similarity (114, 116). The deletion of the *S. macrospora* α domain protein or the small ORF gene does not cause any defects in vegetative growth or sexual reproduction, whereas the PPF and HMG domain proteins are essential for sexual development (74, 115).

In *P. anserina*, *MAT1-2* contains only an HMG domain gene (36). The *MAT1-1* locus contains the α domain, PPF domain, and HMG domain genes and is similar to *MAT1-1* of *N. crassa* (34, 36). In crosses with strains of opposite mating type deletion mutants lacking either the α domain or the HMG domain gene do not result in fertilization but can still produce uniparental asci, indicating that they are needed during fertilization but are not essential for postfertilization development (7, 8, 34). In contrast, the deletion of the PPF domain gene causes an arrest of sexual development after fertilization, suggesting a postfertilization role (7, 34).

As these fungi and their closely related species have similar processes of sexual development but differ in *MAT* locus configuration and reproductive modes, this group of fungi provides a rich resource to study links between the evolution of the *MAT* and reproductive mode. It is unknown whether heterothallism evolved from homothallism or vice versa (82). *S. macrospora*, as well as *Neurospora pannonica* and *Neurospora terricola*, contains a single *MAT* similar to a fusion of the *MAT1-1* and *MAT1-2* idiomorphs in *N. crassa*. However, the homothallic *Neurospora* species *Neurospora africana*, *Neurospora dodgei*, *Neurospora galapagonensis*, and *Neurospora lineolata* have only the *MAT1-1* idiomorph and are unisexual (51, 112), indicating that the fused *MAT* loci of the homothallic species *S. macrospora*, *N. pannonica*, and *N. terricola* most likely are derived from a heterothallic ancestor. A recent *Neurospora* phylogenetic analysis further supports this conclusion and predicts that homothallism might have evolved independently more than six times within the *Neurospora* genus and involved both *MAT* fusion and unisexual reproduction (104). The molecular mechanisms responsible for the fusion event have not been elucidated. As shown in **Figure 3*b***, it is likely that a recombination event occurred between conserved regions of *MAT1-1* and *MAT1-2*, supporting the model that homothallism evolved from heterothallism.

EVOLUTION OF FUNGAL SEX CHROMOSOMES

In plants and animals, sex is often determined by distinguishable sex chromosomes (reviewed in

Figure 3

Sexual development cycles and *MAT* loci in *Neurospora crassa*, *Podospora anserina*, and *Sordaria macrospora*. (*a*) Sexual development begins with the germination of ascospores, followed by the growth of vegetative mycelium with the formation of an ascogonium, which further develops into a perithecium. Inside the perithecium, two nuclei fuse to generate a diploid nucleus, which undergoes meiosis followed by a postmeiotic mitosis, resulting in the formation of eight haploid, linearly arranged ascospores in *N. crassa* and *S. macrospora* and four binucleate ascospores in *P. anserina*. Mating of heterothallic *N. crassa* occurs only between strains of *MATA* and *MATa*. Microconidia (male) of one *MAT* (A in figure; can also be a) are fertilized with ascogonia (female) of the other *MAT* (a in figure; can also be A). Sexual development of pseudohomothallic *P. anserina* initiates from the germination of binucleate ascospores (*MAT*+/−) to form self-fertile, heterokaryotic mycelia carrying nuclei of both mating types. The mycelia of each mating type develop into spermatia or ascogonia, and fertilization occurs between a spermatium and an ascogonium of opposite mating types. *S. macrospora* is homothallic, and its mycelia grow from the germination of uninucleate ascospores. Figures were modified from **Figure 1** with permission from the author Patrick Shiu (132). (*b*) *MAT* in *Aspergillus fumigatus*, *N. crassa*, *P. anserina*, and *S. macrospora*. Abbreviations: HMG, high-mobility group domain; PPF, the domain containing conserved proline, proline, and phenylalanine residues.

Bipolar mating system: mating compatibility is controlled by one locus, and two isolates need to possess opposite mating-type alleles in order for mating to occur

Tetrapolar mating system: mating compatibility is controlled by two unlinked loci, and two isolates need to possess opposite alleles at both loci for mating to occur

27). Sex chromosomes share a common feature: Their recombination is inhibited and restricted to a pseudoautosomal region (26). The sex-determining *MAT* locus in fungi is generally shorter and composed of key cell identity genes, as discussed above. Nevertheless, recent studies have revealed that the chromosome regions determining mating-type identity in several fungi span large chromosomal distances and share more features with the complex sex chromosomes of animals and plants.

