Mycologia, 105(1), 2013, pp. 1–27. DOI: 10.3852/12-253

Issued 8 January 2013

Evolution of fungal sexual reproduction

Joseph Heitman
Sheng Sun

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina 27710

Timothy Y. James

Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan 48109

Abstract: We review here recent advances in our understanding of the genetic, molecular and genomic basis of sex determination and sexual reproduction in the fungal kingdom as a window on the evolution of sex in eukaryotes more generally. In particular, we focus on the evolution of the mating-type locus and transitions in modes of sexual reproduction using examples from throughout the kingdom. These examples illustrate general principles of the origins of mating-type loci/sex chromosomes and the balance between inbreeding and outcrossing afforded by different modes of sexual reproduction involving tetrapolar, bipolar and unipolar sexual cycles.

Key words: evolution, fungi, mating type, meiosis, recombination, sex, sex determination

Introduction to sexual reproduction and the fungal kingdom

The tree of life is split into two broadly successful lineages: the prokaryotes (bacteria, archaea) and the eukaryotes. Recent molecular phylogenetic studies reveal that the eukaryotic tree of life can be divided into five to eight super groups that all descend from a central last eukaryotic common ancestor (LECA) (Wainright et al. 1993, Baldauf and Palmer 1993, Baldauf 2003, Simpson and Roger 2004). We are particularly interested in one of the eukaryotic lineages, the opisthokonts, because it is the lineage containing both the metazoan (animal) and fungal kingdoms. And because the animal and fungal kingdoms last shared a common ancestor as recently as one billion years ago, much more recently than any of the other shared ancestral nodes among other major eukaryotic super groups, fungi are exemplary model systems for the often more complex biology exhibited by their multicellular metazoan compatriots.

Submitted 12 Aug 2012; accepted for publication 18 Aug 2012.
[1] Corresponding author. E-mail: sheng.sun@duke.edu

We do not know precisely what the last common eukaryotic ancestor looked like, but a reasonable hypothesis is that it was a unicellular, aquatic, motile organism with one, or perhaps two, posterior flagella. There are extant opisthokonts that maintain some resemblance to this mythic creature, such as the premetazoan choanoflagellates and the basal fungal Chytridiomycota (King et al. 2008, Stajich et al. 2009). Key features that distinguish eukaryotic from prokaryotic organisms are the presence of the nucleus and other intracellular organelles (mitochondria, chloroplasts, Golgi, endoplasmic reticulum), the emergence of multicellularity and the ability to undergo true sexual reproduction. Given that sexual reproduction is pervasive and extant in all of the major super groups of the eukaryotic tree of life, it is hypothesized the sex emerged once in an ancestral eukaryote and has been preserved and conserved throughout the eukaryotic tree of life. Thus, one can think of the ability to undergo sexual reproduction as a synapomorphy for the eukaryotes. Given the ubiquity of sex, it is remarkable both how conserved the core features are and yet how plastic other aspects appear to be, including how sex is determined and how sexual reproduction is accomplished.

We can illustrate the conserved features of sexual reproduction by comparing the sexual cycles for two of our favorite systems: ourselves (*Homo sapiens*) and the model yeast *Saccharomyces cerevisiae* (Fig. 1). Despite having diverged ~ one billion years ago, the core features of sexual reproduction are conserved. These involve: (i) ploidy changes from diploid to haploid to diploid states, (ii) the production of haploid mating partners or gametes from the diploid state via meiosis which recombines the two parental genomes to produce novel genotypes and halves the ploidy and (iii) cell-cell recognition between the mating partners or gametes followed by cell-cell fusion to generate the diploid zygote and complete the cycle (Fig. 1). Now we typically think of sexual reproduction as involving two genetically divergent parents, to give rise to a diverse repertoire of progeny in which the genetic diversity of the parents has been admixed. And yet we also are aware that inbreeding and selfing forms of sexual reproduction occur, and these involve consanguineous marriages in humans and examples such as mating-type switching in fungi in which a mother cell can switch mating type and mate with a daughter cell to homozygose the entire genome (except the mating-type locus) in what represents an extreme form of inbreeding. We will return to the topic of inbreeding/selfing modes of

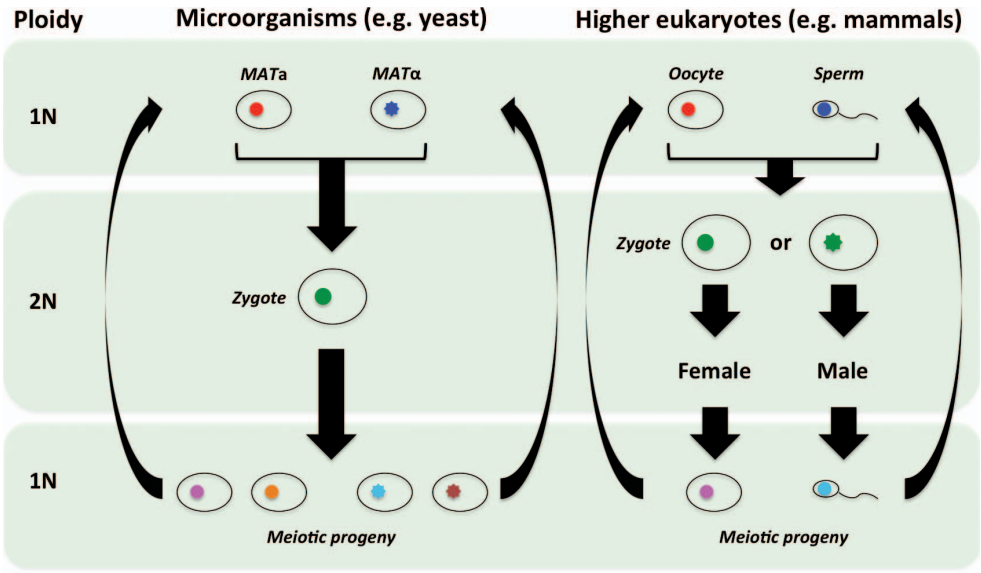

FIG. 1. Common sexual cycles in unicellular and multicellular eukaryotes. The sexual cycles are representative of simpler and more complex eukaryotes, using yeast (left) and human (right) as examples. For lower eukaryotes, the haploid vegetative cells also serve as gametes. Cells of different mating types can fuse to form the diploid zygote. Within the zygote, meiosis occurs and haploid progeny are produced. The haploid progeny then can reproduce either asexually through mitosis or sexually by repeating the sexual life cycle. For sexual higher eukaryotes like mammals, haploid gametes (e.g. sperm and oocyte) fuse to form a diploid zygote. Depending on the zygote's composition of the sex chromosomes, it can develop into either male or female. The male and female individuals then produce gametes of different genetic compositions (at both autosomes and sex chromosomes) through meiosis. These gametes then must fuse to complete the sexual life cycle for reproduction to occur.

sexual reproduction, and their implications, later in this review.

Given the ubiquity of sexual reproduction, combined with the fact that the few known truly asexual lineages appear to be of relatively recent origin and therefore may be doomed to more rapid extinction, it is expected that sex must confer benefits (TABLE I, FIG. 2). For more than a century the basic tenets for the advantages of sexual reproduction have been that it can serve to (i) generate progeny with a diversity of novel genotypes and (ii) purge the genome of deleterious mutations, such as transposable elements, which otherwise would accumulate inexorably via Muller's Ratchet to degrade the integrity of the genome. These are not mutually exclusive, and sex may confer benefits via both mechanisms. Studies in the model yeast *S. cerevisiae* by Goddard et al. (2005) provide a direct experimental test for the potential benefits of sex. These investigators engineered an isogenic pair of yeast strains, one a wild type diploid and the other a mutant for two key genes required for meiotic recombination (*SPO11, SPO13*) such that the mutant strain cannot undergo meiotic recombination but still is able to produce spores. When they grew the two strains under a variety of different stressful conditions they found that the sexual strain always had the competitive edge compared to the asexual strain, providing a direct experimental test for the benefits of sex in a fungal model system.

More recently, an additional hypothesis has been advanced that sex might enable organisms to escape or outrun pathogens. This is termed the Red Queen hypothesis, which is an allusion to Lewis Carroll's novel Through the Looking Glass in which the character the Red Queen must run as fast as she can just to stay in the same place, and in the evolutionary analogy sexual reproduction enables organisms to just keep ahead in the co-evolutionary race with the pathogens that afflict them. This model has been tested recently in two real world biological scenarios. In one, Curt Lively and colleagues from Indiana University studied freshwater snails that live in lakes in New Zealand and discovered that in lakes in which parasites are present the snails are driven to be sexual, but in lakes in which parasites are absent they rapidly evolve to be asexual and triploid (Jokela et al. 2009). This example provides a real world test and verification of the Red Queen hypothesis of sexual reproduction. This theme recently has been extended with a second independent validation of the hypothesis involving studies of the role of sexual reproduction in pathogen evasion by the model nematode *Caenorhabditis elegans* (Morran et al. 2011). Specifically, different lines of *Cae. elegans* that

TABLE I. Comparison between sexual and asexual reproduction

	Sexual reproduction[a]	Asexual reproduction[a]
Energy cost	High a	Low b
Chance of genetic/organelle conflicts	High	Low
Recombination load[b] (i.e. breaking down of co-adapted gene combinations)	High	Low
Genetic diversity	Stable or increase c	Stable or decrease d
Adaptation to changing/fluctuating environment	Fast e	Slow f
Purging deleterious mutations[b]	More efficient	Less efficient
Selection of beneficial mutations	More efficient	Less efficient

[a] a: Require energy for finding and interacting with mating partner; meiosis is more energy consuming than mitosis. b: No energy needed to find and interact with mating partner; mitosis is more energy efficient. c: Stable if the population is in linkage equilibrium; increase if linkage disequilibrium is present in the population. In addition, meiosis may be mutagenic, which can introduce substitutions and ploidy changes at a higher rate than mitosis. d: Stable if there is no selection; decrease if there is clonal expansion. e: In some microorganisms such as fungi, sexual reproduction can also produce spores that can better resist harsh environments, as well as be better dispersed. f: The adaption of asexual population also can be fast, if a favorable genotype emerges and takes over the population by selective sweep.

[b] See FIG. 3.

differed in their patterns of sexuality were tested for their ability to withstand infection by the bacterial pathogen *Serratia marcescens*. Of interest, those lines that were self-fertilizing rapidly became extinct, whereas those that were capable of sexual reproduction survived, thus providing a direct experimental validation of central tenets of the Red Queen hypothesis.

Now sexual reproduction not only confers benefits but also comes with costs (TABLE I, FIG. 1). This includes the well known so-called twofold cost of sex. In most sexual cycles it takes two parents to produce one offspring, resulting in only 50% of any given parent's genes being transmitted to a progeny. This is contrasted with asexual mitotic reproduction in which one parent can produce one progeny, and in which 100% of the parental genes are transmitted to the progeny, thus resulting in a twofold cost of sex vs. mitotic asexual production of progeny. Sexual reproduction also requires an investment of time and energy. Moreover, for some parents or mating partners it takes time to find a partner, and for some of us, this requires more time than others. Finally, one of the lesser appreciated but well established costs of sexual reproduction is that it breaks apart well adapted genomic configurations that have run the gauntlet of adaptive Darwinian selection. This latter cost of sexual reproduction leads to a conundrum for facultatively sexual organisms: Why engage in sex if very few of your progeny might even attain the well adapted genotype of either parent? One way to obviate this cost is to have very many progeny, such that at least one approximates the fitness of the most fit parent. But under diverse or rapidly fluctuating environmental conditions, recombinant progeny may be more fit than either parent, which were optimized for a different environment. We later will return to this theme when we discuss the discovery of novel forms of selfing/inbreeding that involve unipolar, unisexual modes of sexual reproduction.

To introduce and frame our discussions it might help to provide the interested reader with a roadmap for our narration. Much of our discussion will focus on a particular species or group of closely aligned species in the Basidiomycota with the peculiar predilection to cause infections in humans that are a considerable cause of morbidity and mortality. These are *Cryptococcus neoformans* and *C. gattii*, and their associated varieties and molecular types (Idnurm et al. 2005, Heitman et al. 2011). We currently recognize two extant species, but there are likely as many as six to eight taxa in this pathogenic species complex (FIG. 3). Now much of the emphasis on their study has been driven by the field of medical mycology involving studies of their interactions with the host. And yet the groundbreaking work of June Kwon-Chung at the NIH in the 1970s applying classical mycological approaches led to the appreciation of their sexual cycle, demonstration of a classic basidiomycete life cycle and recognition of the teleomorph, *Filobasidiella* (Kwon-Chung 1975, 1976a, b). Our efforts similarly began with a molecular and genetic medical mycology perspective, but we increasingly found ourselves drawn to the mycological. In the course of our investigations, these species emerged as exemplary models for understanding key and general concepts on the evolution of sex in fungi and its importance to the epidemiological structure of the species. These include the transitions from tetrapolar to bipolar mating-type

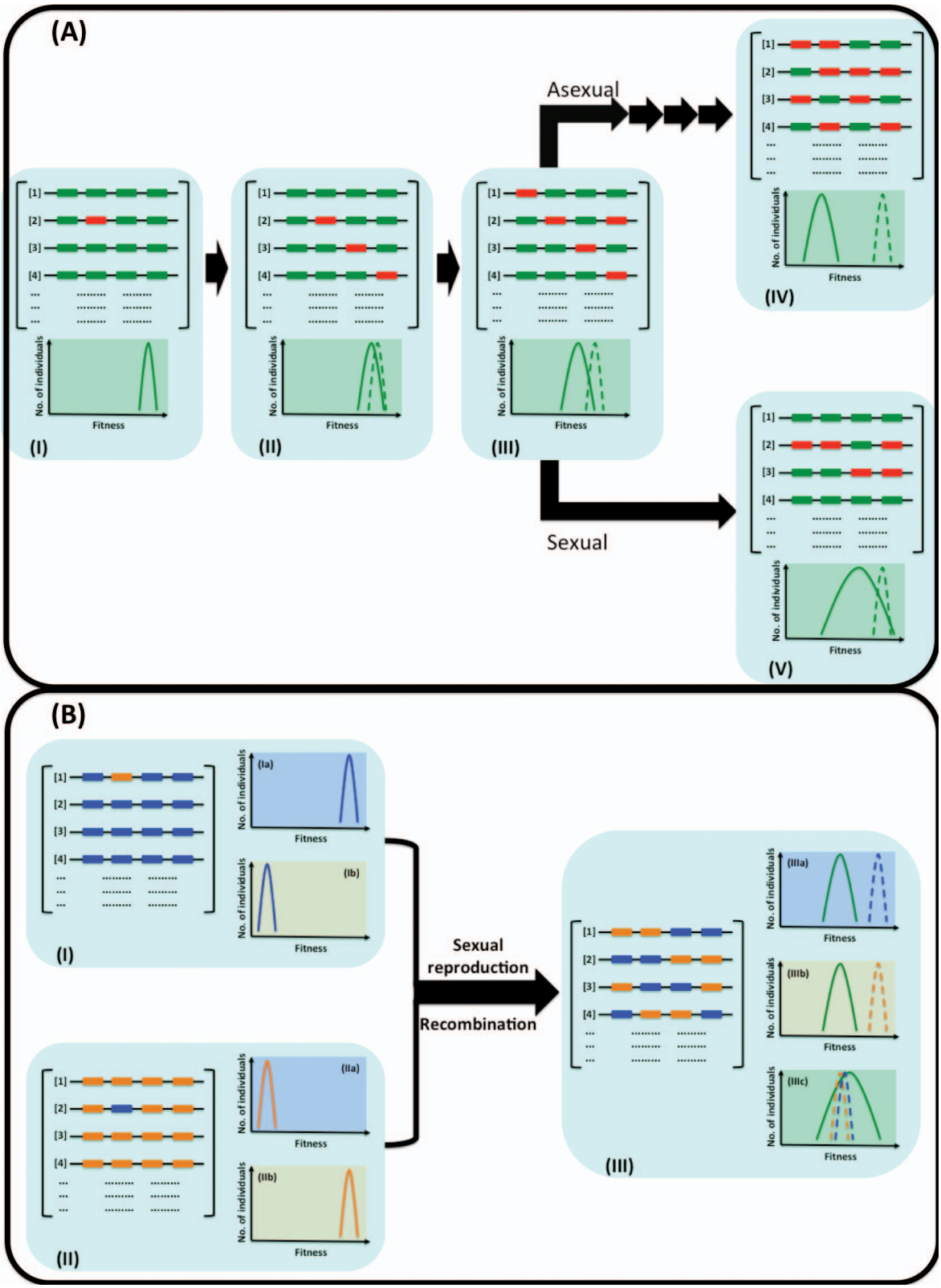

FIG. 2. Benefits and costs of sexual reproduction. A. One of the proposed benefits of meiotic recombination during sexual reproduction—the purging of deleterious mutations. Each line with four blocks represents an individual. For simplicity, only four individuals from the population (represented by the brackets) are shown. The green blocks indicate wildtype genes, and the red blocks indicate genes with deleterious mutations that have gradually accumulated within the population. (i) The

systems, how gene clusters evolve and further transitions to unipolar, unisexual modes of sexual reproduction. In the process, the findings touch on essentially all those that are interesting in the evolution of sex: inbreeding (same-sex mating), sex chromosome evolution and transitions between mating systems (reviewed in Lee et al. 2010, Ni et al. 2011). With this as our backdrop, let us begin our discussions.

