The cryptic sexual strategies of human fungal pathogens

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Abstract | Sexual reproduction is a pervasive attribute of eukaryotic species and is now recognized to occur in many clinically important human fungal pathogens. These fungi use sexual or parasexual strategies for various purposes that can have an impact on pathogenesis, such as the formation of drug-resistant isolates, the generation of strains with increased virulence or the modulation of interactions with host cells. In this Review, we examine the mechanisms regulating fungal sex and the consequences of these programmes for human disease.

Parasexual reproduction
Form of reproduction in which transfer of genetic material and recombination occurs without meiosis or the development of sexual structures.

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Despite the prominence of sexual reproduction in eukaryotic species, the benefits of this reproductive strategy continue to be debated. Current models suggest that the ability of sexual reproduction to promote genetic variation is important for lineage survival. Sexual reproduction can promote adaptation to fluctuating environments and also limit the accumulation of deleterious alleles. However, sexual reproduction is associated with increased 'costs', such that asexual reproduction is predicted to be advantageous under many conditions, particularly as a short-term evolutionary strategy[1–3]. Moreover, sexual reproduction carries the risk of genetic conflicts and the breakdown of well-adapted genetic combinations. There is also the twofold cost of sexual reproduction, in which only 50% of parental alleles are passed on to any single progeny, and two parents are required to make one progeny.

The benefits of sexual versus asexual reproduction are further complicated in pathogenic species. Host–pathogen interactions can lead to cycles of co-adaptation and may result in species that have increased rates of genetic variation. Such an evolutionary 'arms race' between host and pathogen was formulated as the Red Queen hypothesis (BOX 1). In this model, sexual outcrossing in the host promotes adaptation, which enables the host to escape potential destruction by a co-evolving pathogen[4]. Evolution therefore favours faster-evolving lineages, enabling such species to stay ahead in the arms race. Conversely, lack of sexual reproduction results in the host being unable to outrun the pathogen, leading to extinction. Support for the Red Queen hypothesis has come from two experimental models in which sexual populations were found to be more resistant to infection but asexual (that is, self-fertilizing) populations were selected against[5,6] (BOX 1).

Studies show that co-evolution also drives accelerated evolution rates in pathogens[7,8]. Although the host persists by keeping one step ahead, the pathogen also adapts by undergoing co-evolutionary fine-tuning[9]. Moreover, in the case of a fungal plant pathogen, sexual reproduction enabled the rapid evolution of the pathogen and the infection of a more resistant host[10]. Thus, co-evolutionary interactions drive adaptive events both in the host and in the pathogen, and rapid evolution can be driven by sexual reproduction.

Historically, fungal studies have been used to address key questions concerning the molecular evolution of sexual reproduction and its role in promoting genetic diversity. Several prominent pathogenic species were traditionally thought to be clonal and thus restricted to asexual modes of reproduction. This was often due to the inability to observe sexual reproduction under laboratory conditions or to the long time periods that are required for productive mating. The idea that certain species were asexual was challenged by an increasing body of genomic evidence, which, supported by subsequent experimental approaches, has led to the discovery of extant sexual cycles in many clinically relevant species.

In this Review, we describe the sexual programmes of the most prevalent human fungal pathogens. These species are responsible for a wide range of diseases, from oral thrush and skin infections to fungal meningitis and bloodstream infections. Although largely clonal, most of these species have retained the molecular machinery and the ability to undergo sexual reproduction. Sexual or parasexual reproduction is used by these pathogens to shuffle genetic material within the cell, generating recombinant isolates that have altered drug resistance and pathogenicity. Furthermore, we discuss how sexual programmes exhibit high plasticity and the novel

Box 1 | **The Red Queen hypothesis**

The Red Queen model is an allusion to the race with the Red Queen in *Through the Looking-Glass* by Lewis Carroll, in which Alice and the Red Queen are constantly running but remain in the same spot. This model was proposed by Leigh Van Valen in 1973 to indicate that organisms must constantly adapt and evolve to survive a co-evolutionary 'race' with other organisms in an ever-changing environment[1]. In particular, sexual reproduction might enable organisms to escape and 'outrun' pathogens. As the adaptation of a species modifies the environment of neighbouring species and imposes selection onto these, cohabiting species play a 'null sum game' within their ecosystem.