A notable example of fungal sex chromosomes is the ascomycete *Neurospora tetrasperma*, which has a pseudohomothallic sexual life cycle similar to *P. anserina*. Sharing features of sex chromosomes with animals and plants, the sex chromosomes of *N. tetrasperma* fail to recombine with their homologous chromosome over 75% of their length (~7 Mb) (94, 95). By comparing the gene divergence between 35 alleles on the *MATA* and *MATa* chromosomes from a wild-type heterokaryon, Mankis et al. (94) found that there were two successive events involved in the evolution of the *N. tetrasperma* sex chromosomes: (*a*) the suppressed recombination over a very large region (~6.6 Mb) appeared when *N. tetrasperma* split from a last common ancestor with the heterothallic relative *N. crassa*; (*b*) a second smaller region with suppressed recombination (~0.3 Mb) appeared more recently. As the *N. tetrasperma* sex chromosomes evolved less than six million years ago (118), it provides an excellent system to investigate the origin and early evolution of sex chromosomes, as more ancient sex chromosomes are often characterized by a highly degenerated Y (or W) chromosome. By examining preferred codon usage in 290 genes on the *N. tetrasperma* sex chromosomes, Whittle et al. (144) found that the level of degeneration is magnified during evolution of the recombination suppressed region, and these degenerative changes in codon usage might be caused by the altered selection efficiency in the recombinationally suppressed region.

In basidiomycetes, there exist both bipolar mating systems (where mating type is determined by one locus, and two strains must possess different alleles at the locus to be sexually compatible) and tetrapolar mating systems (where mating type is determined by two loci, and two strains must possess different alleles at both loci to be sexually compatible), even among closely related species (45, 96, 122). The number of alleles of the *MAT* locus also varies among species, ranging from two (e.g., the bipolar species *C. neoformans*) to many (e.g., the tetrapolar species *C. cinerea* with thousands of mating types). Based on phylogenetic analyses, the common ancestor of all fungi was most likely bipolar. The tetrapolar mating system, in which the homeodomain (HD) and pheromone/pheromone receptor (P/R) loci are unlinked, and the two gametes must differ at both loci in order to mate, is thus far unique to the Basidiomycota and may have evolved only once. However, approximately 40% of basidiomycetous species are known to have bipolar mating systems and in a few known examples the HD and P/R loci are now linked. How bipolar mating systems evolve from tetrapolar mating systems is an intriguing question. Equally important is whether any extant bipolar species represents a hypothetical ancestral basidiomycete bipolar sexual state.

Raper (120) proposed three hypotheses on the emergence of bipolar mating systems from tetrapolar ancestors in the basidiomycota. In the first model, one of the two original *MAT* loci of the tetrapolar ancestor loses the cell identity function, possibly because of mutations that result in self-compatibility, such that the mating type is now determined only by the other original *MAT* locus. Examples supporting this hypothesis include studies of the mushrooms *C. cinerea* (77, 107, 108), *Pholiota nameko* (1), and *Coprinopsis disseminatus* (70). In *C. disseminatus*, only the HD locus, and not the P/R locus, cosegregates with *MAT* and shows a level of sequence divergence between the *MAT* alleles characteristic of a *MAT* locus. In *C. cinerea*, studies have shown that single amino acid substitutions at the pheromone receptor genes can cause them to lose the cell recognition function or become constitutively

activated, and thus the cells become self-compatible (107, 108). It should be noted that this scenario is not likely to instantaneously establish a complete reproductive barrier between the bipolar and tetrapolar lineages, and subsequent introgression between the two lineages likely still occurs (see discussion below). Because the newly arisen bipolar lineage will be compatible with at least half of the population (compared with the 25% compatibility for each mating type in a biallelic tetrapolar system), it has an advantage in finding a compatible mating partner, and thus has the potential to replace the originally tetrapolar lineage through successive introgression events.

In the second model of Raper (120), the HD and P/R loci of the tetrapolar mating system can become linked. As a result, the two loci now function and are inherited as one single unit, thus giving rise to a bipolar system. One example supporting this hypothesis is the *MAT* locus of *Ustilago hordei*, a fungal pathogen of small-grain cereals (9, 81). *U. hordei* has a bipolar mating system although it is closely related to *Ustilago maydis*, which has a tetrapolar mating system. In *U. hordei*, the two *MAT* loci are located on the same chromosome, and recombination is suppressed across the entire 450–500 kb region between the HD and P/R loci, giving rise to a bipolar mating system. Another example is the human pathogenic fungus *C. neoformans* (see review of its *MAT* locus in 64), in which the HD and P/R loci are physically linked and contained within a large *MAT* locus (>100 kb), giving rise to a bipolar mating system. A recent study discovered an extant sexual cycle in *Cryptococcus heveanensis*, a species that is closely related to *C. neoformans* (96). Further analyses revealed that the *MAT* locus of *C. heveanensis* resembles that of a tetrapolar mating system with an unlinked multiallelic HD locus and at least a biallelic P/R locus, suggesting that the transition from a tetrapolar to a bipolar mating system in *C. neoformans* might have occurred concomitant with the emergence of this pathogenic clade. In addition, current evidence suggests that the physical linkage between the two *MAT* loci, similar to *C. neoformans* and *U. hordei*, likely results from chromosomal translocation or ectopic recombination. The third hypothesis proposed by Raper is that the function of one of the *MAT* loci could be gradually assumed by the other *MAT* locus. Although theoretically possible, there is little empirical evidence to support this last hypothesis.