EVOLUTION OF THE MATING-TYPE LOCUS

We now turn our focus to how sex or mating type is determined in eukaryotes. We know a great deal about how sex is determined in animals such as humans by the X and Y sex chromosomes. Unlike the autosomes, the sex chromosomes are very different in size, the X spans some 200 Mb and the Y chromosome ~ 50 Mb; women have two X chromosomes and men one X and one Y. Among the dramatic differences between these two ''homologous'' chromosomes is a key sex-determining gene, *SRY*, which encodes a high mobility group transcription factor determinant (HMG), which is resident on the Y chromosome and establishes the male fate. Transpositions of the *SRY* gene from the Y to the X chromosome lead to sex reversal phenotypes in both mice and humans, and thus this key gene is sufficient to orchestrate sexual identity (Koopman et al. 1991, Schiebel et al. 1997, Sharp et al. 2005). Because the X and Y chromosomes differ dramatically in size and structure, they are referred to as heteromorphic sex chromosomes.

Although we are all well familiar with this paradigmatic example of sex determination, this process is remarkably plastic throughout the eukaryotes. To provide a sense for this plasticity in other species, including the fish medeka and the plant papaya, the sex-specific region of their sex chromosomes is much more restricted such that the sex chromosomes do not differ appreciably in size from one another, and these are therefore referred to as homomorphic sex chromosomes to reflect that they are not size distinguished (Fraser and Heitman 2005). In contrast to these examples of chromosomal sex determination (CSD) there are other species in which the temperature at which an egg is hatched determines sex (TSD), including crocodiles and turtles, and thus is entirely environmentally rather than genetically determined.

←

population starts with most individuals having wildtype genes. The fitness distribution of the population is approximated by the curve underneath the population (the green background indicates a constant environment favoring green alleles). (ii) Gradually spontaneous substitutions accumulate within the population. Because most of these substitutions tend to be deleterious, the fitness distribution of the current population (solid curve) shifts toward the left (i.e. lower fitness) compared to the original population (dashed curve). (iii) Without recombination and sexual reproduction, the accumulation of deleterious mutations, and the concomitant shift of the population fitness distribution toward the left will continue. The individual without any deleterious mutations (individual [1] in [ii]) can be lost from the population either by further mutation or genetic drift. In additionally, because different mutations occurred in different genes in different individuals, they cannot be purged from the population efficiently by natural selection. (iv) The ultimate fate of a non-recombining population is decline. Eventually the population will be dominated by individuals with multiple deleterious mutations that are collectively maladapted to the original environment. This is called Muller's ratchet effect (i.e. the highest fitness within the population is determined by those with the least deleterious mutations). (v) On the other hand, recombination and sexual reproduction can restore the ''perfect individual'' that does not harbor deleterious mutations. In addition, deleterious mutations occurring in different individuals in different genes can be brought together, thus increasing the fitness variance and increasing the efficiency of natural selection. B. Illustrates one of the possible costs of meiotic recombination during sexual reproduction—disruption of favorable allele combinations. Imagine there are two populations, of which most of the individuals possess blue (I) and orange (II) alleles respectively. This scenario can arise through processes such as temporary geographic isolation as well as in microorganisms by clonal expansion. Now populations (I) and (II) are well adapted to their respective environments (indicated by the high fitness distribution for populations I and II in the blue, curve Ia, and orange, curve IIb, environments respectively), while maladapted in each other's environments (shown by the low fitness distribution of the blue population in the orange environment (curve Ib), and vice versa (curve IIa). If individuals from these two populations engage in sexual reproduction, meiotic recombination will generate progeny that possess mixtures of blue and orange alleles (III). These progeny will have an overall lower fitness compared to the parental populations in the two original populations (curves IIIa, IIIb). Thus, sexual reproduction will disrupt allele combinations that are favored in certain environments. However, on the other hand, if the environment changes, the progeny population generated by meiosis could have a broader fitness distribution than the two parental populations. In addition, some meiotic progeny will have much higher fitness compared to the two parental populations (i.e. transgressive segregation), such as shown in curve IIIc. In curves IIIa–c, the dashed lines indicated the fitness distributions of the parental populations.

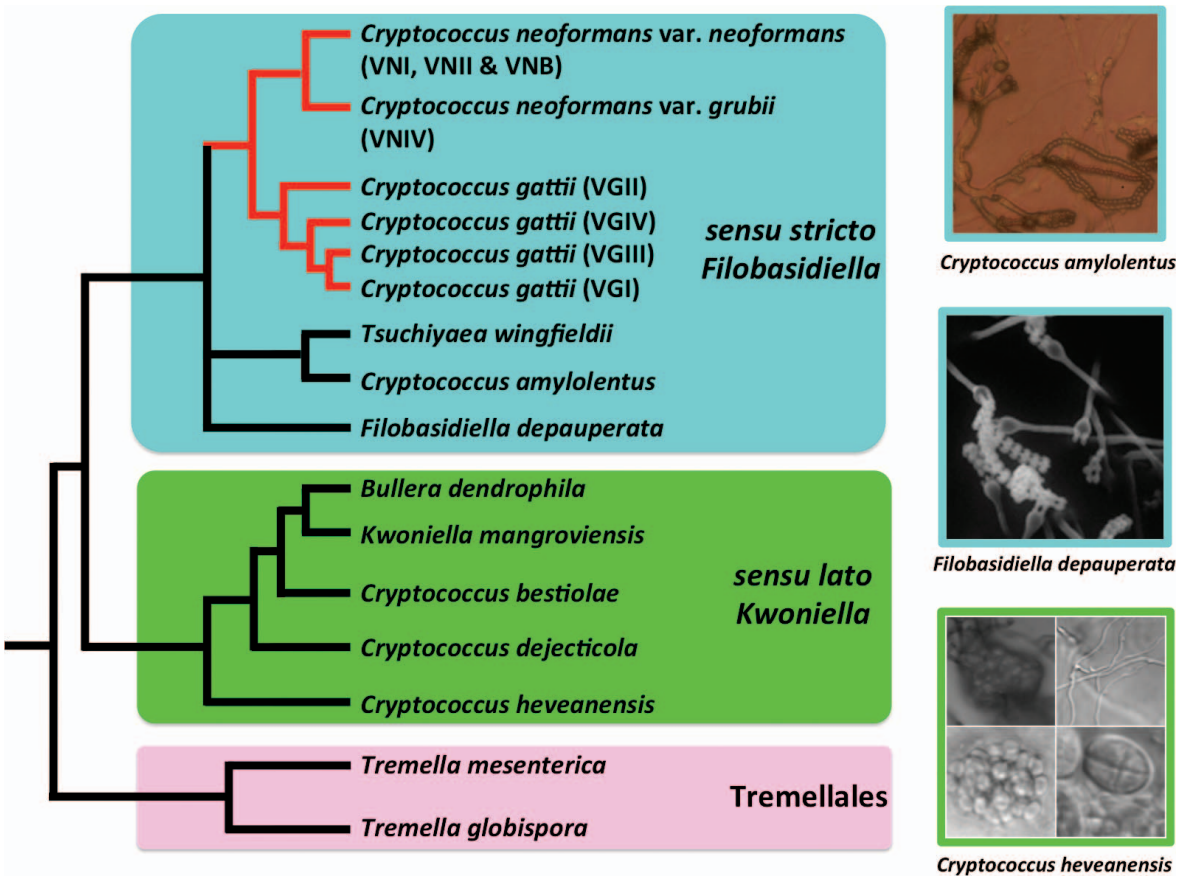

FIG. 3. Phylogeny of the pathogenic *Cryptococcus* species complex and its closely related species. The sensu stricto and sensu lato groups (see text) are shaded blue and green respectively. The phylogeny was adapted from (Findley et al. 2009) with modification. On the right are microscopy images of the hyphae, basidia, as well as basidiospore chains/clusters from three species that are closely related to the pathogenic *Cryptococcus* species complex: *Cryptococcus amylolentus*, *Filobasidiella depauperata* and *Cryptococcus heveanensis*. The images were adapted from (Metin et al. 2010, Rodriguez-Carres et al. 2010, Findley et al. 2012). Please refer to these original publications for detailed descriptions of the mating structures in these species.

Two recent studies involving novel fish species further illustrate the dramatic plasticity of sex determination. First, in the lab model system of zebra fish, it was well known that there are no distinct sex chromosomes, but it was still a mystery how sex determination was established. Bradley et al. (2011) reveal that strikingly sex determination in zebra fish is a quantitative trait and that as many as eight or more distinct genes, each lying on different chromosomes, cooperate to establish whether an individual is male or female. Two of the genes that together account for $\approx$ 25% of the trait have been defined. One is the homolog of the *Dmrt1* sex determinant implicated in sex reversal in humans, and the other is an enzyme involved in sex hormone production. Other genes, and suspected environmental factors, remain to be identified. A second recent striking example of plasticity in sex determination involves the cichlid fishes from Lake Tanganyika in Africa. Remarkably, their genomes abound in dispensable B chromosomes and individuals who inherit an abundance of B chromosomes are more likely to be female and by comparison; those with fewer are more likely to be male (Yoshida et al. 2011). These vignettes serve to illustrate that even though the origins of sex are ancient, the molecular, genetic, and genomic mechanisms that define sexes are remarkably fluid throughout the eukaryotic tree of life. Perhaps this illustrates a role via which this plasticity in sex determination may contribute to speciation or species boundaries.

A specialized region of the genome that is conceptually similar to homomorphic sex chromosomes, termed the mating-type locus, establishes mating type in fungi (Fraser and Heitman 2003). Why do we say that fungi have mating types rather than sexes and as a consequence call these regions mating-type loci instead of sex chromosomes? The

basis for this nomenclature is that most fungi do not have sexes as these are strictly defined, which typically entails gross morphological differences between the individuals of a species that produce each of the two types of gametes (i.e. sperm vs. oocytes). We say that fungi are isogametic (the two gametes look morphologically similar), whereas animals are typically anisogametic (the two gametes look morphologically distinct). There are a few fungi that do have clearly demarcated sexes, and one example is the well established model species *Neurospora crassa* in which the male is the fertilizing partner (via dispersing conidia or hyphal fragments) and the recipient that makes the protoperithecia is designated the female partner (Borkovich et al. 2004). Somewhat curiously, in this species mating type is still determined by a mating-type locus and distinct from the sex of the gamete (Glass et al. 1988), which has led some investigators to conclude that mating types and sexes must be two distinct entities. But from a practical, pragmatic viewpoint, there are also fungi in which the two partners produce mating entities (gametes) that display morphological differences. This includes *Cryptococcus*, in which the **a** mating type often makes enlarged round cells that serve as a target for fusion with an elongating conjugation tube produced by the α partner, reminiscent of the sperm and the egg in anisogametic species (Hull and Heitman 2002, McClelland et al. 2004). Thus, to some the distinction between sexes and mating types is more semantic than substantive whereas to others this is dogma that is not open to debate.

We know a great deal about the mating-type locus from studies of the model budding yeast *S. cerevisiae*, a hemiascomycetous yeast (Herskowitz 1989). In this species, a small (fewer than 1000 bp) region of the genome that encodes only one or two key cell identity determinants that establish mating type. There are two haploid mating types, known as **a** and α, which can fuse to produce the diploid **a**/α cell type. The **a** mating-type locus encodes a single gene product, **a**1, which is a homeodomain transcription factor. The α mating-type locus encodes two gene products, α2, which is a homeodomain transcription factor heterodimeric partner of **a**1, and α1, which is an α-domain transcription factor that activates α specific cell type genes. This species is an example of a bipolar mating-type system, because there are just two mating types, and any given cross produces progeny of just these two mating types. In nature, there is a roughly equal proportion of the two mating types produced (a majority of the population is **a**/α diploid), and thus there is an ~ 50% chance of inbreeding among sibling progeny of a diploid genotype and a 50% chance of outcrossing during encounters with other members of the population.

While the bipolar mating-type system is common in fungi, there are other fungi that have even more exotic mating-type determination systems that result in literally thousands of different mating types (Raper 1966). These species are found within a different phylum of the fungal kingdom, the Basidiomycota. A representative example is *Ustilago maydis*, a pathogen of maize. In this species there are two different mating-type loci, termed *a* and *b*, which lie on different chromosomes and therefore are unlinked (Schulz et al. 1990, Bölker et al. 1992). In this species, the *b* locus encodes a pair of divergently oriented homeodomain proteins, called *bE* and *bW*, which are homologous to the α2 and **a**1 factors of *S. cerevisiae*. The *a* locus encodes pheromone and pheromone receptors. Both loci must differ for a productive, fertile interaction to occur (the loci are not self-activating, but are cross active with an allele of compatible mating type). In *U. maydis* the *b* locus is multi-allelic, whereas the *a* locus is bi-allelic. Recent studies reveal that the bi-allelic version of the *a* locus in *U. maydis* is a derived state and descends from a cluster of smut species in which many are tri-allelic for the *a* locus (Schirawski et al. 2005, Kellner et al. 2011).

Now in other model species in this phylum, such as *Schizophyllum commune* and *Coprinopsis cinereus*, both mating-type loci are multi-allelic, leading to literally thousands and thousands and thousands of different mating types, as many as > 20 000 (Pukkila 2011). In these species, mating of an isolate of *A1B1* with another isolate of *A2B2* mating type can give rise to progeny of four different mating types: *A1B1*, *A2B2*, *A1B2* and *A2B1*, when we consider the pool of F1 progeny produced, which is a combination of parental ditype, non-parental ditype, and tetratype meiotic events. As a consequence, this is termed a tetrapolar mating-type system. Any given progeny can only mate with 25% of its sibling progeny, and thus this type of mating-type system results in a relative twofold depression of inbreeding potential from the 50% observed for bipolar species. It is important to note here that, when an isolate of *Cop. cinereus* encounters another member of its general population, in contrast to interactions with its siblings, almost every interaction (> 98%) results in sexual fertility and thus the multi-allelic tetrapolar mating-type system achieves a high level of outbreeding potential, higher than the bipolar system (Kües et al. 2011). The major differences between these systems are the twofold increase in inbreeding potential in bipolar systems, the ~ twofold increase in outbreeding potential of tetrapolar systems, and the genetic uncoupling of the major processes

occurring during Basidiomycete mating (nuclear migration and synchronous nuclear division) in tetrapolar species. We posit that transitions between the unipolar, bipolar and tetrapolar systems observed in nature are due to the relative importance of inbreeding and outbreeding in the life cycle.

Before beginning the discussion on the evolution of the mating-type genes we must clarify what is meant by inbreeding and selfing and discuss evidence supporting their occurrence in fungi. Inbreeding can be defined simply as sexual reproduction that brings together more related genomes than random mating. Obvious inbreeding examples in heterothallic fungi include intra-tetrad mating in *Microbotryum violaceum*, mating-type switching in *Saccharomyces* and pseudo-homothallic reproduction in *Agaricus bisporus*, where two compatible nuclei are packaged in the same spore. In all these examples, population genetic analysis of these species provides evidence for inbreeding as a form of reduction in heterozygosity when viewed in the diploid state (Giraud 2004, Johnson et al. 2004, Foulongne-Oriol et al. 2009). Selfing is the most severe form of inbreeding, and with fungi it can occur at two scales, which we may distinguish as occurring at the level of haploid genotypes (in the case of homothallism) or occurring as a form of mating among the gametes produced by a single diploid genotype.