The Red Queen hypothesis has received empirical support, which highlights that organisms capable of sexual reproduction have better chances of survival under stressful conditions than asexual species[5,6]. One study compared sexual and asexual snail lineages in an environment that contained natural parasites. Although initially more resistant to infection, the asexual lineage was soon outcompeted and the initial fitness advantage was lost. By contrast, sexual lineages persisted throughout the study, which supports the hypothesis that sexual reproduction gives animals an advantage in the battle against co-evolving parasites[5]. This was also the case in an interaction between *Caenorhabditis elegans* and a natural bacterial parasite, as all of the obligately selfing populations were driven to extinction, whereas the outcrossing population persisted[6].

mechanisms that have evolved between species to control this developmental pathway. Overall, studies of sexual reproduction in pathogenic fungi have redefined the paradigms concerning the life cycles of these species, their evolution and their ability to adapt to the mammalian host.

Discovery of extant sexual cycles

Among eukaryotes, sexual reproduction is ubiquitous, and exclusively asexual lineages are often evolutionary 'dead ends' owing to the accumulation of deleterious mutations; this is known as Muller's ratchet. It was therefore surprising that prominent fungal pathogens such as *Candida albicans* and *Aspergillus fumigatus* were, for many years, considered to be obligate asexual species. Analysis of genome sequences revealed that these species had retained many genes associated with mating and meiosis. Furthermore, they contained a mating-type (*MAT*) locus, which is a genetic locus encoding the transcription factors that are the master regulators of sexual reproduction[11–14]. Despite these discoveries, the exclusive use of genome sequences to establish the sexual fecundity of a species has proven to be difficult. Although a core set of conserved genes is associated with sexual reproduction in many species (for example, the 'meiosis detection toolkit' (REF. 15)), some genes that are described as markers of sexual activity (for example, *DMC1* and *HOP1*) are absent in species that are now known to be sexual[13,16]. These exceptions have challenged the idea that a set of conserved genes is shared by all sexual species, suggesting considerable plasticity in sexual strategies between species.

In the case of the three most important human fungal pathogens — *C. albicans*, *A. fumigatus* and *Cryptococcus neoformans* — the existence of sexual or parasexual cycles has now been established. Strikingly, each species seems to restrict access to this mode of reproduction, generating mostly clonal populations in nature. Why these species have retained the machinery for sexual reproduction despite the associated fitness costs, and the importance of sexual programmes for mediating interactions with the mammalian host, are now beginning to be revealed.

Mating-type loci and mate recognition

Fungi often exhibit bipolar or tetrapolar mating systems that are controlled by transcription factors encoded at the *MAT* locus (Supplementary information S1 (figure)). Bipolar systems have a single bi-allelic locus and include pathogenic ascomycetes such as *C. albicans* and *A. fumigatus* (Supplementary information S2 (figure)) as well as the model yeast *Saccharomyces cerevisiae*. Tetrapolar systems have two unlinked, multiallelic sex loci as exemplified by many basidiomycetes. Bipolar systems enable mating with half of the sibling offspring, thereby enabling both inbreeding (50% of offspring) and outbreeding (50% of offspring). By contrast, tetrapolar systems favour outbreeding by restricting mating to 25% of the sibling offspring (Supplementary information S1 (figure)).

Mate recognition occurs by mating-pheromone signalling. For bipolar systems, the expression of pheromone and pheromone-receptor genes is regulated by *MAT*-encoded transcription factors[17]. For tetrapolar systems, one *MAT* locus encodes regulatory homeodomain transcription factors, whereas the other locus encodes pheromones and pheromone receptors; the correct combination of pheromone, pheromone receptor and transcription factors is necessary for successful mating. Following stimulation, pheromone receptors turn on a highly conserved MAPK (mitogen-activated protein kinase) pathway, which results in the induction of a transcriptional mating response[18].