Fraser et al. (47) proposed that recombination could occur within either the HD or P/R locus, leading to self-compatibility of the recombinant locus, and thus to the transformation of a tetrapolar to a bipolar mating system. This hypothesis is similar to the first hypothesis that was proposed by Raper (see discussion above). Further studies are necessary to test this specific molecular hypothesis.

Because of introgression, the transition from a tetrapolar to a bipolar mating system may not be a sharply demarcated process, and mating systems representing intermediate stages could exist. This is supported by the findings in which a tetrapolar mating system was genetically engineered from the bipolar species *C. neoformans* by relocating the HD genes to a different chromosome from the remaining *MAT* locus (63). Although the modified tetrapolar strains can undergo meiosis and produce viable, fertile spores, when they were crossed with the original bipolar strains, 50% of the progeny were sterile, suggesting that a partial postzygotic reproductive barrier is established between the two systems.

The red yeast *Sporidiobolus salmonicolor* has a bipolar mating system; however, a recent study revealed that its mating system has certain features that resemble the tetrapolar system, namely that the *MAT* locus is multiallelic, and recombination can occur within *MAT* to generate novel *MAT* alleles (28). Additionally, the *MAT* locus of *S. salmonicolor* is large (>800 kb), which is possibly why recombination can occasionally occur within *MAT*. It also possibly represents a stage where the HD and P/R loci have been recently joined together through recombination or chromosomal translocation, or it could represent an extant example of

an ancestral bipolar state of the *MAT* locus. Further chromosomal rearrangements (e.g., inversions and translocations) could reduce the size of the *MAT* locus to further suppress recombination occurring within this pseudobipolar *MAT* locus.

Interestingly, a recent study of the mushroom *Schizophyllum commune* details a mating system that differs from both bipolar and tetrapolar mating systems (106). Specifically, *S. commune* has a mating system similar to a typical tetrapolar mating system, with the *matA* and *matB* loci located on different chromosomes. However, the *matA* locus of *S. commune* is unusually large, and the two *matA* subunits are separated by approximately 550 kb on chromosome I. Given the large distance between the *matA* α and β subloci, it is possible that recombination could occur between them to generate novel mating specificities, which is consistent with results from previous recombination analyses that predicted as many as 32 mating specificities for this locus (120). Therefore, *S. commune* may have taken another step from the tetrapolar mating system to evolve a system that further promotes outcrossing because of the increased likelihood of encountering a mating partner.

SEX AND MITOCHONDRIAL INHERITANCE: UNIPARENTAL VERSUS BIPARENTAL

Among fungal species that have been examined, a majority exhibit uniparental mitochondrial inheritance, i.e., the progeny all possess a mitochondrial genome inherited from only one of the two mating parents, similar to maternal inheritance of mitochondria in mammals. Examples of species in which uniparental mitochondrial inheritance is observed include *A. nidulans*, *N. crassa*, *C. albicans*, *C. cinerea*, *Agaricus bisporus*, *C. neoformans*, and *U. maydis*. In contrast, in some fungal species, including *S. cerevisiae* and *S. pombe*, inheritance of mitochondria is biparental. In these species, mating between isogamous sexual partners results in an equal contribution of organelles from the two gametes into the zygote, and the coexistence of two different mitochondria often results in recombination (13, 39). Even in cases of biparental inheritance, homoplasy is rapidly established after the initial heteroplasmic zygote, such that each daughter cell possesses the mitochondrion of one parental genotype or a recombinant of the two parental genotypes (10, 13).

The predominance of uniparental mitochondrial inheritance suggests that coexistence of two different mitochondria within one cell may be disadvantageous, possibly due to conflicts or competition between mitochondria with different genotypes. Indeed, avoiding potential conflicts between genetically different mitochondria has been proposed as one selection pressure that maintains uniparental inheritance of organelles in many fungal species, as well as in other organisms, including plants and animals. Another possible and not mutually exclusive hypothesis is that uniparental inheritance of mitochondria (as well as other organelles such as chloroplasts) could prevent the spread of selfish or deleterious genetic elements arising within these organelles in the population (54, 60, 61, 68, 79). It should be noted that the selection pressure for uniparental inheritance of organelles could vary among different fungal species because of a variety of biological factors. For example, during inbreeding modes of sexual reproduction, genetic conflicts and competition between mitochondria from the two mating partners would be less likely to occur. Even in these scenarios, uniparental mitochondrial inheritance may still be favored to limit the spread of potentially selfish deleterious mitochondrial mutations during sexual reproduction.