Evolution of the mating-type locus in the Cryptococcus *pathogenic species complex.*—We now turn our focus to a pathogenic species within the Basidiomycota called *Cryptococcus neoformans* (FIG. 4). This species is a common, globally distributed pathogen of humans. We all have been exposed by the inhalation of desiccated yeast cells or spores, which are small enough to penetrate the alveoli in the lung. This results in an initial pulmonary infection, which can become latent or disseminate to infect most prominently the central nervous system, including both the meninges and parenchyma of the brain to cause meningoencephalitis. The species is distributed globally in association with pigeon guano and certain arboreal niches, and we are exposed to airborne infectious propagules. The magnitude of impact on human health caused by this pathogen is considerable. Recent studies from the Centers for Disease Control document that more than 1 000 000 infections occur annually, largely in the context of the HIV/AIDS pandemic, resulting in more than 600 000 attributable deaths each year, and ~ one-third of all AIDS-associated deaths, now surpassing tuberculosis as the cause of AIDS-associated deaths in Africa (Park et al. 2009). Moreover, the sibling species *C. gattii* molecular type VGII is causing an ongoing and expanding outbreak in the Pacific Northwest (Byrnes III et al. 2011). This outbreak began on Vancouver Island in 1999 and has spread to both the Canadian mainland and to the United States in Washington and Oregon and possibly also northern California. This molecular type lineage of *C. gattii* does not typically infect HIV/AIDS patients, and roughly half of those infected during the outbreak were otherwise healthy before presentation. The absolute number of cases has been modest (~ 400) by comparison to the global health burden caused by *C. neoformans*. But because it is airborne, widely distributed in the environment in association with trees and soil and immunocompetent individuals are at risk the outbreak remains a serious public health concern.

Because sexual reproduction is the process that produces infectious spores, there is an association of mating type with virulence and the population is also largely unisexual, studies in the field have focused on both the structure, function and evolution of the *Cryptococcus* mating-type locus and the detailed mechanisms involved in sexual reproduction. We will consider both topics here.

While this pathogenic species is a member of the same phylum of fungi with complex tetrapolar mating systems, it has the simpler bipolar mating-type system like *S. cerevisiae* with just two mating types, **a** and α (FIG. 4). We cloned and sequenced the **a** and α mating-type locus from *C. neoformans* and found that it is dramatically expanded compared to the small, compact mating-type locus of *S. cerevisiae*, spanning more than 100 kb and encoding more than 25 genes (Lengeler et al. 2002, Fraser et al. 2004, Byrnes et al. 2011) (FIG. 5). Embedded within this contiguous mating-type locus are the genes that normally reside in the two unlinked *MAT* loci of tetrapolar fungi, at the left end encoding one or the other homeodomain protein and at the other end encoding the pheromones and pheromone receptor. Thus, to a first approximation, it appears that a translocation event has brought the two unlinked *MAT* loci into linkage, incorporated additional genes and resulted in a contiguous derived *MAT* locus that now defines a bipolar species. It is worth stressing that this is one of three models that John Raper originally proposed to explain how bipolar species might be derived from tetrapolar ones, in his classic text titled Genetics of Sexuality of Higher Fungi (Raper 1966).

To understand how this complex gene cluster involved in establishing cell type identity might have been formed, we took a comparative genomics approach. We cloned and sequenced the **a** and α mating-type locus from three closely aligned varieties or species from the *Cryptococcus* pathogenic species complex (Karos et al. 2000, Lengeler et al. 2002, Fraser et al. 2004, Byrnes et al. 2011). This analysis

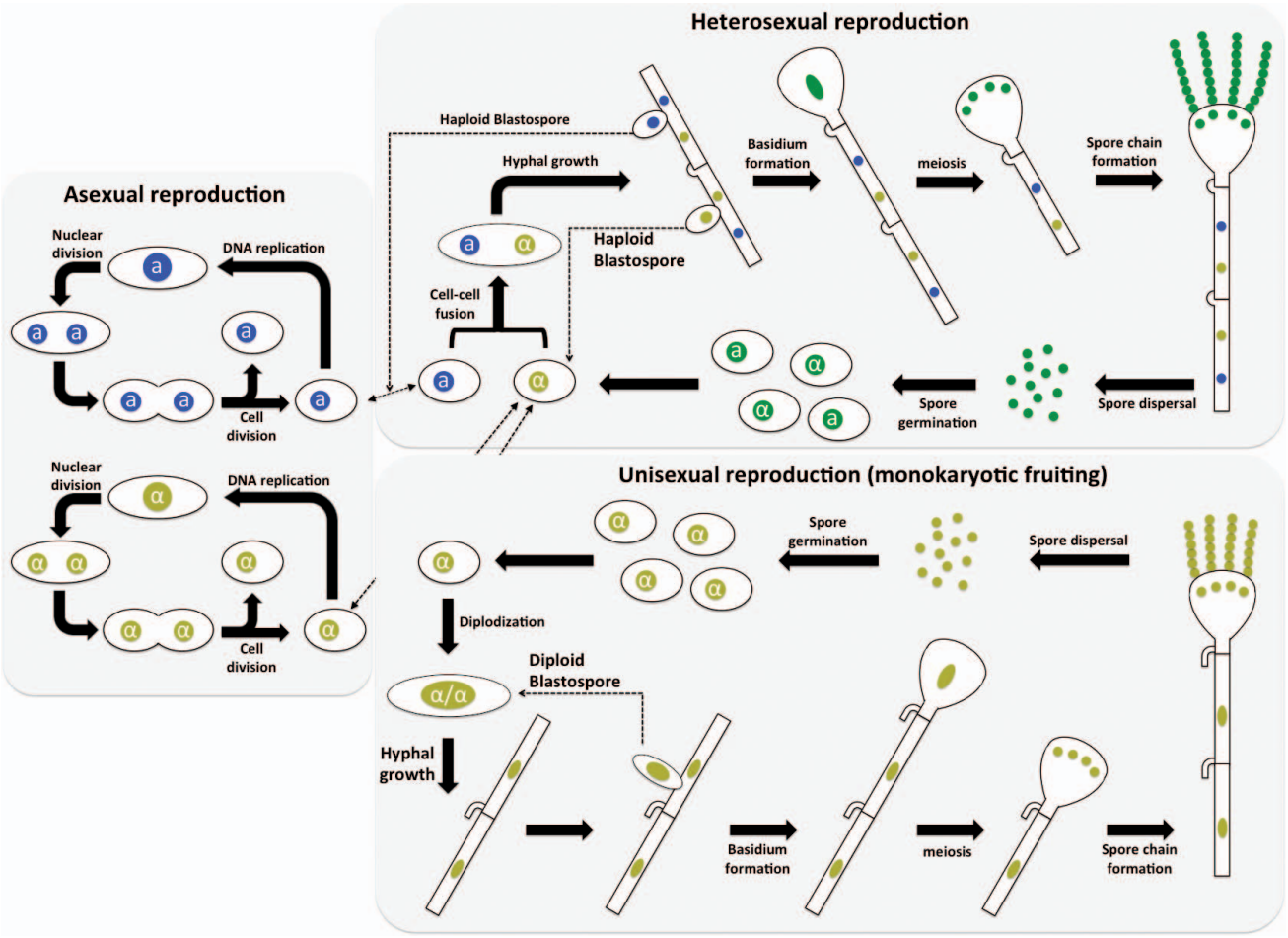

FIG. 4. Modes of reproduction of *Cryptococcus neoformans*. *Crytococcus neoformans* has three modes of reproduction: asexual, heterosexual (**a**-α bipolar mating system), and unisexual (α-α monokaryotic fruiting). Asexual reproduction is similar to mitotic reproduction of a typical budding yeast, in which the haploid cell first undergoes DNA replication, followed by nuclear division and cell division to produce two haploid daughter cells. Heterosexual reproduction begins when two cells of opposite mating types fuse and initiate hyphal growth. The hyphae are dikaryotic with fused clamp connections. Eventually the tip of the hypha expands and forms a basidium. Within the basidium, the two nuclei fuse and the ensuing meiosis produces four haploid products, which then undergo repeated rounds of mitosis to generate basidiospores that emerge on the surface of the basidium and form four basidiospore chains. The dispersed basidiospores then can germinate under suitable conditions and re-enter the life cycle. Unisexual reproduction so far has been observed mostly in *C. neoformans* cells of the α mating type. It starts when a diploid α-α cell is formed either by cell-cell fusion or endoduplication. This diploid cell undergoes monokaryotic hyphal growth with unfused clamp connections. Similar to heterosexual reproduction, eventually a basidium is formed at the tip of the hypha and meiosis occurs within the basidium. The four meiotic products then undergo repeated rounds of mitosis and produce basidiospores that emerge on the surface of the basidium and form four basidiospore chains. The basidiospores then disperse and germinate when the environment is suitable and re-enter the life cycle. For both heterosexual and unisexual reproduction, haploid or diploid blastospores can be generated around the clamp connection.

revealed that the size of the *MAT* locus in general is conserved, spanning 100–120 kb, and is flanked by highly syntenic regions with conserved genes and gene order. But within the *MAT* locus, there have been marked rearrangements of the gene order but few examples of gene loss, possibly because the presence of five essential genes constrains the rearrangements that can occur without loss of any of these essential genes that punctuate the locus. While this analysis reveals that the size of the locus has been conserved across this group of species over the ~ 10, to 20 to 40 million years that separate them from their last shared common ancestor, it doesn't provide insight into how this large gene cluster might have been formed from an ancestral tetrapolar species. We next looked in detail at the sequences of the genes resident within the *MAT* locus. By assaying the rate of synonymous substitutions (dS)

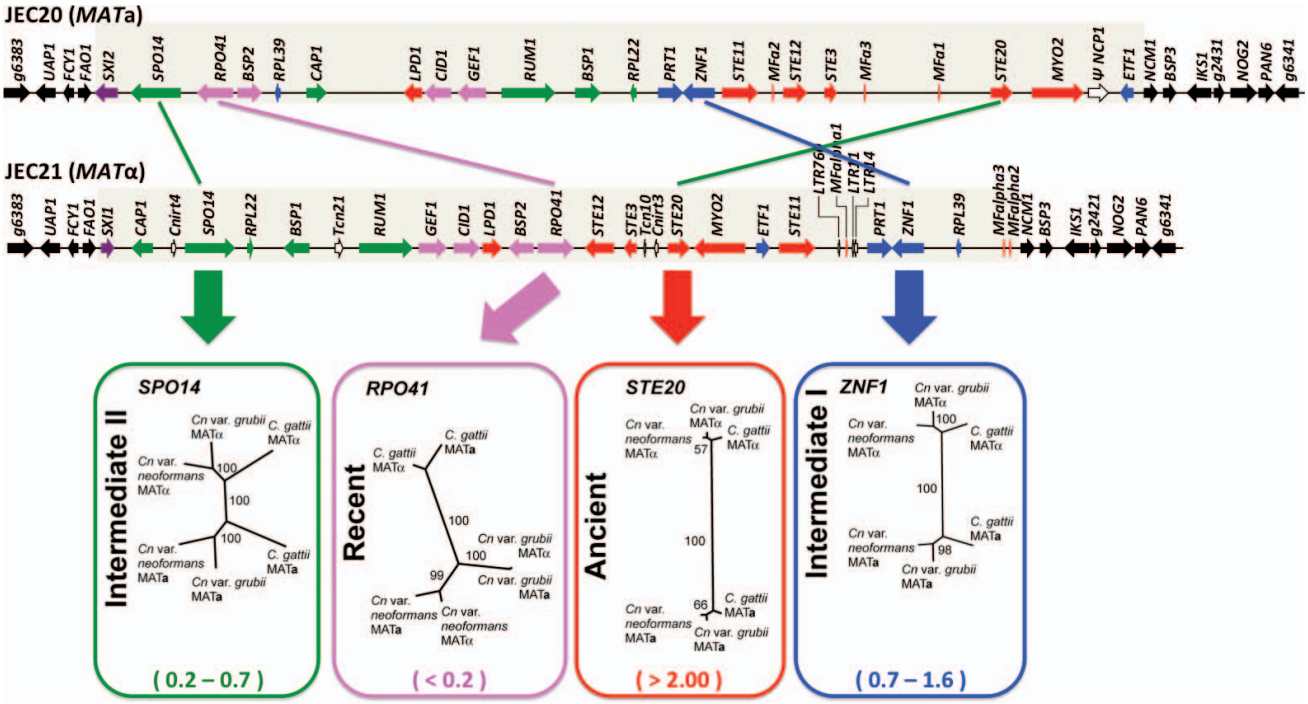

FIG. 5. Gene strata within the *Cryptococcus neoformans MAT* locus. On top are the illustrations of the *MAT* alleles of the two opposite mating types, *MAT***a** (JEC20) and *MAT*α (JEC21) respectively. Black highlights the flanking genes; genes within the ancient stratum are in red; genes within the intermediate I and II strata are in blue and green respectively; genes within the recent stratum are highlighted in pink. At the bottom are phylogenies of the genes representing each of the four strata respectively. When *C. neoformans* and *Cryptococcus gattii* are considered together, genes within the ancient stratum all have highly mating type-specific phylogenies, genes within the intermediate strata also have a mating type-specific phylogeny, but to a lesser extent, while genes within the recent stratum all have a species-specific phylogeny. Below each phylogeny, the number within the parentheses indicates the observed rate of synonymous substitutions for the genes within that specific stratum. Data and phylogeny are adapted from (Fraser et al. 2004) with modification.

between the **a** and α gene alleles as a molecular clock, we observed that four strata of genes of different evolutionary ages are present within *MAT* (FIG. 5). First, there are the most ancient genes, recognized by their higher dS rate. Next, there are two strata of genes of more intermediate evolutionary age. Finally, there is a strata of the most closely related genes, which have the lowest dS rate. These are either the youngest genes within the *MAT* locus or their evolutionary age might have been reset as a consequence of more recent gene conversions that have punctuated the evolutionary trajectory of the *MAT* locus. Generation of gene trees further corroborated these findings. The oldest strata of genes formed very diverged bipartite gene trees in which the **a** and α alleles formed distant groups. Next, there are the two intermediate strata, in which the genes are still clearly mating type-specific but less diverged than the more ancient strata of genes. Finally, there is the stratum of the most closely related genes, which turn out to be not mating type-specific but instead species specific, even though they are embedded within the otherwise mating type-specific region of the genome. In this case the gene trees form a tripartite rather than bipartite phylogeny, in which the **a** and α alleles from each species are more closely related to each other than to their mating-type counterparts from the other species (FIG. 5).