Interestingly, the most prominent human fungal pathogens exhibit bipolar mating-type systems. This is the case for *Cryptococcus gattii* and *C. neoformans*, even though the ancestor to these species had a tetrapolar mating system[19]. Studies have suggested that the ancestral pheromone locus and transcription factor locus gradually expanded by sequential rounds of gene acquisition, ultimately fusing into a single *MAT* locus that is >100 kb and encodes >20 genes[19–21] (Supplementary information S2 (figure)). Recombination at this locus led to the acquisition of several essential genes that restricted further large-scale rearrangements[19,22]. Recent studies identified recombination hot spots both flanking the *C. neoformans MAT* locus and within this locus[23,24]. These observations suggest that there is ongoing gene flow between the two mating types and that gene conversion can occur at genomic regions that were previously thought to be cold spots for recombination[23,24].

The mating-type-like (*MTL*) locus in *C. albicans* has also undergone expansion since diverging from the related hemiascomycete, *S. cerevisiae*. The *C. albicans MTL* locus includes three genes that are not present in the *S. cerevisiae MAT* locus, two of which are essential for growth and have roles that are unrelated to sexual reproduction[13,16,25] (Supplementary information S2 (figure)). The *C. albicans* **a**1 and α2 transcription factors encoded at the *MTL* locus, control sexual mating by

Muller's ratchet
The accumulation of deleterious mutations in an asexual population, which becomes so great that it leads to the extinction of the population.

Ascomycetes
The largest division in the fungal kingdom; they are commonly known as sac fungi. Their name stems from their defining sexual feature, ascus (in the form of an ascocarp or cleistothecium), which is where nuclear fusion and meiosis take place, resulting in the formation of ascospores.

Basidiomycetes
One of the two large phyla of Fungi that are typically known as higher fungi. They are most commonly filamentous fungi that reproduce sexually by forming round-shaped cells known as basidia, which bear external basidiospores.

Homeodomain
A 60 amino acid protein domain that folds into a helix–turn–helix compact structure and binds to DNA. Homeodomain folds are commonly found in transcription factors and they are found exclusively in eukaryotes, where they often induce cellular differentiation.