In most fungal species, mating occurs between either two isogametes or between two compatible mycelia. Unlike in plants and animals, where size differences between the gametes (i.e., anisogamy) ensure unequal organellar contributions to the zygote and thus facilitate uniparental organelle inheritance, several mechanisms have evolved in fungi to actively avoid mitochondrial heteroplasy during

sexual reproduction. In filamentous fungi, mating between two compatible mycelia is achieved by the mutual migration of the two nuclei while all of the cytoplasm, including the organelles, is left behind, and the inheritance of two different mitochondria is avoided (59, 92). In unicellular fungi in which mating occurs between two isogametous mating partners, there is evidence indicating that active degradation of organelles from one gamete occurs, thus ensuring homoplasy in the zygote. One example is the human pathogenic fungus *C. neoformans*, where mitochondria are uniparentally inherited from the **a** parent. Further studies provide evidence that the homeodomain genes located in the *MAT* locus, *SXI1*α in *MAT*α and *SXI2***a** in *MAT***a**, are both required to ensure uniparental inheritance of mitochondria during mating (148, 149). Another example in which *MAT* controls mitochondrial inheritance is *U. maydis* (42). *U. maydis* has a tetrapolar mating system constituted by the *a* and *b* loci. The biallelic *a* locus (*a1* and *a2*) is involved in pheromone and pheromone receptor-based cell recognition and fusion, whereas the multiallelic *b* locus encodes the homeodomain transcription factors. In *U. maydis*, mitochondrial inheritance is governed by the *a2*-specific genes *lga2* and *rga2* (42). Although evidence supports an active *lga2*- and *rga2*-mediated selective elimination process, the finding that deletion of *rga2* reverses the inheritance in favor of the *a1*-type mitochondria (rather than resulting in a biparental pattern) indicates that an *rga2*-independent mechanism may also be involved in the control of mitochondrial inheritance (42).

It should be noted that in heterokaryons of dimorphic ascomycetes and basidiomycetes, biparental inheritance of the mitochondrion, as well as mitochondrial recombination, frequently occurs (33, 135, 141). Additionally, even in species where the mitochondrion is typically uniparentally inherited, mitochondrial leakage (i.e., inheritance of the mitochondrion from the parent whose mitochondrion is typically excluded) and mitochondrial recombination can still occur, although at a lower level (4, 125, 147–149).

GENOME DEFENSE MECHANISMS DURING SEX

Mobile genetic elements populate the genomes of virtually all eukaryotic organisms. Although transposable elements can confer beneficial effects for their hosts, unchecked transposon activity can be detrimental by challenging genetic integrity. Many organisms have developed control mechanisms that effectively limit the activity of these selfish DNA elements and thus establish a more peaceful symbiotic relationship between transposons and their hosts. Transposon control is especially critical during sexual reproduction, a time at which transposons could be more threatening because meiosis may trigger new transposon exchange between genomes or lead to heterologous chromosome alignment and recombination or translocation due to the presence of ectopic copies of preexisting transposons (17, 71). Therefore, it is not surprising that many organisms have evolved specific sex-related silencing mechanisms that are activated during the sexual cycle to suppress transposable elements.

Early, strong evidence for active sex-related silencing emerged from studies of the filamentous fungus *N. crassa*. This organism has developed a number of complex genome defenses operating at different stages of its life cycle, including DNA methylation, quelling, repeat-induced point mutation (RIP), and meiotic silencing of unpaired DNA (MSUD) (29, 48, 50, 71, 127). Among these, quelling is an RNAi-dependent posttranscriptional gene-silencing pathway that is active in the vegetative phase of the life cycle, whereas RIP and MSUD function during the sexual cycle but via different molecular mechanisms. RIP acts premeiotically and efficiently detects duplicated sequences present in the haploid genomes destined to participate in meiosis and inactivates these sequences by extensive C to T (G to A) base mutations (128). Up to 30% of the GC base pairs in duplicated sequences can be altered to AT pairs via RIP after a single sexual cycle (21). Therefore, RIP serves as a powerful genome defense mechanism against

Transposons: sequences of DNA that can move or transpose themselves to new positions within the genome

Heterologous chromosomes: chromosomes that do not belong to the same pair; e.g., Chr6 and Chr8 form a heterologous pair

RIP: repeat-induced point mutation

MSUD: meiotic silencing of unpaired DNA

RNAi: RNA interference

repetitive transposable elements, as evidenced by the presence of numerous nonfunctional transposon relics in the *N. crassa* genome (48).