This analysis provides the insight that the *MAT* locus is something like a patchwork quilt sewn from different pieces of cloth and that it evolved through a series of steps. Based on this insight, we developed a model (FIG. 6) in which we posited that the extant bipolar *MAT* locus evolved from a simpler, standard tetrapolar system by a series of steps that involved acquisition of genes that function in sexual reproduction into the ancestral *A* and *B MAT* loci, forming expanded gene clusters. Next, the two gene clusters fused to form one contiguous mating-type locus in one mating type, whereas the other remained unlinked, resulting in an intermediate transitory state we term the tripolar system. Then, the tripolar intermediate state was converted to a fully linked bipolar state by recombination or gene conversion, linking the remaining two

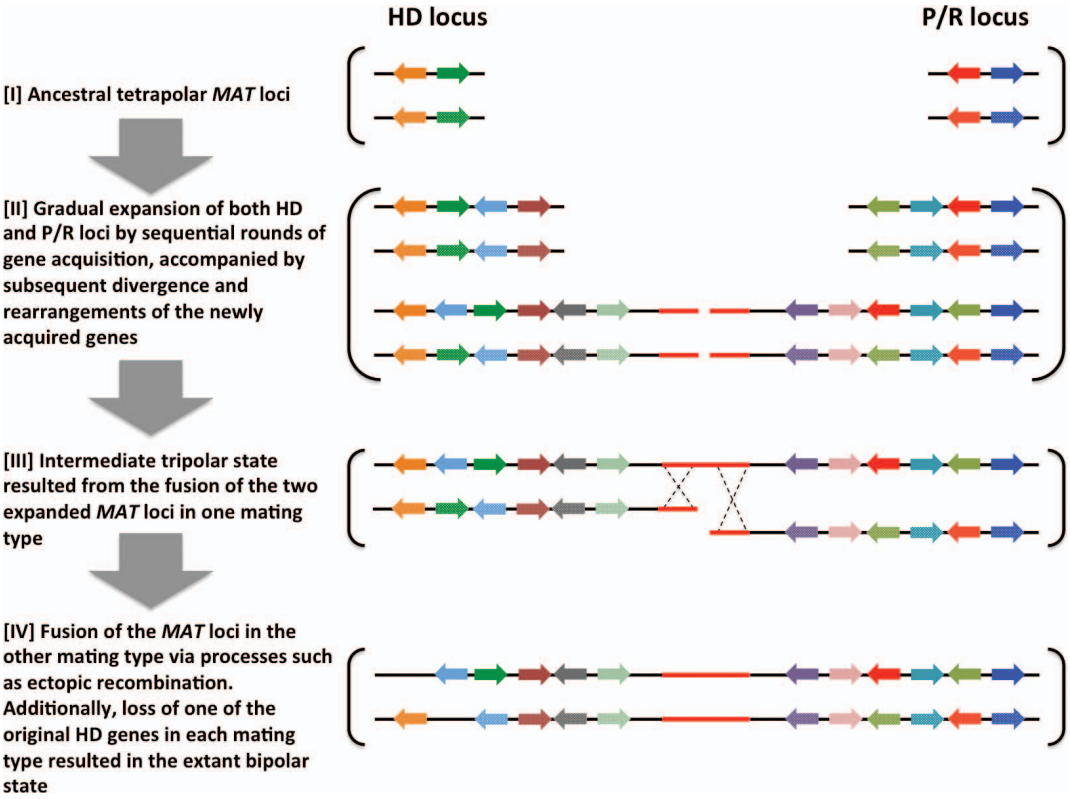

FIG. 6. Evolution of the bipolar mating type locus of species in the pathogenic *Cryptococcus* species complex. I. The evolution of the bipolar *MAT* locus originated from an ancestral tetrapolar mating system with unlinked HD and P/R loci. II. The two unlinked ancestral *MAT* loci underwent gradual expansion by sequential rounds of gene acquisition, recruiting components such as the pheromone signaling MAPK cascade, as well as genes that are hypothesized to be involved in dikaryon formation or meiosis. At the same time, chromosomal rearrangements shuffled the newly acquired genes within the expanded *HD* and *P/R* loci. III. Fusion of the expanded *HD* and *P/R* loci in one mating type, which could have occurred by processes such as chromosomal translocation or ectopic recombination mediated by elements such as transposons or centromeric sequences (illustrated here by the red lines), resulting in an intermediate tripolar state. IV. Intercrosses between individuals with a fused *MAT* locus and individuals with separate tetrapolar *MAT* loci allow further recombination around the repetitive sequences (indicated with red lines), which could result in fusion of the alleles from the unlinked *MAT* loci, generating a contiguous *MAT* locus for the other mating type. At the same time, one of the two HD transcription factors was lost in each of the two contiguous *MAT* alleles. In addition, this newly formed *MAT* locus configuration could have established a certain level of reproductive isolation, because intercrosses between individuals with fused and unfused *MAT* loci would produce progeny with abnormal chromosomal compositions at much higher frequencies than mating between individuals with the fused *MAT* locus or between individuals with unfused *MAT* loci. Ongoing chromosomal rearrangements (e.g. inversions and translocations) as well as gene conversion within the contiguous *MAT* locus eventually gave rise to the extant bipolar *MAT* alleles observed today.

mating-type alleles in the other mating type. A series of additional recombination and gene conversion events then occurred to give rise to the extant *MAT* alleles observed today. By deleting the homeodomain genes in *MAT*, and relocating them to an unlinked genomic location (*URA5*), we artificially constructed strains that mimic this proposed ancestral tetrapolar state and intermediate tripolar state, providing direct experimental evidence for these aspects of the model (Hsueh et al. 2008). This effort provided a plausible model by which the *MAT* locus in the pathogenic species complex might have evolved. We next sought to test this model experimentally.

Transitions in sexual reproduction from tetrapolar to bipolar: the mating-type locus and extant sexual cycles for Cryptococcus heveanensis *and* C. amylolentus.— To investigate the evolution of the *Cryptococcus* pathogenic species complex *MAT* locus, we took a molecular phylogenomic approach. First, we constructed a robust phylogenetic tree for the 15 species that span and encompass the pathogenic species complex (Findley et al. 2009) (FIG. 3). This effort was greatly facilitated by key advice from Rytas Vilgalys on which species to focus this analysis upon, and the ongoing fungal tree of life project (AFTOL) that provided a broader intellectual framework. In fact,

our efforts used multilocus sequence analysis of the six genes central to the AFTOL program (James et al. 2006a). We rooted our tree with the species *Tremella mesenterica*, whose genome was sequenced by the DOE-JGI fungal genome initiative (Floudas et al. 2012). Our analysis revealed two groups of species, which we term the sensu stricto and sensu lato complexes (FIG. 3). The sensu stricto complex includes the three pathogenic *Cryptococcus* lineages (*C. neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, *C. gattii*). Three other species populate this group, *Cryptococcus amylolentus*, *Tsuchiyaea wingfieldii* and *Filobasidiella depauperata*, which are most closely related to the pathogenic species. The sensu lato group, which is more distantly related to the pathogenic species complex compared to the sensu stricto species, includes several species such as *Cryptococcus heveanensis* and *Kwoniella mangroviensis*, as well as others. It is important to stress that other than those species that are part of the pathogenic species complex, none of these other species are pathogens but instead are associated with trees or insect debris (frass), or possibly are mycoparasitic. It also is important to stress that none of these are model systems and they have been subject to very little genetic or molecular analysis beyond their mycological description as species. Moreover, for some of these very few isolates are available, which remains a challenge. Caveats aside, these species are molecular windows on the evolution of the *MAT* locus and sexual cycles of the pathogenic species complex, with which they share a most recent last common ancestor.

Our next step was to focus on representatives of these species (Rodriguez-Carres et al. 2010), and we will focus on two of these here for which we have cloned the mating-type locus and discovered and characterized extant sexual cycles (Metin et al. 2010, Findley et al. 2012). First, for the sensu lato species *C. heveanensis*, we cloned and sequenced the mating-type loci (Metin et al. 2010). This analysis revealed two gene clusters corresponding to the *A* and *B MAT* loci, organized similarly to a canonical tetrapolar mating-type system. The *A* locus encodes the pheromone and pheromone receptor with a linked *STE12* gene that also is present in the *MAT* locus of the *C. neoformans* species complex. The *B* locus spans two divergently oriented homeodomain genes, similar to other canonical fungi with tetrapolar mating systems, such as the *bE* and *bW* genes of the *b* locus of *U. maydis*. Second, for the sensu stricto species *C. amylolentus* (and also *T. wingfieldii*), we cloned and sequenced the mating-type loci (Findley et al. 2012). We again found two distinct gene clusters corresponding to the *A* and *B MAT* loci that lie on different chromosomes, based on pulsed field gel electrophoresis and chromoblot analysis. In this case, the *B* locus again contains two divergently oriented homeodomain genes, similar to *C. heveanensis* and other tetrapolar fungal species, while the *A* locus appears to have been expanded even further compared to *C. heveanensis* and spans a considerable distance (this is currently being resolved by whole genome analysis). Thus, these two species represent extant examples of two of the hypothesized evolutionary intermediates from an ancestral tetrapolar state to the derived bipolar one. In both cases acquisition of genes observed in the bipolar *C. neoformans MAT* into the vicinity of the *A* locus of the tetrapolar species has occurred, perhaps corresponding to the intermediate age genes type 1 and type 2 found within the extant derived bipolar state. This analysis supports our evolutionary model and provides further insights into the specific nature of the events that might have given rise to a large gene complex linked to virulence.

To fully elucidate the nature and function of the *MAT* loci-related regions in *C. heveanensis* and *C. amylolentus*, we sought to discover extant sexual cycles such that we might be able to correlate the molecular *MAT* loci analyses with their functions in mating. For *C. heveanensis*, there were three isolates available via culture collections. We were unable to find any evidence of mating when any of these isolates were co-cultured, either pairwise or all three together. Further molecular analysis suggested that while one is the type strain of *C. heveanensis* (CBS569), the other two represent isolates of two closely related but distinct species. Through prodigious efforts and extensive review of a broad range of primary literature sources, Banu Metin found a chapter describing a set of *C. heveanensis* isolates obtained from insect frass or flowers in Thailand. These isolates were deposited in a public strain repository in Thailand, from which we were able to obtain cultures by request. Molecular analysis revealed that all eight of these isolates were in fact bona fide isolates of *C. heveanensis*. Subsequently we discovered that several of these isolates were fertile with the type strain CBS569, generating beautiful dikaryotic hyphae, basidia (with cruciate septa) and associated spores (FIG. 3). We named the teleomorph for this species *Kwoniella heveanensis*. We next characterized the molecular nature of the *A* and *B MAT* loci in these strains and found evidence that the *A* locus is at least bi-allelic, the *B* locus is multi-allelic and both mating assays and population genetic analysis provide evidence for an extant tetrapolar mating-type system. We explicitly note that access to well validated and accessible strains from culture collections was integral to this effort and thus we applaud the efforts of mycologists to collect, docu-

ment and store fungal isolates that provide the key materials for subsequent genetic, molecular and genomic analysis.

We next turned our efforts to *C. amylolentus*, and after considerable effort we also were successful in defining an extant sexual cycle for this species (Findley et al. 2012). Only two isolates are available for this species, and they have been preserved at the ATCC for several decades. We obtained these isolates, and our molecular analysis suggested that they could be representatives of the same species, whereas the single isolate that is available for *T. wingfieldii* appears to be a singleton isolate from a closely related but likely sibling taxon. While *C. amylolentus* grows as a yeast, both available strains produce hyphae when grown on a variety of media that induce mating of *C. neoformans* or *C. gattii*, complicating recognition of mating by morphological criteria. We focused on conditions that enhance mating of the closely aligned pathogenic species (V8 medium pH 5, in the dark, without parafilm, with the agar side of the Petri dish facing up), and Keisha Findley discovered that unique sectors arise out of the peripheral hyphae from cocultured strains. Upon microscopic examination, these unique sectors contain dikaryotic hyphae with fused clamp connections, basidia, and four very long spore chains of round spores (FIG. 3). We found that we could readily dissect these spores following digestion with lytic enzymes and using a standard microdissection fiber-optic cable dissecting needle and micromanipulator. Analysis of germinated basidiospores provided evidence that progeny of four mating types are produced from the *A1B1* × *A2B2* parental genotypes, resulting in *A1B1*, *A2B2*, *A1B2* and *A2B1* progeny. One caveat is that a substantial fraction of the progeny was sterile in crosses with either parent, or with their siblings, illustrating a potential danger of sex with respect to fecundity. Another interesting fact was that many of the fertile progeny were biased toward one of the two parental mating types, which might represent a precursor to the unipolar mating of *C. neoformans* that we will discuss below. These findings provide evidence that this sensu stricto species exhibits key features of the tetrapolar mating-type system. We named the teleomorph for this species *Filobasidiella amylolenta*.

Taking these studies together, we can now return to our phylogenetic tree and root it with a closely aligned species, a more distantly related species and an outgroup species, each of which has a tetrapolar mating-type system. This analysis lets us conclude that the most parsimonious model is that the last common ancestor of this species complex was tetrapolar and that the cluster of pathogenic *Cryptococcus* species that all feature a bipolar mating-type system represent a derived state. We can further suggest that this transition to bipolarity occurred once in the origins of the pathogenic complex, given the conserved nature of the extant mating type-locus alleles observed in the three recognized pathogenic species/varieties. Key to our analysis was a combination of molecular analysis wedded with classical mycology to discover extant sexual cycles for two species that heretofore had been classified as asexual. It is especially gratifying to find extant sexual cycles for both; this is not the type of discovery that happens every day, even to the most accomplished mycologists, and in this case was fully attributable to the tenacity and prodigious efforts of highly talented graduate students and fellows (Keisha Findley, Banu Metin, Sheng Sun).

Transitions in sexual reproduction from tetrapolar to bipolar have occurred repeatedly and independently in the Basidiomycota.—We now appreciate that these transitions from ancestral tetrapolar states to a derived bipolar state have occurred repeatedly and independently in the Basidiomycota (FIG. 7). Three examples feature the paradigm illustrated above for the pathogenic species complex, including *Ustilago hordei* and *Malassezia restricta/Malassezia globosa*. *Ustilago hordei* is a plant pathogen related to *U. maydis* but which infects barley or rye rather than corn. In contrast to *U. maydis*, which is tetrapolar, *U. hordei* has a bipolar mating system. Guus Bakkeren and Jim Kronstad demonstrated that the *U. hordei MAT* locus has two alleles in which the *A* and *B MAT* loci have been fused and are separated by a large 450–500 kb intervening region that has a relative paucity of genes and an abundance of repetitive sequences (Bakkeren and Kronstad 1994, Lee et al. 1999, Bakkeren et al. 2006, Bakkeren et al. 2008). Similarly, the research team at Proctor & Gamble, together with international collaborators, sequenced the genomes of the human skin associated commensal *Malassezia* species that are linked to causing dandruff. They discovered a *MAT* region, similar to the fused locus of *U. hordei* in which the *A* and *B MAT* loci lie at the ends of a contiguous region, separated by an ~ 167 kb internal region (Xu et al. 2007). The fact that there is no similarity between the intervening regions of *U. hordei* and *Malassezia restricta* and *M. globosa*, combined with other phylogenetic evidence, argues that these are independent origins of the derived bipolar state, which are themselves independent from that which arose in the *Cryptococcus* lineage (FIG. 7) (Bakkeren et al. 2008, Hsueh and Heitman 2008).

That these transitions from a tetrapolar outcrossing system to a derived bipolar system, which could facilitate inbreeding or have arisen due to inbreeding

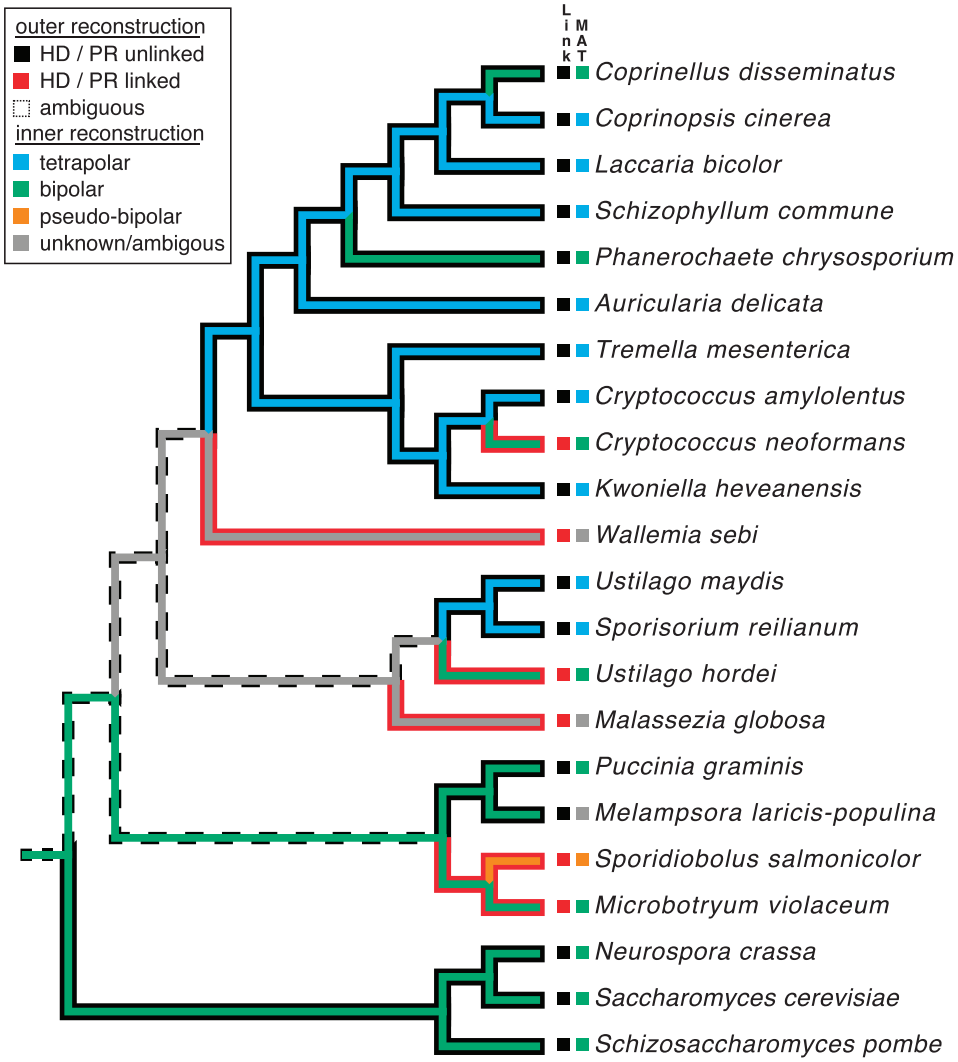

FIG. 7. Phylogenetic reconstruction of the evolution of mating systems and linkage between *HD* and *P/R* loci in the Basidiomycota. On the outer layer of the branches is the ancestral state reconstruction under parsimony of the physical linkage between *HD* and *P/R* loci. In some cases (e.g. *Melampsora larici-populina*), absence of linkage is inferred by association of *HD* and *P/R* loci to different scaffolds of draft genome sequences, and this may not be equivalent to chromosomal linkage. The inner portion of the branches reconstructs ancestral mating systems, where known. The character states of species included in the tree are given to the left of the names, where ''Link'' refers to linkage of *HD* and *P/R* and ''*MAT*'' refers to mating system. The phylogeny is based on (Aime et al. 2006, Hibbett 2006, Matheny et al. 2006, Kellner et al. 2011).

have occurred repeatedly and independently in three different successful pathogenic lineages of fungi that infect plants or animals, suggests that such transitions might be adaptive for pathogens, perhaps as they become specialized to unique niches or hosts. A bipolar mating system may be favored in situations where long distance dispersal of a tetrad occurs, such as in the case of the smut teliospore. Multiple levels of mating system transitions serve to increase the potential for inbreeding, including the transition from tetrapolar to bipolar, from multi-allelic to bi-allelic, and the loss of one or the other paired homeodomain that has occurred in the *Cryptococcus* pathogenic species. In tetrapolar species that have paired homeodomain genes, there are two opportunities to produce an active heterodimeric homeodomain complex, whereas in the derived bipolar pathogenic lineages there is a single homeodomain encoded by each *MAT* locus and thus these must productively interact to form an active heterodimer or a given cross will be infertile.