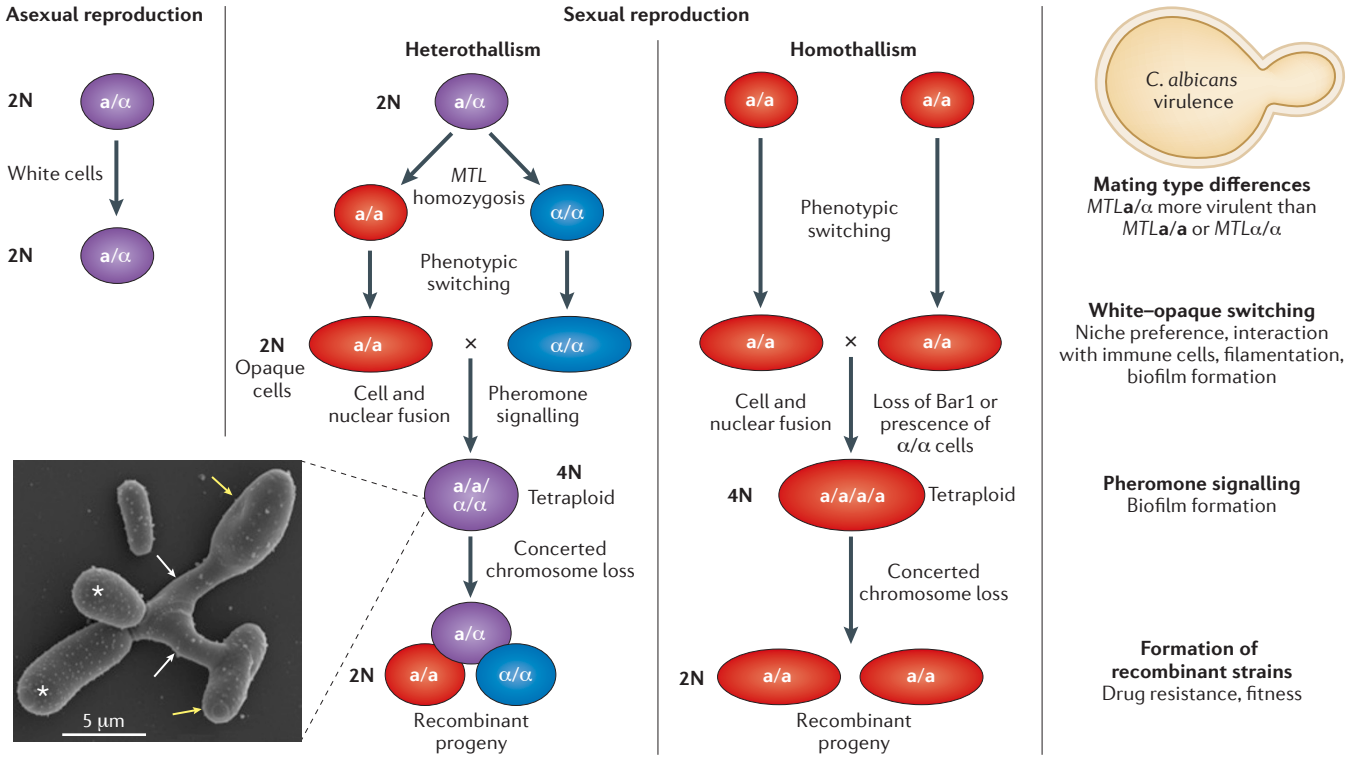


Figure 1 | **Asexual and parasexual reproduction in *Candida albicans*.** *Candida albicans* cells are diploid (2N) and can divide asexually or can undergo heterothallic or homothallic mating. Mating type-like locus **a** (*MTL***a**) and *MTL*α cells must switch from white to opaque to become mating-competent. Opaque cells secrete pheromones that result in the formation of conjugation tubes, and subsequently, cell and nuclear fusion occur to form tetraploid (4N) cells. Homothallic mating also occurs and can be driven by loss of Bar1 protease in *MTL***a** cells. Mating products can be induced to undergo concerted chromosome loss to return to the diploid state. The relationship between virulence and parasexual reproduction is indicated on the right-hand side of the figure. Inset, a scanning electron micrograph of a *C. albicans* mating zygote is shown. Parental opaque cells (yellow arrows) form mating projections (white arrows) and subsequently fuse and generate daughter cells (white asterisk). Scanning electron micrograph courtesy of M. P. Hirakawa, Brown University, Rhode Island, USA.

inhibiting a unique phenotypic switch that is necessary for conjugation (discussed below). Thus, both *C. albicans* and *C. neoformans* have evolved to have mechanisms that may restrict efficient outbreeding.

Studies on the evolution of sex-determining regions in fungi also have implications for the development of sex chromosomes in higher eukaryotes. Analogous to the case in *C. neoformans*, sex determinants that are located on one chromosome may have accrued additional sex regulatory genes via genome rearrangements, eventually forming sex chromosomes that are dimorphic between the two sexes[3,26].

Homothallism versus heterothallism

Fungi can have heterothallic sexual cycles — in which mating occurs between partners of different sexes and promotes outbreeding — or homothallic sexual cycles — in which organisms are self-fertile, promoting inbreeding. Transitions between heterothallic and homothallic lifestyles are common during fungal evolution; the switch from outcrossing to selfing enables the expansion of a species to niches in which encounters with opposite mating types are rare[3].

Limiting outbreeding might be particularly beneficial in human pathogens. Studies in protozoan parasites first indicated that eukaryotic pathogens generate highly clonal populations that undergo limited recombination[27]. Homothallic reproduction may help to preserve genomic configurations that are well adapted for growth in the host, while still enabling strains to undergo occasional recombination and reshuffling of their genomes[12].

Studies in the model yeast *S. cerevisiae* have also shown differences among different populations in their tendency to undergo outbreeding. In particular, human-associated isolates (including clinical and vineyard isolates) showed evidence of sexual outbreeding followed by long periods of clonality[28]. In this case, the limitation of sexual activity was linked to decreased sporulation and increased pseudohyphal growth. Although both responses are stimulated by nutrient limitation, pseudohyphal growth was hypothesized to have a more beneficial response in human-associated environments.

In *C. albicans*, both heterothallic and homothallic mating have been observed. Heterothallic mating occurs between diploid **a** cells and α cells to generate tetraploid **a**/α cells and is mediated by pheromone signalling between the two mating types (FIG. 1). However, in cells that lack the Bar1 protease, same-sex **a**–**a** mating can occur[29]. Bar1 normally degrades α-pheromone but, in the absence of this protease, **a** cells do not destroy

MAPK
(Mitogen-activated protein kinase). A serine/threonine-specific protein kinase that signals cellular responses to a wide range of stimuli, including pheromones, mitogens, osmotic or heat stress.