MSUD is another genome defense mechanism occurring during meiosis that operates as a failsafe mechanism against any transposons that escape RIP. In contrast to RIP, MSUD detects and silences ectopic sequences and newly transposed elements that are present as only a single copy. This process is mediated by *trans*-sensing that identifies the unsuccessful pairing of discrete DNA regions during homolog pairing (132). The failure to sense an equivalent region in the opposite chromosome triggers an RNAi-mediated silencing mechanism that posttranscriptionally silences all genes contained in the loop of unpaired DNA (71, 132). Given these rigorous genome defense mechanisms, it is not surprising that duplicated elements and active transposons have been largely eradicated in the *N. crassa* genome (48). As a result, *N. crassa* serves as an exemplary model system to study sex-related silencing pathways.

Transposon families are typically heterogeneous. RNAi, an evolutionarily conserved mechanism in which gene silencing is orchestrated by small RNAs (~20 to ~30 nt) in a sequence-specific manner (53), could recognize transposons and lead to transcriptional or posttranscriptional silencing based on even imperfect sequence homology. Indeed, small RNA pathways play a prominent role in transposon control in many eukaryotic organisms (91). A surprisingly large number of small RNAs mapping to specific transposable elements have been identified in plants, insects, nematodes, and most recently in the budding yeast *Saccharomyces castellii* through the application of next-generation sequencing technologies (6, 38, 86). These processes frequently share obvious mechanistic overlap involving RNAi pathways and are considered to function in parallel with or to have evolved as specialized adaptions of RNAi pathways. For example, MSUD in *N. crassa* is induced by siRNAs and requires core RNAi components, including Argonaute, Dicer-like proteins, and an RNA-dependent RNA polymerase (80, 132). In addition, a similar requirement for factors linked to RNAi during meiotic silencing has also been demonstrated in ciliates, *Caenorhabditis elegans*, and mammals (5, 24, 134).

Recently, a novel sex-induced RNAi genome defense system has been reported in the human fungal pathogen *C. neoformans* (143). A transgene-induced RNAi-dependent gene silencing process occurs at ~250-fold higher frequency during the sexual cycle than in vegetative mitotic growth, and hence this phenomenon was named sex-induced silencing (SIS). Abundant small RNAs were mapped to repetitive transposable elements, and a group of retrotransposons was found to be highly expressed during the mating of RNAi mutant strains and an increased transposition/mutation rate was detected in their progeny, indicating that the RNAi-mediated SIS pathway squelches transposon activity during the sexual cycle. Most interestingly, RNAi machinery components were more abundant during mating, supporting a model in which increased expression of RNAi machinery may function to silence potentially overexpressed transposons during mating (143). Although the mechanistic details of the initiation of SIS are not yet understood, this discovery brings a fresh perspective to meiotic silencing involving the upregulation of RNAi pathways as a strategy to guard genomic integrity during the sexual cycle. Similar mechanisms may be conserved and operate in other fungal species, especially in those that contain the RNAi component machinery yet lack known meiotic silencing pathways (71), such as the fission yeast *S. pombe*. In fungi such as *U. maydis* and *S. cerevisiae* in which the RNAi machinery has been lost, novel RNAi-independent silencing pathways may remain to be discovered, possibly involving long dsRNA mechanisms.

SEX AND ANEUPLOIDY

Aneuploidy refers to changes in chromosome copy number that do not correspond to increases or decreases of the entire haploid or diploid genome suite of chromosomes. In

SIS: sex-induced silencing

Retrotransposons: transposons that have RNA intermediates

Transposition/mutation rate: the frequency at which transposons move or jump to new positions within the genome, which can result in mutations

Aneuploidy: changes in chromosome copy number that do not correspond to increases or decreases of the entire haploid or diploid genome

humans, aneuploidy can frequently occur during meiosis in both oogenesis and spermatogenesis (67) and also in the genesis of cancer cells (83). Aneuploidy is also generated during sexual reproduction in several fungal species. For example, *C. albicans* mates but has no recognized meiotic process (reviewed in 82); instead, *C. albicans* undergoes a parasexual cycle to reduce the genome from 4n to 2n, or close to 2n (2n+1, 2n+2, 2n+3) (46). During this process, Spo11-dependent recombination occurs between homologous chromosomes. However, many of the progeny are trisomic for one or more chromosomes, conferring phenotypic and genotypic plasticity (46). In contrast, *Candida lusitaniae* undergoes meiosis, but one-third of the progeny generated are aneuploid (n+1) or diploid (2n) (121). Aneuploidy is generated during both same- and opposite-sex mating in *C. neoformans* (M. Ni, M. Feretzaki, W. Li, Y-L. Chen, A. Floyd, unpublished results). During same-sex mating, approximately seven percent (6 of 90) of the progeny exhibit phenotypic changes such as temperature-sensitive growth, drug sensitivity or resistance, or enhanced melanin production. Comparative genomic hybridization analyses revealed that many of these variant progeny are aneuploid and carry an extra chromosome. Aneuploidy appears to be responsible for the observed phenotypic variance, as the return to euploidy following chromosome loss restores wild-type phenotypes (M. Ni, M. Feretzaki, W. Li, Y-L. Chen, A. Floyd, unpublished results). In the haploid plant pathogen *Mycosphaerella graminicola*, approximately 15% to 20% of sexual progeny lack one or more chromosomes that were present in the parents (145). The generation of aneuploidy during sexual reproduction may be a common feature in fungi, and genotypic analysis of progeny using advanced genomic techniques in more species is necessary to explore this hypothesis.