How might these recombination events between mating-type loci on two different chromosomes have occurred repeatedly to bring the two sex determinants into one contiguous chromosomal region? Given that mating-type loci and sex chromosomes

are sheltered from recombination, transposable elements and other repetitive sequences tend to accumulate and might have served to foment recombination events leading to chromosomal translocations and transition from a tetrapolar to a bipolar system. While this is a reasonable hypothesis, recent genetic observations by one of us (Sheng Sun) have led us to consider models in which the *A* and *B MAT* loci may be linked to the centromeres on their respective chromosomes. In *C. neoformans*, we know that the candidate centromeric regions of its 14 linear chromosomes each harbor at least one copy of the two different transposable elements, *Tcn5* and *Tcn6* (Loftus et al. 2005). Thus, one plausible working hypothesis is that intercentromeric recombination could have driven the linkage of the originally unlinked sex determinants of the tetrapolar mating-type system. If so, then in some examples such as *U. hordei* the centromere still may be apparent and even lie between the two ends of the now contiguous mating-type locus. Approaches to test this model will involve dissection of spores from individual basidia of *C. amylolentus* to examine the patterns of marker segregation, which can discern centromere linkage, and whole genome analysis to examine the chromosomal context of the *A* and *B* loci with respect to their respective centromeres on their host chromosomes.

Unlike the earlier diverging subphyla, the Agaricomycotina is largely characterized by tetrapolar species (Whitehouse 1949, Raper and Flexer 1971) (FIG. 3). Because of similarity in the genetic and physiological manifestations of the tetrapolar mating system throughout the group, the complex genetic architecture of the tetrapolar mating-type loci and the phylogenetically widespread distribution of tetrapolar and bipolar species across the Agaricomycetes (mushrooms), Raper (1966) posited that the ancestor of the Agaricomycetes was tetrapolar and that both bipolar and homothallic species had arisen multiple times independently from tetrapolar ancestors, much in the way that was observed in the Ustilaginomycotina and Tremellomycetes (Raper 1966). One major difference between Agaricomycetes and all other basidiomycetes, the paraphyletic group previously known as the heterobasidiomycetes, is the presence of a high diversity of alleles at the pheromone receptor *B* mating-type locus in the former, whereas no more than three alleles are observed in the latter. Surprisingly few studies have investigated the phylogenetic patterns of mating system evolution in the Agaricomycetes, but one exceptional study (Hibbett and Donoghue 2001) provides support for Raper's hypotheses that tetrapolarity is ancestral in Agaricomycetes and that bipolarity has arisen multiple times from tetrapolarity while the opposite transition is not observed. Going beyond the Agaricomycetes, there has been no convincing evidence that bipolarity has given rise to tetrapolarity more than once, yet the recent demonstration of pseudo-bipolar systems (Coelho et al. 2010) and the paucity of data in the Pucciniomycotina suggests that this pattern ultimately will be broken. Currently it is unclear whether the last common ancestor of the Basidiomycota was tetrapolar or bipolar. Phylogenetic reconstruction of the trait suggests ancestral bipolarity; however, this interpretation is again hampered by a paucity of data at the base of the phylum and would benefit from a closer analysis of putatively tetrapolar rust fungi including genome sequences (FIG. 7) (Lawrence 1980, Narisawa et al. 1994).

The single mating-type locus of a number of bipolar Agaricomycete fungi has now been investigated and a general trend has emerged that differs from the observations of the tetrapolar to bipolar transition in the heterobasidiomycetes. A phylogenetically diverse set of bipolar species, *Coprinellus disseminatus* (Agaricales), *Pholiota nameko* (Agaricales), *Phanerochaete chrysosporium* (polyporoid clade) and *Heterobasidion annosum* (Russulales), all display a similar mating-type locus architecture, comprising either one or two pairs of homeodomain encoding pairs (Aimi et al. 2005; James et al. 2006b, 2011; Olson et al. 2012). The genomes of each of these species also encode homologues of the P/R genes observed and descended from mating-type genes of tetrapolar species, but the genes are not genetically linked to the *HD* locus or sufficiently polymorphic to encode different mating types. In the case of *Coprinellus disseminatus*, these non-mating-type specific pheromone receptors were analyzed further by transformation into a heterologous host (*Coprinopsis cinerea*). We observed that the pheromone receptors of *C. disseminatus* were able to confer regular *B* mating-type pathway development in *C. cinerea*, suggesting that the pheromone receptors of *C. disseminatus* were either auto-activating or auto-activated (James et al. 2006b). In another tip of the hat to John Raper's insights, these findings confirm a second of his hypothesized mechanisms by which a bipolar mating system might originate from a tetrapolar one: through the evolution of a non-discriminating, self-compatible *B* mating type. Such bipolar patterns of mating essentially had been reconstructed in the lab from self-compatible *B* mutants of *Schizophyllum commune* (Parag 1962; Casselton and Olesnicky 1998; Olesnicky et al. 1999, 2000; Casselton 2002). These examples of reversion to a bipolar system in the Agaricomycetes might provide some insights into how the ancestral bipolar basidiomycete mating genes appeared because the arrangement bears similarity to that of the Ascomycota.

UNISEXUAL (UNIPOLAR) REPRODUCTION: THE *CRYPTOCOCCUS* PARADIGM

We have discussed in detail the transitions from tetrapolar to bipolar mating systems, and we now consider how the *Cryptococcus* pathogenic species complex has taken this one step further to result in a unipolar mating system in which a partner of opposite mating type is no longer obligate and unisexual reproduction can occur. A central conundrum in the field was that studies of both environmental and clinical isolates revealed that the *Cryptococcus* population is in many aspects largely unisexual. For example, in the predominant pathogenic lineage (*C. neoformans* var. *grubii* serotype A) that is globally distributed and causing $> 1\,000\,000$ infections per year, we and others typed ~ 3000 isolates and found that 2997 were of the α mating type, and only three were of the rarer **a** mating type (Lengeler et al. 2000, Keller et al. 2003, Viviani et al. 2003). And for the one **a** mating-type isolate we studied most extensively, it was only fertile with three of 150 possible α isolate partners (Nielsen et al. 2003). Thus, it seems that most global isolates that are predominantly α mating type will rarely encounter an isolate of opposite **a** mating type. If so, how are infectious spores produced and how might diversity be maintained? One possible answer to this conundrum is that they are not and that the organism is asexual and mitotically reproducing globally. In fact, a decade or so ago it was commonly held that not only this pathogenic eukaryotic microbe but also many other pathogenic fungi, and even the pathogenic parasites (*Leishmania*, *Giardia*, Trypanosomes), were asexual and clonal, and that this reproduction strategy had been selected concomitant with emergence of successful pathogens (Tibayrenc et al. 1990, 1991).

But we considered an alternative hypothesis that at the time might have been considered somewhat heretical, which suggested that a sexual cycle might occur involving just one of the two mating types, α, and in this model **a** cells were not required for sexual reproduction to occur. As a fellow, Xiaorong Lin in fact made a series of observations that revealed that *Cryptococcus* has the capacity for two types of sexual reproduction, one involving both the α and **a** opposite mating types and the other involving just cells of the α mating type (Lin et al. 2005) (FIG. 4). In this modified sexual cycle, α cells transition from haploid to diploid via either cell-cell fusion with themselves or another α isolate from the population (or undergo endoreplication), and these diploid isolates form a monokaryotic hyphae with unfused clamp connections, ultimately produce terminal basidia and produce four chains of spores. In other strain backgrounds, karyogamy may occur late, in the basidium, similar to opposite sex mating, but the ultimate outcome is similar: a diploid nucleus in the basidium undergoes meiosis to produce haploid basidiospores that are all of the α mating type (FIG. 4). From a mycological perspective, this is at one level simply a new form of homothallism—an isolate in solo culture can undergo sexual reproduction all on its own. But unlike other forms of homothallism that we already know about, there is no mating-type switching involved here and there is just one mating-type locus in the genome and not a fused *MAT* locus or both *MAT* alleles, as is the case in some other homothallic fungi (Lin and Heitman 2007).

Now, at first it might seem odd that a fungal species could harbor both extant heterothallic, opposite mating-type and homothallic, same mating-type sexual cycles. And yet we need look no further than the model yeast *S. cerevisiae* to find a similar example. Many natural isolates of *S. cerevisiae* are homothallic because they express the Ho endonuclease that creates the breaks that provoke mating-type switching. However, among natural isolates about 25% are naturally occurring *ho*– mutants. These isolates are not homothallic, yet they retain their ability to undergo heterothallic mating with suitable partners. Thus in this well defined species, both homothallic and heterothallic isolates are part of the naturally occurring interbreeding set of isolates we consider the species. Another way to consider this is that even homothallic fungal species are capable of outcrossing with a suitable partner and yet they are also uniquely endowed with the ability to self. It may be that these represent examples of a species hedging its bets, because one or the other mating strategy might prove more adaptive in response to different types of environments, selective pressures or the absence of an appropriate partner of opposite mating type. This plasticity may be a form of environmental sex determination/orientation similar to the earlier examples in fishes.

We have exerted considerable effort in the laboratory comparing and contrasting the features of both opposite-sex and same-sex mating. We found, for example, that ploidy changes from haploid to diploid to haploid occur in both. We also found that key meiotic genes, including *SPO11*, which encodes the endonuclease that makes DNA double strand breaks that provoke meiotic recombination, and *DMC1*, which encodes a meiotic recombinase, are both required for the efficient production of viable, fertile spores. Self-fertile strains lacking either produce stunted spore chains, in some cases only two instead of four spore chains, and the germination frequency

of the fewer spores produced was dramatically reduced. In addition, we found that the frequency of meiotic recombination was similar and approximately equivalent in both opposite-sex and same-sex mating (this was established by constructing a special α/α diploid strain with distinguishable genetic markers to generate a meiotic map of its progeny). Furthermore, we found that genes involved in pheromone production and sensing appear to contribute to both types of sexual reproduction, perhaps reflecting a role for cell-cell fusion or stimulated nuclear-nuclear fusion, during same sex mating. Finally, one key genetic difference we found was that the homeodomain proteins that are required for opposite-sex mating are dispensable for same-sex mating. In one case there is an obvious explanation: Because the *SXI2***a** gene is specific to **a** cells, it is not present in α cells undergoing same-sex mating. But it also turns out that *SXI1*α is also not required for same-sex mating in several strains in which this has been tested. Given that the α mating type is the numerically dominant one and thus encounters with **a** cells are likely to be rare in the environment, the fact that we have not been able to find a role for *SXI1*α in unisexual mating for this conserved gene seems puzzling. It may be that there is some other mitotic function or a more subtle aspect of same-sex mating we have not yet been able to score experimentally.

Recent studies have revealed two other transcription factors that function in both opposite-sex and unisexual reproduction. One is the high mobility group (HMG) factor called Mat2 that has been implicated as the factor responsible for activating pheromone responsive genes downstream of the pheromone activated MAP kinase cascade (Lin et al. 2010, Kruzel et al. 2012). *MAT2* was identified via an *Agrobacterium* insertional mutagenesis screen for genes required for unisexual reproduction. The second factor identified is a zinc finger transcription factor, Znf2, which cooperates with Mat2 to operate signaling circuits that let cells engage in sexual reproduction in the absence of the homeodomain heterodimeric complex that normally would be required. *ZNF2* was identified via a transcriptional profiling approach to identify genes that were markedly induced during hyphal growth (Lin et al. 2010).

One additional question is frequently posed: If α cells can undergo unisexual reproduction, what about **a** cells? It turns out that both mating types can undergo unisexual reproduction, but in a quantitative trait analysis of genes that contribute to the fecundity of unisexual reproduction we found evidence for up to five QTL loci from a screen of ~ 25% of the genome, and thus as many as 20 or more QTL loci may contribute (Lin et al. 2006). Among the five QTL loci identified in this study, the *MAT* locus was found to be the one contributing the most to the trait (enhanced hyphal growth) and the α allele of *MAT* was better endowed to support unisexual reproduction than the **a** allele. Put another way, in a background in which many of the QTL alleles are those favorable for unisexual reproduction, the status of the *MAT* locus will be less important and **a** cells are self-fertile, whereas in backgrounds where some of the QTL loci are those less favorable for unisexual reproduction, the status of the *MAT* locus is more important and only those that are α mating type will be fertile. There is a Poisson distribution of strain phenotypes, and the mean of those that are α mating type is more skewed toward hyphal growth, whereas the mean of the **a** mating-type isolates is skewed towards less hyphal growth.

Now these are all studies of same-sex mating under laboratory conditions, and two criticisms that can be leveled are whether this occurs only in the laboratory and whether it is relevant to biology of the species in nature. Studies over the past several years have provided a series of findings that suggest that same-sex mating could be a frequent and possibly even the predominant form of mating in nature. First, recent studies document that spores are bona fide infectious propagules, and given that the vast majority of isolates in nature are α mating type, a parsimonious model is that this may be the route via which infectious spores are produced in nature. Second, analysis of diploid serotype AD intervarietal hybrid strains revealed an unusual type that, instead of descending from mating between isolates of opposite mating type (**a**ADα or α**a**D**a**), were produced by unisexual mating between two α isolates and are therefore αADα hybrids (Lin et al. 2007). Third, a series of studies applying population genetics approaches reveals that populations of isolates from trees in India or from infected animals in Australia (all of which are mating-type α) are nonetheless recombining populations and in some cases also harbor α/α diploids that appear to be intermediates/products of same-sex mating (Bui et al. 2008, Hiremath et al. 2008, Saul et al. 2008). Fourth, studies have revealed a novel cell type that is present in the lungs of mice or humans infected with *Cryptococcus* that are termed giant or titan cells (Okagaki et al. 2010, Zaragoza et al. 2010). Quite remarkably, whereas the infecting yeast cells are ~ 5 μm diam, these giant cells are as large as ~ 100 μm. In addition, the starting cells are haploid, but the giant cells have been found to be octoploid. Genetic studies with marked strains suggest they are produced via endoreplication, although it is hard to exclude models

in which closely opposed mother and daughter cells might be fusing. These giant octoploid cells bud to produce daughter cells that are haploid or diploid. Essentially nothing is known about how ploidy increases and then reduces again in a stepwise fashion, but we submit that this might represent a modified form of same-sex mating occurring in the context of infection of the mammalian host and that ploidy reduction might involve meiotic processes also occurring in the infected lung.

WHY HAVE SEX WITH YOURSELF?