Why do fungi generate aneuploidy at a high rate during meiosis? Several studies have suggested that aneuploidy may provide phenotypic and genotypic plasticity for natural selection during evolution. Rancanti et al. (119) found that aneuploidy facilitated rapid adaptive evolution of yeast cells lacking a conserved cytokinesis motor protein. In addition, aneuploidy has been found to evoke transcriptomic and proteomic changes that orchestrate phenotypic diversity in *S. cerevisiae* (109, 139). A single nucleotide mutation in a deubiquitinating enzyme of *S. cerevisiae*, which arose during the evolution of an aneuploid isolate, leads to improved proliferation of some, but not all, aneuploid strains (138). A potential explanation for this is that the mutation leads to the activation of the proteosome and the degradation of protein subunits present in unbalanced ratios, thereby contributing to the restoration of normal growth. Aneuploid isolates of *C. albicans* and *C. neoformans* can be more fit than their euploid counterparts in stressful conditions, such as when exposed to antifungal drugs (129, 133). Therefore, aneuploidy generated from sexual development may yield a diverse genetic pool upon which natural selection acts. Compared with spontaneous mutations, aneuploidy may be beneficial because its effects can be rapidly reversed through the loss or gain of entire chromosomes, thereby returning to the euploid state when the environment is again favorable.

ANALOGIES BEYOND FUNGI

Why do many organisms maintain sexual reproduction? The advantages of sex have been among the most debated questions in biology. The advantage of asexual development is to propagate rapidly while expending less energy, whereas the advantage of sexual development is to generate genetic diversity to accelerate adaptation to novel and changing environments.

There are many obligately sexual species, including mammals, plants, and possibly even fungi such as *S. macrospora*, *P. anserina*, *N. tetrasperma* (as discussed above), and *Filobasidiella depauperata* (122). Certain fungi and plants are able to reproduce both asexually and sexually, and choose one or the other strategy depending on the environment. For instance, the filamentous fungus *A. nidulans* undergoes asexual development in favorable conditions but favors sexual development in harsh conditions,

e.g., low levels of moisture, oxygen, or light (99).

Are there any obligately asexual species? Many fungal species, such as *A. fumigatus*, *A. oryzae*, and *A. niger*, had long been thought to be obligately asexual organisms because a sexual cycle had not been observed. However, recent genomic studies have revealed that all contain the *MAT* locus and the entire suite of genes needed for sexual development (49, 88, 102, 110, 113). Furthermore, sexual development of these fungi has been shown to occur under unusual lab conditions, e.g., extremely long (~six months) incubation (62, 105). The most common pathogenic fungus, *C. albicans*, had been thought to be completely asexual for more than a century; yet whole genome sequencing revealed that it possesses a *MAT* locus homologous to those in *S. cerevisiae* (65). Further lab experiments discovered that *C. albicans* could undergo a parasexual cycle, during which Spo11-dependent recombination can occur (12, 46, 66, 89). In addition to heterothallic mating, same-sex mating has been reported in both *C. neoformans* and *C. albicans* (2, 3, 85). Interestingly, aneuploidy is generated at a high rate during same-sex mating in *C. neoformans*, providing additional genetic diversity for this α mating type–dominant (>99% in the natural population) species (M. Ni, M. Feretzaki, W. Li, Y-L. Chen, A. Floyd, unpublished results). For a more comprehensive treatise of cryptic sex in fungi, please refer to the review by Kück & Pöggeler (75). An interesting question remains as to whether any obligately asexual fungi exist that are entirely lacking the *MAT* locus or mating pathway genes, or key meiotic genes, and if so, how and why were they lost? In an other view, certain unknown loci may be responsible for sexual development. One possible example is that *Lodderomyces elongisporus* lacks a *MAT*-like locus (*MTL)* and an **a**-factor pheromone, receptor, and transporter but is thought to have an extant homothallic sexual cycle based on the production of asci harboring single spores (monads) (20). Further study is necessary to establish whether this is a true sexual cycle or an asexual mode of sporulation.

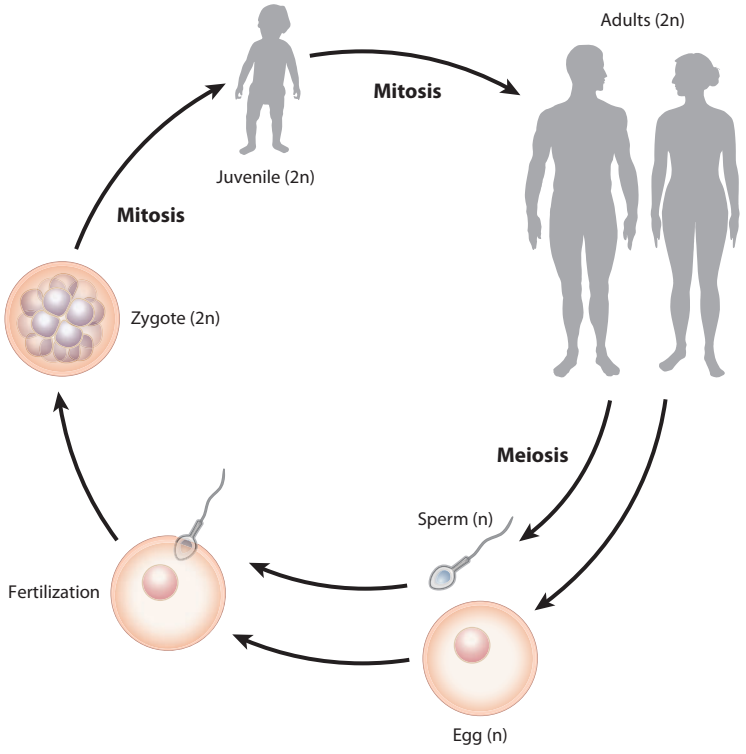
CONCLUSION AND FUTURE DIRECTIONS

Sex in fungi is still mysterious in many aspects and open questions remain. Did the ancestral mating process involve filamentous fungi or yeast? What are the driving forces for the evolution of *MAT* loci, and why are the molecular components so plastic throughout the fungal kingdom? This may require a better understanding of the ecology and population genetics of different fungi species. Is the ancestral sexual state homothallic or heterothallic, or possibly both? Are there mating systems that differ from both bipolar and tetrapolar mating systems that exist, and if so how common are they? Are there any obligately asexual fungal species? Further study of sex in fungi provides fertile ground to explore and solve these and other mysteries and thereby advance our understanding of the evolution of sex in both unicellular and multicellular eukaryotes (**Figure 4**).

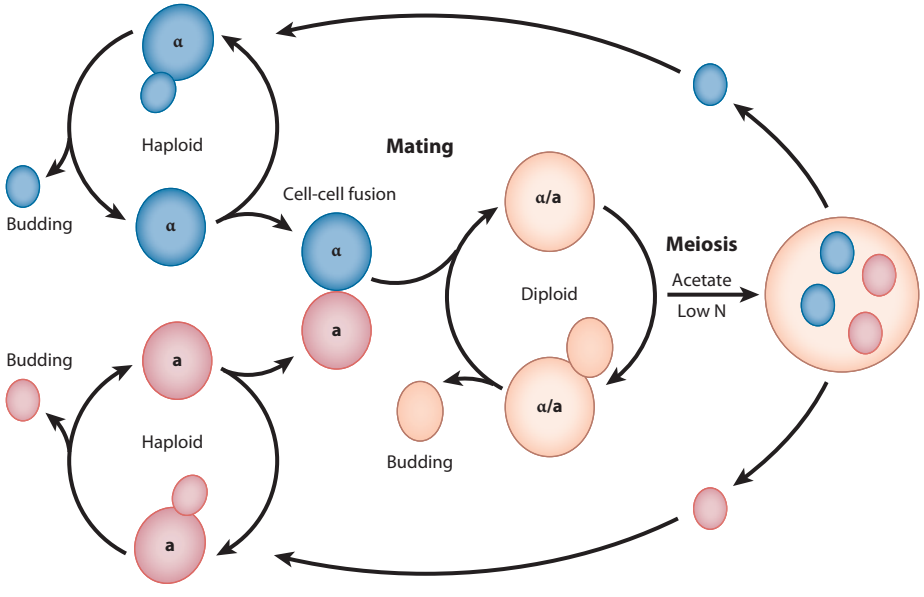
Figure 4

Modes of sexual reproduction. Sex typically involves two genetically divergent partners of opposite sex or mating type. (*a*) Obligate sexual reproduction, e.g., human sexual cycle. Specialized cells (2n) in adult gonads undergo meiosis to form haploid (n) gametes (sperm or egg). Haploid gametes fuse to form the diploid zygote (2n), which undergoes repeated mitosis, differentiation, and growth to become multicellular organisms (juvenile, 2n). The mature organism is diploid; gametes are the only haploid cells. (*b*) Facultative sexual reproduction, e.g., yeast life cycle. *Saccharomyces cerevisiae* can grow as haploid yeasts by asexual budding. Mating occurs between strains of *MAT***a** and *MAT*α to form diploid cells, which can undergo meiosis to generate haploid spores. The mature organism can be diploid or haploid; gametes are haploid cells. Many fungi maintain haploid life cycles, and only become diploid following fertilization.