From a broad survey of the ploidy of almost 500 isolates of *C. neoformans*, we found that almost 10% were diploid (Lin et al. 2009). This was surprising because the dogma was that this species is haploid. And while diploids had been identified previously in the context of intervarietal AD hybrids, the majority of the diploids we identified were not and instead were AA hybrids produced by intravarietal fusion. Examination of the mating-type locus configuration further revealed that almost all were αAAα isolates produced via same-sex mating. One class was clearly heterozygous for different genetic markers, and thus the result of fusion of two genetically distinct parental isolates, similar to opposite-sex mating. However, the majority were apparently homozygous at all the genetic loci we examined, suggesting they resulted from either endoreplication or mother-daughter cell fusion. This finding perplexed us for some time. At the root of this is the question: Why mate with yourself if there is no genetic diversity to admix in your progeny? We considered a number of possible explanations, including that this might be a route to produce spores, which might be hardier in the environment and also airborne and therefore more readily dispersed. We considered that the hyphae produced by same-sex mating and diploid isolates might be better able to invade the growth substratum and extract nutrients. We even considered that same-sex mating might be a form of practice. In this model, isolates that undergo periodic rounds of same-sex mating might have enhanced fitness compared to isolates that do not and evolve to be asexual more quickly. Thus, same-sex mating serves to preserve fecundity and confers an evolutionary advantage compared to isolates that instead evolve to be asexual.

But at some level these models all seemed to be ad hoc explanations. This led us to consider whether there might be another underlying reason, and we again returned to question the central premises for the function and nature of sex: to admix pre-existing genetic diversity of two different parental isolates. But what if the function of sex could be to generate genetic diversity de novo, rather than to simply admix it. In such a model sex would be a mutagen and serve to introduce novel genetic diversity for genetic selection to act upon (TABLE I).

Are there precedents for this type of mutagenic process that we know about in biology? Yes! First, you probably are aware that B lymphocytes of the immune system, which are the antibody producing cells, go to great trouble to assemble an active antibody encoding gene via a process termed VDJ recombination. And then they do something odd: They riddle the resulting antibody gene they have just so carefully constructed with mutations, in a process termed somatic hypermutation. The purpose is to diversify the antibody repertoire, and in fact it recently has been found that this process is able to generate high affinity neutralizing antibodies against HIV, but this often takes years to occur and thus is not sufficiently efficient or rapid to control HIV earlier during infection (Chen et al. 2010, Wu et al. 2011). A second example involves the phenomena of mutators that arise in bacterial pathogens (Miller 1996, Oliver et al. 2000). These are often mutations that arise in the mismatch repair system, the genome is sprinkled with mutations, yet ultimately the mutator is selected against because of longer term deleterious effects. But we can recognize the effect of the mutator by the sequence of the starting and evolved strain and the presence of a multitude of mutations that have been introduced genome wide and exhibit the sequence bias signatures of the mutator that was responsible. Finally, a third example is the intrinsic error rate of DNA polymerases, without which humans might not even be here as a species, given that these errors contribute to adaptive evolution. More accurate polymerase mutants can be selected, but the cost they incur is that they spend too much time contemplating which nucleotide they have inserted, fail to replicate the genome in time for cell division to occur and as a consequence slow growth dramatically and are selected against (Reha-Krantz 1998). Ultimately a balance between accuracy and efficiency is struck, and a certain level of mutations is not only tolerated, but in some cases adaptive. Thus, mutations, while clearly detrimental under many circumstances, also can be beneficial.

We thus considered whether unisexual reproduction might be mutagenic and serve to generate genetic diversity de novo. One of the great advantages of working in mycology is that, if you have an interesting model, it can be the case that some other investigator has tested aspects of this in a different organism. This turned out to be the case here. Rolf Hoekstra's lab conducted a beautiful series of experiments in *Aspergillus nidulans* that sought to

address the effect of ploidy on adaptive evolution (Schoustra et al. 2007). They constructed isogenic strains that were haploid and diploid and subjected them to repeated rounds of laboratory passage, selecting for faster growing variants. They found that these faster growing variants arose much more frequently from the diploid compared to the haploid background. But remarkably, with further analysis they found that all faster growing variants that arose from a diploid background were instead haploid. Through a series of crosses and genetic analyses, they deduced that the reason for this is a phenomenon termed reverse epistasis. In essence, recessive mutations arose in the diploid background that would be deleterious on their own but when combined are beneficial. These mutations do not arise in the haploid because the intermediate single mutants are less fit. But they propose that the diploid serves as a capacitor for evolution, letting multiple different recessive mutations arise in the shelter of the diploid genome. Subsequently, these mutations are released into the haploid state via the parasexual cycle of *A. nidulans* and in the haploid state together interact to enhance growth compared to the starting haploid or diploid wild type background.

Clearly here the parasexual cycle can serve to generate genetic diversity de novo, and our leap of faith was to consider that sexual cycles might operate similarly. We have been testing this experimentally with a strain (XL280) that undergoes robust unisexual reproduction during solo culture on V8 or MS medium (Ni and Heitman unpubl). We generated 96 progeny by mitotic asexual growth and 96 progeny that were derived by germinating spores produced by unisexual reproduction. We next assessed them for phenotypic and genotypic plasticity. The progeny produced by mitotic growth are all boring and look exactly like the parent in a panel of phenotypic assays. In contrast, from a screen for variants in five different phenotypic tests, we found that ~ 5% of progeny produced by unisexual reproduction differ from the parental isolate. Thus, unisexual reproduction can generate considerable phenotypic diversity, even though there is no genetic diversity to admix from two different parents because there is only one parent. We then subjected these variant progeny to a series of genotypic tests, including comparative genome hybridization (CGH), pulsed field gel analysis with band CGH for any variant chromosomes identified and next generation sequencing. We initially thought that we might find a high rate of single nucleotide polymorphisms. In fact, we found few from three progeny sequenced to high coverage spanning the ~ 20 Mb genome. What we did find were examples of intracentromeric deletions, chromosomal translocations and high aneuploidy. In fact, all our variant progeny were aneuploid for one of several different chromosomes. When isolates with a wild-type phenotype were recovered, we invariably found that they now exhibited a euploid karyotype, providing direct evidence that aneuploidy can significantly contribute to the altered phenotypes observed.

At this stage you are probably thinking that aneuploidy is a bad thing, and you would be correct. We need look no further than human biology, in which we know that only three trisomies in humans (2N + 1) can survive to birth and these result in Edwards, Patau and Down syndrome, in which there are profound health consequences. But there is both a yin and yang to aneuploidy. Studies over the past several years have brought aneuploidy to the fore in studies of drug resistance and adaptive evolution in fungi. In *Candida albicans*, treatment with fluconazole leads to azole resistant isolates, and many of these turn out to harbor an unusual isochromosome 5 in which the left arm has been duplicated via recombination involving centromeric flanking repeats (Selmecki et al. 2006). As a consequence, the genes encoding the direct target of fluconazole (Erg11) and the transcription factor that drives expression of drug efflux pumps (Tac1) that are normally harbored as single copies on the left arm of chromosome 5 were now found in four instead of two copies in the aneuploid strains. There is thus a direct and adaptive benefit conferred by aneuploidy, and this is a significant cause of drug resistance in patients treated with the most broadly used azole in our antifungal drug armamentarium. Recent studies reveal in *C. neoformans* that an extra copy of chromosome 1 (disomy, 1N + 1 aneuploid) is common and similarly confers resistance to fluconazole (Hu et al. 2008, Sionov et al. 2010).

Recent studies in *S. cerevisiae* have documented that aneuploidy for any chromosome results in a common signature of phenotypes, likely by leading to imbalances in the ratio of subunits of multiprotein complexes (Torres et al. 2007, 2010). Moreover, aneuploidy has been implicated in driving phenotypic diversity and enabling adaptive evolution of strains compromised via loss of proteins (such as molecular motors) normally required for proper cell division and viability (Pavelka et al. 2010, Rancati et al. 2008). Taken together, these studies in both pathogenic and model yeasts bring aneuploidy to the forefront of thinking about genotypic plasticity that can rapidly generate phenotypic diversity even in the absence of outcrossing. And the fact that sexual reproduction and meiosis can readily lead to aneuploidy by promoting a variety of types of chromosome nondisjunction and occur at an elevated rate in some fungi,

such as *Candida lusitaniae* (Reedy et al. 2009), suggest that sex in nature may be messier than we typically give it credit for, given that much of the focus in the field has been on strains that undergo robust meiosis, producing well behaved tetrads in which all spores germinate. It may even turn out to be the case that genetic determinants that promote aneuploidy during sex and meiosis remain to be identified.

UNISEXUAL REPRODUCTION: BEYOND *CRYPTOCOCCUS*

Thus far we have been considering unisexual reproduction of just one species, *Cryptococcus*, and a central question is whether other fungal species exhibit a similar mode of homothallic selfing. Recent studies from Kevin Alby, a graduate student working with Richard Bennett at Brown University, have shown that unisexual reproduction also occurs in *Candida albicans*, another representative of the three most common systemic human fungal pathogens (Alby et al. 2009). For more than a century it was thought that *Ca. albicans* was asexual. But with the discovery of the *Ca. albicans* mating-type locus by Christina Hull when she was a graduate student with Sandy Johnson at UCSF (Hull and Johnson 1999) and then the discovery of mating under laboratory conditions (Hull et al. 2000, Magee and Magee 2000), we now appreciate that there is a conserved **a**-α opposite-sex parasexual cycle in *Ca. albicans* (Miller and Johnson 2002; Bennett and Johnson 2003, 2005; Forche et al. 2008). The key advance of Alby and Bennett was to discover conditions under which **a** cells can mate with **a** cells in *Ca. albicans*. First, they and others found that **a** cells transcriptionally express both mating pheromones, and when the Bar1 protease that destroys the α factor is mutated, **a** cells produce sufficient α mating pheromone, auto-respond, form unusual wrinkled colonies, and mate with other **a** cells. This suggests that natural conditions may exist in which Bar1 is inactivated by mutation or specific conditions. For example, the *S. cerevisiae* homolog is less active under acidic conditions, and thus in the acidic pH of the vaginal mucosa, where the pH is ~ 4 due to high acetic acid, unisexual mating of *Ca. albicans* might occur. Another possible way in which Bar1 might be inhibited is by production of pseudo substrate mimics, or even pheromones from other related species that bind to and inhibit the protease. The second condition found to support unisexual mating of *Ca. albicans* involves special mating conditions in which three types of partners are present, two **a** strains and then a limiting number of α cells that serve as pheromone donors that promote like-with-like cell fusions, a so-called ménage a trios mating reaction, which was described first in *C. neoformans* (Hull et al. 2002, Hull and Heitman 2002, Lin et al. 2005). The mechanisms via which same-sex mating is accomplished differ between *C. neoformans* and *Ca. albicans*, indicating that these are independent origins of unisexual reproduction in the two species that lie in different fungal phyla and are diverged by 500–1000 million years. It is quite striking that two of the three most successful systemic human fungal pathogens have not only retained sexual cycles, but also the ability to complete two distinct types of sexual reproduction involving heterothallic opposite-sex mating and homothallic same-sex mating.

There is an expression in Japanese that things that happen once happen once, but things that happen twice will happen a third time. We therefore are very interested in which species will be the third example of unisexual reproduction. Given that there are an estimated 1.5–5 million fungal species, we may have a prodigious task to find the next example. On the other hand, *C. neoformans* and *Ca. albicans* are among the best-studied fungi of the entire kingdom and thus the phenomenon may be universal but universally overlooked because it does not fit into the standard fungal genetics paradigm. But given that the first two examples are successful human fungal pathogens, one way to focus the search involves examining other fungi that infect humans. One that we are currently focusing on is *Trichophyton rubrum*, the fungus that causes athletes foot and other skin and nail infections. The population is clonal, unisexual, and thus far has no known sexual cycle. Another approach is to scour the literature for any examples of this type of sexual behavior. It turns out that there are several *Neurospora* species (*N. africana*, *N. lineolata*, *N. galapagosensis* and *N. dodgei*) that harbor only the *MAT1-1* idiomorph but nonetheless exhibit homothallic sexual reproduction (Glass et al. 1988, 1990; Glass and Smith 1994; Gioti et al. 2012). While clearly not human pathogens, these represent other candidates for the emergence of a novel form of homothallism, unipolar reproduction, in which one mating partner suffices for sex to occur.

While beyond the scope of this Karling Lecture review, that by virtue of its nature and forum has a focus on mycology, it is worth considering whether these same types of unusual modes of sexual reproduction may operate in other eukaryotic microorganisms. In fact, recent studies focused on an entirely different, broad group of microbial pathogens of humans, namely the protozoan parasites, has revealed that, like the pathogenic fungi, these organisms have retained sexual cycles that in many cases are cryptic and also involve selfing modes of sexual reproduction. In contrast to fungi, in which the mating-type locus is an extremely well established

molecular paradigm, we know almost nothing about how mating types are established at a molecular level in any of the pathogenic protozoan parasites of humans. But what we have come to appreciate is that many of these organisms have unusual sexual cycles.

Let us consider *Giardia* as one paradigmatic example. This organism infects the GI tract and is a common cause of diarrheal disease globally. It was thought to be asexual, until its genome was sequenced and genes involved in meiosis were found to be present (Ramesh et al. 2005). Then population geneticists found evidence that the population is recombining (Cooper et al. 2007). Finally, Zac Cande's lab at UCSF discovered a novel form of sexual reproduction involving nuclear fusion and genetic exchange in this binucleate organism (something like the dikaryotic hyphae of the Basidiomycota) (Poxleitner et al. 2008). They also found that key meiotic genes are induced when the trophozoite matures into the cyst stages and these proteins also localize to the nucleus, including homologs of Spo11 that makes DSB to stimulate meiotic recombination and Dmc1, the meiotic recombinase (Poxleitner et al. 2008). We are still missing conditions that enable isolates to fuse in nature, which seems a natural prerequisite for genetic recombination in the population, but clearly there is the capacity for a selfing genetic cycle that can generate recombinant genotypes between the two nuclear genotypes. Similar findings have emerged for *Leishmania*, *Trypanosoma brucei* and *T. cruzi*. As for *Toxoplasma gondii*, well known to undergo its sexual cycle in the GI tract of cats and other felids, recent studies revealed a profound impact of selfing on the generation of infectious zoospores that cause outbreaks in both humans and other animals (Wendte et al. 2010). These parallels between sexual reproduction in pathogenic fungi and pathogenic parasites have been reviewed in considerable detail, and the interested reader is referred to these other sources (Heitman 2006, 2010).

WHY HAS UNISEXUAL REPRODUCTION ARISEN INDEPENDENTLY IN DIFFERENT LINEAGES?

Why has unisexual reproduction arisen repeatedly and independently, both in pathogenic fungi, possibly model fungi, and pathogenic protozoans? Our contention is that unisexual reproduction has arisen multiple times because it can mitigate several of the costs associated with sex that we introduced at the beginning of this treatise. First, no longer are only 50% of any given parent's genes transmitted to progeny, and instead 50 to as much of 100% of parental genes are transmitted to progeny by unisexual reproduction, and even more than 100% if you consider aneuploidy! Second, the time and energy to find a mating partner are eliminated if you simply mate with yourself. Third, sex no longer breaks apart well adapted genomic configurations but instead preserves them while still introducing a much more limited genetic diversity, superimposed upon a well adapted genotype that has run the gauntlet of adaptive Darwinian selection. And this may well emerge as a general mechanism for adaptation in pathogenic microbes as well as those in the environment.

Let us summarize then what we have learned. First, sex can be unisexual. Second, sex is mutagenic and can generate diversity de novo. Third, unisexual reproduction mitigates costs of sex and has evolved repeatedly and independently. Fourth, pathogenic fungi are not asexual but can be cryptically sexual or unisexual. Finally, our musings about an unusual form of sexual reproduction in fungi lead us to consider that unisexual reproduction could have been the ancestral form of sex in the eukaryotic tree of life. Perhaps when sex first evolved, it was a selfing mode of reproduction that was inherently mutagenic and diversity generating. This may reflect the view that early on, sex was likely about DNA repair, and if serious errors occurred during replication, there was another copy available to use to correct. And it might be that the more complex versions involving mating types and sexes came later and were embellishments upon a simpler form of unisexual reproduction. If so, then the recent transitions of some species from opposite-sex mating to unisexual reproduction simply recapitulate more ancient transitions in the ancestral evolution of sex itself.

Now we have considered fungi and protozoan parasites, but what about other eukaryotes, such as plants and animals? Do they have anything like unisexual reproduction? Plants are a highly successful super group within the eukaryotic tree of life. Moreover, we know that plants are capable of both self-pollination and cross-pollination. In fact, transitions from cross- to self-pollination are common in plants and are thought to underlie the transition from provincial niches to enabling species to become cosmopolitan. In fact, in the model plant *Arabidopsis thaliana*, it is self-pollinating $\sim$ 99% of the time and only cross-pollinating 1% of the time, and this may underlie the ability of this plant to colonize most of the planet, given that it does not have to find a mating partner to reproduce.