a **Obligate sexual reproduction**



b **Facultative sexual reproduction**



SUMMARY POINTS

1. Sex is initiated by secretion of peptide pheromones or chemical compounds and following mate recognition cells and nuclei fuse, and meiosis and sporulation occur.
2. Mating-type switching evolved independently in *S. cerevisiae* and *S. pombe*. *K. lactis* mating-type switching mechanisms diverged from *S. cerevisiae* by disposing of the Ho endonuclease and acquiring the Mts1 transposase.
3. Homothallism and heterothallism are present in all phyla of the fungal kingdom, and transitions from one pattern to the other are common, and can even coexist in the same species.
4. In basidiomycetes, both bipolar and tetrapolar mating systems exist. In addition, recent studies suggest the existence of mating systems that represent possible transitions between the two.
5. In fungi, both biparental and uniparental mitochondrial inheritance occur. Current evidence suggests the underlying mechanisms of uniparental mitochondrial inheritance differ in different species.
6. Sex-related silencing mechanisms function during fungal sexual development, suppressing transposon activity and defending genome integrity.
7. Aneuploidy generated during mating enables genotypic plasticity and rapid adaption.

FUTURE ISSUES

1. Does the ancestral mating process involve filamentous fungi or yeast?
2. Is the ancestral sexual state homothallic or heterothallic?
3. What is the nuclease that induces mating-type switching in *S. pombe*?
4. Are there mating systems that differ from both bipolar and tetrapolar mating systems?
5. What are the driving forces for the evolution of *MAT* and the genes resident therein?
6. What are the mechanisms underlying uniparental mitochondrial inheritance?
7. How is sex-induced RNAi-dependent silencing initiated?
8. Are there any obligately asexual fungal species?

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Annual Review of Genetics

Volume 45, 2011

Contents

Comparative Genetics and Genomics of Nematodes: Genome Structure, Development, and Lifestyle
Ralf J. Sommer and Adrian Streit 1

Uncovering the Mystery of Gliding Motility in the Myxobacteria
Beiyan Nan and David R. Zusman 21

Genetics and Control of Tomato Fruit Ripening and Quality Attributes
Harry J. Klee and James J. Giovannoni 41

Toxin-Antitoxin Systems in Bacteria and Archaea
Yoshihiro Yamaguchi, Jung-Ho Park, and Masayori Inouye 61

Genetic and Epigenetic Networks in Intellectual Disabilities
Hans van Bokhoven 81

Axis Formation in Hydra
Hans Bode 105

The Rules of Engagement in the Legume-Rhizobial Symbiosis
Giles E.D. Oldroyd, Jeremy D. Murray, Philip S. Poole, and J. Allan Downie 119

A Genetic Approach to the Transcriptional Regulation of *Hox* Gene Clusters
Patrick Tschopp and Denis Duboule 145

V(D)J Recombination: Mechanisms of Initiation
David G. Schatz and Patrick C. Swanson 167

Human Copy Number Variation and Complex Genetic Disease
Santhosh Girirajan, Catarina D. Campbell, and Evan E. Eichler 203

DNA Elimination in Ciliates: Transposon Domestication and Genome Surveillance
Douglas L. Chalker and Meng-Chao Yao 227

Double-Strand Break End Resection and Repair Pathway Choice
Lorraine S. Symington and Jean Gautier 247

CRISPR-Cas Systems in Bacteria and Archaea: Versatile Small RNAs for Adaptive Defense and Regulation
Devaki Bhaya, Michelle Davison, and Rodolphe Barrangou 273

Human Mitochondrial tRNAs: Biogenesis, Function, Structural Aspects, and Diseases
Tsutomu Suzuki, Asuteka Nagao, and Takeo Suzuki 299

The Genetics of Hybrid Incompatibilities
Shamoni Maheshwari and Daniel A. Barbash 331

Maternal and Zygotic Control of Zebrafish Dorsoventral Axial Patterning
Yvette G. Langdon and Mary C. Mullins 357

Genomic Imprinting: A Mammalian Epigenetic Discovery Model
Denise P. Barlow 379

Sex in Fungi
Min Ni, Marianna Feretzaki, Sheng Sun, Xuying Wang, and Joseph Heitman 405

Genomic Analysis at the Single-Cell Level
Tomer Kalisky, Paul Blainey, and Stephen R. Quake 431

Uniting Germline and Stem Cells: The Function of Piwi Proteins and the piRNA Pathway in Diverse Organisms
Celina Juliano, Jianquan Wang, and Haifan Lin 447

Errata

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