What about animals? Several years ago, a series of reports about virgin births in sharks emerged. In essence, astute aquaria keepers noted that females housed in tanks in the absence of males became pregnant and gave birth to live young. They ruled out

that these were some type of intersex individual or that sharks store sperm for years after mating in the ocean. Genetic analyses revealed that these are examples of parthenogenesis (Chapman et al. 2007). Because sharks have an XY sex-determining system, all offspring are daughters. Concurrently reports emerged that Komodo dragons that had not been fertilized lay eggs capable of hatching to produce live young. Zookeepers had known for years that Komodo dragons lay eggs but always assumed these would be sterile. But some curious zookeepers decided to watch these eggs and observed they can hatch. This turns out to be another example of parthenogenesis (Watts et al. 2006), and because Komodo dragons have the more unusual ZW sex-determining system (where female is ZW and males are ZZ), they can have male offspring. One suggestion was that a lone Komodo dragon female could swim up on a deserted island, give birth to a live male young, and then restart an opposite sex sexual cycle, albeit with a genetic bottleneck as the result of parthenogenesis. What about in mammals? We know that imprinting normally serves as a barrier to parthenogenesis by genetically marking the sperm and egg differently marks. But in mice with mutations in the imprinting system, pups born via parthenogenesis can survive to birth and beyond (Kono et al. 2004). And in humans, while there are no reports of parthenogenetic births, there is a case in which ES cells were derived from embryos via parthenogenesis (Kim et al. 2007).

Thus, unusual patterns of sexual reproduction initially discovered in kingdom Fungi turn out to have profound implications for modes of sexual reproduction in other eukaryotic microbes and some of these principles even extend to plants and in some cases animals. Moreover, fungi, especially yeast, has served as the best models for understanding the evolutionary importance of sex, ploidy and mate recognition using experimental approaches (Zeyl and Bell 1997, Anderson et al. 2004, Xu 2005, Gerstein et al. 2011). Finally, the pheromone signaling pathway of yeast stands as the best worked example of signal transduction and gene interactions in all eukaryotes, which is fortunate for mycologists because all Dikarya appear to use the same pheromone-based mechanism for reproduction. Yet, both outside and within Dikarya there are many more biological questions and mysteries that remain to be solved from the perspective of the fungal kingdom.

ACKNOWLEDGMENTS

This review is based, in part, on the Karling lecture delivered at the Mycological Society of America meeting in Fairbanks, Alaska, 1–6 Aug 2011, which was dedicated to John and Cardy Raper for their pioneering work on sex in fungi.

LITERATURE CITED

Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, Piepenbring M, McLaughlin DJ, Szabo LJ, Begerow D, Sampaio JP, Bauer R, Weiß M, Oberwinkler F, Hibbett D. 2006. An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. Mycologia 98:896–905, doi:10.3852/mycologia.98.6.896

Aimi T, Yoshida R, Ishikawa M, Bao D, Kitamoto Y. 2005. Identification and linkage mapping of the genes for the putative homeodomain protein (*hox1*) and the putative pheromone receptor protein homologue (*rcb1*) in a bipolar basidiomycete, *Pholiota nameko*. Curr Genet 48: 184–194, doi:10.1007/s00294-005-0012-7

Alby K, Schaefer D, Bennett RJ. 2009. Homothallic and heterothallic mating in the opportunistic pathogen *Candida albicans*. Nature 460:890–893, doi:10.1038/nature08252

Anderson JB, Sirjusingh C, Ricker N. 2004. Haploidy, diploidy and evolution of antifungal drug resistance in *Saccharomyces cerevisiae*. Genetics 168:1915–1923, doi:10.1534/genetics.104.033266

Bakkeren G, Jiang G, Warren RL, Butterfield Y, Shin H, Chiu R, Linning R, Schein J, Lee N, Hu G, Kupfer DM, Tang Y, Roe BA, Jones S, Marrac M, Kronstad JW. 2006. Mating factor linkage and genome evolution in basidiomycetous pathogens of cereals. Fungal Genet Biol 43:655–66, doi:10.1016/j.fgb.2006.04.002

———, Kamper J, Schirawski J. 2008. Sex in smut fungi: structure, function and evolution of mating-type complexes. Fungal Genet Biol 45(Suppl 1):S15–S21, doi:10.1016/j.fgb.2008.04.005

———, Kronstad JW. 1994. Linkage of mating-type loci distinguishes bipolar from tetrapolar mating in basidiomycetous smut fungi. Proc Natl Acad Sci USA 91: 7085–7089, doi:10.1073/pnas.91.15.7085

Baldauf SL. 2003. The deep roots of eukaryotes. Science 300:1703–1706, doi:10.1126/science.1085544

———, Palmer JD. 1993. Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins. Proc Nat Acad Sci USA 90:11558–11562, doi:10.1073/pnas.90.24.11558

Bennett RJ, Johnson AD. 2003. Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. EMBO J 22:2505–2515, doi:10.1093/emboj/cdg235

———, ———. 2005. Mating in *Candida albicans* and the search for a sexual cycle. Ann Rev Microbiol 59:233–255, doi:10.1146/annurev.micro.59.030804.121310

Bölker M, Urban M, Kahmann R. 1992. The a mating-type locus of *U. maydis* specifies cell signaling components. Cell 68:441–450, doi:10.1016/0092-8674(92)90182-C

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N, Plamann M, Goodrich-Tanrikulu M, Schulte U, Mannhaupt G, Nargang FE, Radford A, Selitrennikoff C, Galagan JE, Dunlap JC, Loros JJ, Catcheside D, Inoue H, Aramayo R, Polymenis M, Selker EU, Sachs MS, Marzluf GA, Paulsen I, Davis R, Ebbole DJ, Zelter

A, Kalkman ER, O'Rourke R, Bowring F, Yeadon J, Ishii C, Suzuki K, Sakai W, Pratt R. 2004. Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108, doi:10.1128/MMBR.68.1.1-108.2004

Bradley KM, Breyer JP, Melville DB, Broman KW, Knapik EW, Smith JR. 2011. An SNP-based linkage map for zebrafish reveals sex determination loci. G3 1:3–9.

Bui T, Lin X, Malik R, Heitman J, Carter D. 2008. Isolates of *Cryptococcus neoformans* from infected animals reveal genetic exchange in unisexual, alpha mating-type populations. Eukaryot Cell 7:1771–80, doi:10.1128/EC.00097-08

Byrnes EJ III, Bartlett KH, Perfect JR, Heitman J. 2011. *Cryptococcus gattii*: an emerging fungal pathogen infecting humans and animals. Microbes Infect 13:895–907, doi:10.1016/j.micinf.2011.05.009

———, Li W, Ren P, Lewit Y, Voelz K, Fraser JA, Dietrich FS, May RC, Chaturvedi S, Chaturvedi V, et al. 2011. A diverse population of *Cryptococcus gattii* molecular type VGIII in southern Californian HIV/AIDS patients. PLoS Pathog 7:e1002205, doi:10.1371/journal.ppat.1002205

Casselton LA. 2002. Mate recognition in fungi. Heredity 88:142–147, doi:10.1038/sj.hdy.6800035

———, Olesnicky NS. 1998. Molecular genetics of mating recognition in Basidiomycete fungi. Microbiol Molec Biol Rev 62:55–70.

Chapman DD, Shivji MS, Louis E, Sommer J, Fletcher H, Prodöhl PA. 2007. Virgin birth in a hammerhead shark. Biol Lett 3:425–427, doi:10.1098/rsbl.2007.0189

Chen W, Zhu Z, Liao H, Quinnan GV, Broder CC, Haynes BF, Dimitrov DS. 2010. Cross-reactive human IgM-derived monoclonal antibodies that bind to HIV-1 envelope glycoproteins. Viruses 2:547–565, doi:10.3390/v2020547

Coelho MA, Sampaio JP, Goncalves P. 2010. A deviation from the bipolar-tetrapolar mating paradigm in an early diverged basidiomycete. PLoS Genetics 6:e1001052, doi:10.1371/journal.pgen.1001052

Cooper MA, Adam RD, Worobey M, Sterling CR. 2007. Population genetics provides evidence for recombination in Giardia. Curr Biol 17:1984–1988, doi:10.1016/j.cub.2007.10.020

Findley K, Rodriguez-Carres M, Metin B, Kroiss J, Fonseca A, Vilgalys R, Heitman J. 2009. Phylogeny and phenotypic characterization of pathogenic *Cryptococcus* species and closely related saprobic taxa in the *Tremellales*. Eukaryot Cell 8:353–361, doi:10.1128/EC.00373-08

———, Sun S, Fraser JA, Hsueh Y-P, Averette AF, Li W, Dietrich FS, Heitman J. 2012. Discovery of a modified tetrapolar sexual cycle in *Cryptococcus amylolentus* and the evolution of *MAT* in the *Cryptococcus* species complex. PLoS Genet 8:e1002528, doi:10.1371/journal.pgen.1002528

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martínez AT, Otillar R, Spatafora JW, Yadav JS, Andrea Aerts, Benoit I, Boyd A, Carlson A, Copeland A, Coutinho PM, de Vries RP, Ferreira P, Findley K, Foster B, Gaskell J, Glotzer D, Górecki P, Heitman J, Hesse C, Hori C, Igarashi K, Jurgens JJ, Kallen N, Kersten P, Kohler A, Kües U, Kumar TKA, Kuo A, LaButti K, Larrondo LF, Lindquist E, Ling A, Lombard V, Lucas S, Lundell T, Martin R, McLaughlin DJ, Morgenstern I, Morin E, Murat C, Nagy LG, Nolan M, Ohm RA, Patyshakuliyeva A, Rokas A, Ruiz-Dueñas FJ, Sabat G, Salamov A, Samejima M, Schmutz J, Slot JC, St John F, Stenlid J, Sun H, Sun S, Syed E, Tsang A, Wiebenga A, Young D, Pisabarro A, Eastwood DC, Martin F, Cullen D, Grigoriev IV, Hibbett DS. 2012. The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719, doi:10.1126/science.1221748

Forche A, Alby K, Schaefer D, Johnson AD, Berman J, Bennett RJ. 2008. The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biol 6:e110, doi:10.1371/journal.pbio.0060110

Foulongne-Oriol M, Spataro C, Savoie J-M. 2009. Novel microsatellite markers suitable for genetic studies in the white button mushroom *Agaricus bisporus*. Appl Microbiol Biotechnol 84:1125–1135, doi:10.1007/s00253-009-2030-8

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 Biology 2:e384, doi:10.1371/journal.pbio.0020384

———, Heitman J. 2003. Fungal mating-type loci. Curr Biol 13:R792–5, doi:10.1016/j.cub.2003.09.046

———, ———. 2005. Chromosomal sex-determining regions in animals, plants and fungi. Curr Opin Genet Develt 15:645–651, doi:10.1016/j.gde.2005.09.002

Gerstein AC, Cleathero LA, Mandegar MA, Otto SP. 2011. Haploids adapt faster than diploids across a range of environments. J Evol Biol 24:531–540, doi:10.1111/j.1420-9101.2010.02188.x

Gioti A, Mushegian AA, Strandberg R, Stajich JE, Johannesson H. 2012. Unidirectional evolutionary transitions in fungal mating systems and the role of transposable elements. MolecBiol Evol 29:3215–3226.

Giraud T. 2004. Patterns of within population dispersal and mating of the fungus *Microbotryum violaceum* parasitising the plant *Silene latifolia*. Heredity 93:559–565, doi:10.1038/sj.hdy.6800554

Glass NL, Metzenberg RL, Raju NB. 1990. Homothallic Sordariaceae from nature: the absence of strains containing only the **a** mating-type sequence. Exp Mycol 14:274–289, doi:10.1016/0147-5975(90)90025-O

———, Smith ML. 1994. Structure and function of a mating-type gene from the homothallic species *Neurospora africana*. Mol Gen Genet 244:401–409, doi:10.1007/BF00286692

———, Vollmer SJ, Staben C, Grotelueschen J, Metzenberg RL, Yanofsky C. 1988. DNAs of the two mating-tye alleles of *Neurospora crassa* are highly dissimilar. Science 241:570–573, doi:10.1126/science.2840740

Goddard MR, Godfray HC, Burt A. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. Nature 434:636–640.

Heitman J. 2006. Sexual reproduction and the evolution

of microbial pathogens. Curr Biol 16:R711–R725, doi:10.1016/j.cub.2006.07.064

———. 2010. Evolution of eukaryotic microbial pathogens via covert sexual reproduction. Cell Host Microbe 8:86–99, doi:10.1016/j.chom.2010.06.011

———, Kozel TR, Kwon-Chung JK, Perfect JR, Casadevall A. 2011. *Cryptococcus*: from human pathogen to model yeast. Washington DC: ASM Press. 576 p.

Herskowitz I. 1989. A regulatory hierarchy for cell specialization in yeast. Nature 342:749–757, doi:10.1038/342749a0

Hibbett DS. 2006. A phylogenetic overview of the Agaricomycotina. Mycologia 98:917–925, doi:10.3852/mycologia.98.6.917

———, Donoghue MJ. 2001. Analysis of character correlations among wood decay mechanisms, mating systems and substrate ranges in Homobasidiomycetes. Syst Biol 50:215–242, doi:10.1080/10635150151125879

Hiremath SS, Chowdhary A, Kowshik T, Randhawa HS, Sun S, Xu J. 2008. Long-distance dispersal and recombination in environmental populations of *Cryptococcus neoformans* var. *grubii* from India. Microbiology 154:1513–1524, doi:10.1099/mic.0.2007/015594-0

Hsueh Y-P, Fraser JA, Heitman J. 2008. Transitions in sexuality: recapitulation of an ancestral tri- and tetrapolar mating system in *Cryptococcus neoformans*. Eukaryot Cell 7:1847–1855, doi:10.1128/EC.00271-08

———, Heitman J. 2008. Orchestration of sexual reproduction and virulence by the fungal mating-type locus. Curr Opin Microbiol 11:517–524, doi:10.1016/j.mib.2008.09.014

Hu G, Liu I, Sham A, Stajich JE, Dietrich F, Kronstad JW. 2008. Comparative hybridization reveals extensive genome variation in the AIDS-associated pathogen *Cryptococcus neoformans*. Genome Biol 9:R41, doi:10.1186/gb-2008-9-2-r41

Hull CM, Davidson RC, Heitman J. 2002. Cell identity and sexual development in *Cryptococcus neoformans* are controlled by the mating-type-specific homeodomain protein Sxi1α. Genes Dev 16:3046–3060, doi:10.1101/gad.1041402

———, Heitman J. 2002. Genetics of *Cryptococcus neoformans*. Ann Rev Genet 36:557–615, doi:10.1146/annurev.genet.36.052402.152652

———, Johnson AD. 1999. Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans*. Science 285:1271–1275, doi:10.1126/science.285.5431.1271

———, Raisner RM, Johnson AD. 2000. Evidence for mating of the 'asexual' yeast *Candida albicans* in a mammalian host. Science 289:307–310, doi:10.1126/science.289.5477.307

Idnurm A, Bahn YS, Nielsen K, Lin X, Fraser JA, Heitman J. 2005. Deciphering the model pathogenic fungus *Cryptococcus neoformans*. Nat Rev Microbiol 3:753–64, doi:10.1038/nrmicro1245

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE,37, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R. 2006a. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822, doi:10.1038/nature05110

———, 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 10:249–261, doi:10.1128/EC.00212-10

———, Srivilai P, Kues U, Vilgalys R. 2006b. Evolution of the bipolar mating system of the mushroom *Coprinellus disseminatus* from its tetrapolar ancestors involves loss of mating-type-specific pheromone receptor function. Genetics 172:1877–1891, doi:10.1534/genetics.105.051128

Johnson LJ, Koufopanou V, Goddard MR, Hetherington R, Schäfer SM, Burt A. 2004. Population genetics of the wild yeast *Saccharomyces paradoxus*. Genetics 166:43–52, doi:10.1534/genetics.166.1.43

Jokela J, Dybdahl MF, Lively CM. 2009. The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. The Am Nat 174:S43–S53.

Karos M, Chang YC, McClelland CM, Clarke DL, Fu J, Wickes BL, Kwon-Chung KJ. 2000. Mapping of the *Cryptococcus neoformans MAT*α locus: presence of mating type-specific mitogen-activated protein kinase cascade homologs. J Bacteriol 182:6222–6227, doi:10.1128/JB.182.21.6222-6227.2000

Keller SM, Viviani MA, Esposto MC, Cogliati M, Wickes BL. 2003. Molecular and genetic characterization of a serotype A *MAT***a** *Cryptococcus neoformans* isolate. Microbiology 149:131–142, doi:10.1099/mic.0.25921-0

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, doi:10.1371/journal.pgen.1002436

Kim K, Ng K, Rugg-Gunn PJ, Shieh J-H, Kirak O, Jaenisch R, Wakayama T, Moore MA, Pedersen RA, Daley GQ. 2007. Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer. Cell Stem Cell 1:346–352, doi:10.1016/j.stem.2007.07.001

King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Li W, Lyons JB, Morris A, Nichols S, Richter DJ, Salamov A, Sequencing JGI, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Igor V, Grigoriev IV,

Rokhsar D. 2008. The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. Nature 451:783–788, doi:10.1038/nature06617

Kono T, Obata Y, Wu Q, Niwa K, Ono Y, Yamamoto Y, Park ES, Seo J-S, Ogawa H. 2004. Birth of parthenogenetic mice that can develop to adulthood. Nature 428:860–864, doi:10.1038/nature02402

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. 1991. Male development of chromosomally female mice transgenic for Sry. Nature 351:117–121, doi:10.1038/351117a0

Kruzel EK, Giles SS, Hull CM. 2012. Analysis of *Cryptococcus neoformans* sexual development reveals rewiring of the pheromone response network by a change in transcription factor identity. Genetics 191:435–449, doi:10.1534/genetics.112.138958

Kües U, James TY, Heitman J. 2011. Mating type in basidiomycetes: unipolar, bipolar and tetrapolar patterns of sexuality. In: Pöggeler S, Wöstemeyer J, eds. Evolution of fungi and fungal-like organisms. Mycota XIV:97–160, doi:10.1007/978-3-642-19974-5_6

Kwon-Chung KJ. 1975. A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. Mycologia 67: 1197–1200, doi:10.2307/3758842

———. 1976a. Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. Mycologia 68:821–833, doi:10.2307/3758800

———. 1976b. A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. Mycologia 68:943–946.

Lawrence GJ. 1980. Multiple mating-type specificities in the flax rust *Melampsora lini*. Science 209:501–503, doi:10.1126/science.209.4455.501

Lee N, Bakkeren G, Wong K, Sherwood JE, Kronstad JW. 1999. The mating-type and pathogenicity locus of the fungus *Ustilago hordei* spans a 500 kb region. Proc Natl Acad Sci USA 96:15026–15031, doi:10.1073/pnas.96.26.15026

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, doi:10.1128/MMBR.00005-10

Lengeler KB, Fox DS, Fraser JA, Allen A, Forrester K, Dietrich FS, Heitman J. 2002. Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. Eukaryot Cell 1:704–718, doi:10.1128/EC.1.5.704-718.2002

———, Wang P, Cox GM, Perfect JR, Heitman J. 2000. Identification of the *MAT***a** mating-type locus of *Cryptococcus neoformans* reveals a serotype A *MAT***a** strain thought to have been extinct. Proc Natl Acad Sci USA 97:14555–14460, doi:10.1073/pnas.97.26.14455

Lin X, Heitman J. 2007. Mechanisms of homothallism in fungi—transitions between heterothallism and homothallism. In: Heitman J, Kronstad JW, Taylor JW, Casselton LA, eds. Sex in Fungi: molecular determination and evolutionary implications. Washington DC: ASM Press. p 35–58.

———, Huang J, Mitchell T, Heitman J. 2006. Virulence attributes and hyphal growth of *C. neoformans* are quantitative traits and the *MAT*α allele enhances filamentation. PLoS Genet 2:e187, doi:10.1371/journal.pgen.0020187

———, Hull CM, Heitman J. 2005. Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature 434:1017–1021, doi:10.1038/nature03448

———, Jackson JC, Feretzaki M, Xue C, Heitman J. 2010. Transcription factors Mat2 and Znf2 operate cellular circuits orchestrating opposite- and same-sex mating in *Cryptococcus neoformans*. PLoS Genet 6:e1000953, doi:10.1371/journal.pgen.1000953

———, 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 3:1975–1990.

———, 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, doi:10.1371/journal.ppat.1000283

Loftus BJ, Fung E, Roncaglia P, Rowley D, Amedeo P, Bruno D, Vamathevan J, Miranda M, Anderson IJ, Fraser JA, Allen JE, Bosdet IE, Brent MR, Chiu R, Doering T, Donlin MJ, D'Souza CA, Fox DS, Grinberg V, Fu J, Fukushima M, Haas BJ, Huang JC, Janbon G, Jones SJM, Koo HL, Krzywinski MI, Kwon-Chung KJ, Lengeler KB, Maiti R, Marra MA, Marra RE, Mathewson CA, Mitchell TG, Pertea M, Riggs FR, Salzberg SL, Schein JE, Shvartsbeyn A, Shin H, Shumway M, Specht CA, Suh BB, Tenney A, Utterback TR, Wickes BL, Wortman JR, Wye NH, Kronstad JW, Lodge JK, Heitman J, Davis RW, Fraser CM, Hyman RW. 2005. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science 307:1321–1324, doi:10.1126/science.1103773

Magee BB, Magee PT. 2000. Induction of mating in *Candida albicans* by construction of MTLa and MTLα strains. Science 289:310–313, doi:10.1126/science.289.5477.310

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo J-M, Ge Z-W, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98:982–995, doi:10.3852/mycologia.98.6.982

McClelland CM, Chang YC, Varma A, Kwon-Chung KJ. 2004. Uniqueness of the mating system in *Cryptococcus neoformans*. Trends Microbiol 12:208–212, doi:10.1016/j.tim.2004.03.003

Metin B, Findley K, Heitman J. 2010. The mating type locus (*MAT*) and sexual reproduction of *Cryptococcus heveanensis*: insights into the evolution of sex and sex-determining chromosomal regions in fungi. PLoS Genetics 6: e10000961, doi:10.1371/journal.pgen.1000961

Miller JH. 1996. Spontaneous mutators in bacteria: insights into pathways of mutagenesis and repair. Annu Rev Microbiol 50:625–643, doi:10.1146/annurev.micro.50.1.625

Miller MG, Johnson AD. 2002. White-opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell 110:293–302, doi:10.1016/S0092-8674(02)00837-1

Morran LT, Schmidt OG, Gelarden IA, Parrish RC, Lively CM. 2011. Running with the red queen: Host-parasite coevolution selects for biparental sex. Science 333:216–218, doi:10.1126/science.1206360

Narisawa K, Yamaoka Y, Katsuya K. 1994. Mating type of isolates derived from the spermogonial state of *Puccinia coronata* var. *coronata*. Mycoscience 35:131–135, doi:10.1007/BF02318489

Ni M, Feretzaki M, Sun S, Wang X, Heitman J. 2011. Sex in fungi. Annu Rev Genet 45:405–430, doi:10.1146/annurev-genet-110410-132536

Nielsen K, Cox GM, Wang P, Toffaletti DL, Perfect JR, Heitman J. 2003. Sexual cycle of *Cryptococcus neoformans* var. *grubii* and virulence of congenic **a** and α isolates. Infect Immun 71:4831–4841, doi:10.1128/IAI.71.9.4831-4841.2003

Okagaki LH, Strain AK, Nielsen JN, Charlier C, Baltes NJ, Chrétien F, Heitman J, Dromer F, Nielsen K. 2010. Cryptococcal cell morphology affects host cell interactions and pathogenicity. PLoS Pathog 6:e1000953, doi:10.1371/journal.ppat.1000953

Olesnicky NS, Brown AJ, Dowell SJ, Casselton LA. 1999. A constitutively active G-protein-coupled receptor causes mating self-compatibility in the mushroom *Coprinus*. EMBO J 18:2756–2763, doi:10.1093/emboj/18.10.2756

———, ———, Honda Y, Dyos SL, Dowell SJ, Casselton LA. 2000. Self-compatible B mutants in *Coprinus* with altered pheromone-receptor specificities. Genetics 156:1025–1033.

Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science 288:1251–1253, doi:10.1126/science.288.5469.1251

Olson Å, Aerts A, Asiegbu F, Belbahri L, Bouzid O, Broberg A, Canbäck B, Coutinho PM, Cullen D, Dalman K, et al. 2012. Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytol 194:1001–1013, doi:10.1111/j.1469-8137.2012.04128.x

Parag Y. 1962. Mutations in the *B* incompatibility factor of *Schizophyllum commune*. Proc. Natl Acad Sci USA 48:743–750, doi:10.1073/pnas.48.5.743

Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23:525–530, doi:10.1097/QAD.0b013e328322ffac

Pavelka N, Rancati G, Zhu J, Bradford WD, Saraf A, Florens L, Sanderson BW, Hattem GL, Li R. 2010. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. Nature 468:321–325, doi:10.1038/nature09529

Poxleitner MK, Carpenter ML, Mancuso JJ, Wang CJ, Dawson SC, Cande WZ. 2008. Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. Science 319:1530–1533, doi:10.1126/science.1153752

Pukkila PJ. 2011. *Coprinopsis cinerea*. Curr Biol 21:R616–R617, doi:10.1016/j.cub.2011.05.042

Ramesh MA, Malik SB, Logsdon JM Jr. 2005. A phylogenomic inventory of meiotic genes: evidence for sex in Giardia and an early eukaryotic origin of meiosis. Curr Biol 15:185–91.

Rancati G, Pavelka N, Fleharty B, Noll A, Trimble R, Walton K, Perera A, Staehling-Hampton K, Seidel CW, Li R. 2008. Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. Cell 135:879–893, doi:10.1016/j.cell.2008.09.039

Raper J, Flexer A. 1971. Mating systems and evolution of the Basidiomycetes. In: Petersen R, ed. Evolution in the higher basidiomycetes. Knoxville: Univ Tennessee Press. p 149–167.

Raper JR. 1966. Genetics of sexuality in higher fungi. New York: Ronald Press Co. 283 p.

Reedy JL, Floyd AM, Heitman J. 2009. Mechanistic plasticity of sexual reproduction and meiosis in the *Candida* pathogenic species complex. Curr Biol 19:891–899, doi:10.1016/j.cub.2009.04.058

Reha-Krantz LJ. 1998. Regulation of DNA polymerase exonucleolytic proofreading activity: studies of bacteriophage T4 'antimutator' DNA polymerases. Genetics 148:1551–1557.

Rodriguez-Carres M, Findley K, Sun S, Dietrich FS, Heitman J. 2010. Morphological and genomic characterization of *Filobasidiella depauperata*: a homothallic sibling species of the pathogenic *Cryptococcus* species complex. PLoS ONE 5:e9620, doi:10.1371/journal.pone.0009620

Saul N, Krockenberger M, Carter D. 2008. Evidence of recombination in mixed-mating-type and alpha-only populations of *Cryptococcus gattii* sourced from single eucalyptus tree hollows. Eukaryot Cell 7:727–734, doi:10.1128/EC.00020-08

Schiebel K, Winkelmann M, Mertz A, Xu X, Page DC, Weil D, Petit C, Rappold GA. 1997. Abnormal XY interchange between a novel isolated protein kinase gene, *PRKY*, and its homolog, *PRKX*, accounts for one third of all (Y+)XX males and (Y−)XY females. Human Mol Genet 6:1985–1989, doi:10.1093/hmg/6.11.1985

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 4:1317–1327, doi:10.1128/EC.4.8.1317-1327.2005

Schoustra SE, Debets AJ, Slakhorst M, Hoekstra RF. 2007. Mitotic recombination accelerates adaptation in the fungus *Aspergillus nidulans*. PLoS Genet 3:e68, doi:10.1371/journal.pgen.0030068

Schulz B, Banuett F, Dahl M, Schlesinger R, Schafer W, Martin T, Herskowitz I, Kahmann R. 1990. The b alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell 60:295–306, doi:10.1016/0092-8674(90)90744-Y

Selmecki A, Forche A, Berman J. 2006. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. Science 313:367–70, doi:10.1126/science.1128242

Sharp A, Kusz K, Jaruzelska J, Tapper W, Szarras-Czapnik M, Wolski J, Jacobs P. 2005. Variability of sexual phenotype

in 46,XX(SRY+) patients: the influence of spreading X inactivation versus position effects. J Med Genet 42: 420–427, doi:10.1136/jmg.2004.022053

Simpson AGB, Roger AJ. 2004. The real 'kingdoms' of eukaryotes. Curr Biol 14:R693–R696, doi:10.1016/j.cub.2004.08.038

Sionov E, Lee H, Chang YC, Kwon-Chung KJ. 2010. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. PLoS Pathog 6: e1000848, doi:10.1371/journal.ppat.1000848

Stajich JE, Berbee ML, Blackwell M, Hibbett DS, James TY, Spatafora JW, Taylor JW. 2009. The Fungi. Curr Biol 19: R840–R845, doi:10.1016/j.cub.2009.07.004

Tibayrenc M, Kjellberg F, Arnaud J, Oury B, Breniere SF, Darde ML, Ayala FJ. 1991. Are eukaryotic microorganisms clonal or sexual? A population genetics vantage. Proc Natl Acad Sci USA 88:5129–5133, doi:10.1073/pnas.88.12.5129

———, ———, Ayala FJ. 1990. A clonal theory of parasitic protozoa: the population structures of Entamoeba, Giardia, Leishmania, *Naegleria*, *Plasmodium*, *Trichomonas* and *Trypanosoma* and their medical and taxonomical consequences. Proc Natl Acad Sci USA 87:2414–2418, doi:10.1073/pnas.87.7.2414

Torres EM, Dephoure N, Panneerselvam A, Tucker CM, Whittaker CA, Gygi SP, Dunham MJ, Amon A. 2010. Identification of aneuploidy-tolerating mutations. Cell 143:71–83, doi:10.1016/j.cell.2010.08.038

———, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, Amon A. 2007. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. Science 317:916–924, doi:10.1126/science.1142210

Viviani MA, Nikolova R, Esposto MC, Prinz G, Cogliati M. 2003. First European case of serotype A *MAT***a** *Cryptococcus neoformans* infection. Emerg Infect Dis 9: 1179–1180, doi:10.3201/eid0909.020770

Wainright P, Hinkle G, Sogin M, Stickel S. 1993. Monophyletic origins of the metazoa: an evolutionary link with fungi. Science 260:340–342, doi:10.1126/science.8469985

Watts PC, Buley KR, Sanderson S, Boardman W, Ciofi C, Gibson R. 2006. Parthenogenesis in Komodo dragons. Nature 444:1021–1022, doi:10.1038/4441021a

Wendte JM, Miller MA, Lambourn DM, Magargal SL, Jessup DA, Grigg ME. 2010. Self-mating in the definitive host potentiates clonal outbreaks of the apicomplexan parasites *Sarcocystis neurona* and *Toxoplasma gondii*. PLoS Genet 6:e1001261, doi:10.1371/journal.pgen.1001261

Whitehouse HLK. 1949. Multiple-allelomorph heterothallism in the fungi. New Phytol 48:212–244, doi:10.1111/j.1469-8137.1949.tb05120.x

Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, Chen X, Longo NS, Louder M, McKee K, et al. 2011. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science 333:1593–1602, doi:10.1126/science.1207532

Xu J. 2005. Cost of interacting with sexual partners in a facultative sexual microbe. Genetics 171:1597–1604, doi:10.1534/genetics.105.045302

Xu J, Saunders CW, Hu P, Grant RA, Boekhout T, Kuramae EE, Kronstad JW, DeAngelis YM, Reeder NL, Johnstone KR, et al. 2007. Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc Natl Acad Sci USA 104:18730–18735, doi:10.1073/pnas.0706756104

Yoshida K, Terai Y, Mizoiri S, Aibara M, Nishihara H, Watanabe M, Kuroiwa A, Hirai H, Hirai Y, Matsuda Y, et al. 2011. B chromosomes have a functional effect on female sex determination in Lake Victoria Cichlid fishes. PLoS Genet 7:e1002203, doi:10.1371/journal.pgen.1002203

Zaragoza O, García-Rodas R, Nosanchuk JD, Cuenca-Estrella M, Rodríguez-Tudela JL, Casadevall A. 2010. Fungal cell gigantism during mammalian infection. PLoS Pathog 6:e1000945, doi:10.1371/journal.ppat.1000945

Zeyl C, Bell G. 1997. The advantage of sex in evolving yeast populations. Nature 388:465–468, doi:10.1038/41312