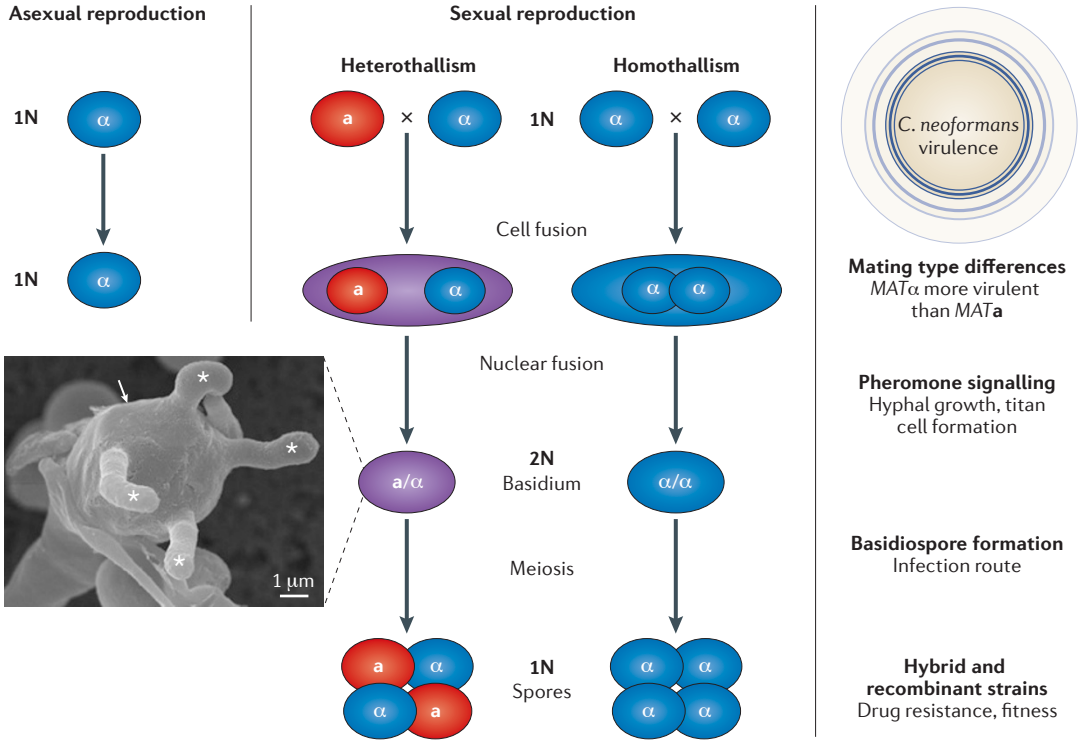


Figure 2 | **Sexual and asexual reproduction in *Cryptococcus neoformans*.** *Cryptococcus neoformans* cells are haploid (1N) and can divide asexually or enter a heterothallic or homothallic mating cycle. In heterothallic mating, pheromone signalling between **a** cells and α cells results in cell–cell fusion. Nuclei do not fuse but form a filamentous dikaryon. The tips of the filamentous cells differentiate into basidia, in which nuclear fusion and meiosis occur. Additional rounds of mitotic division produce multiple, haploid basidiospores, which results in the formation of four long chains. The consequences of sexual reproduction for virulence in *C. neoformans* are shown on the right-hand side of the figure. Inset, a scanning electron micrograph shows a *Cryptococcus gattii* basidium (white arrow) with four emerging basidiospores (white asterisk). *MAT*, mating-type locus. Scanning electron micrograph reproduced from REF. 185.

the α-pheromone that they produce and the resulting autocrine feedback loop drives same-sex mating (FIG. 1). Homothallic mating is also observed in *ménage à trois* matings in which α-pheromone secreted by α cells induces productive mating between two **a** cells[29]. *C. neoformans* has similarly been shown to undergo both heterothallic and homothallic mating. Although conventional mating between *C. neoformans* **a** cells and α cells had been well documented[30–32], a surprising unisexual mating cycle between *C. neoformans* α cells has been described[33,34] (discussed below and shown in FIG. 2). Unisexual α–α mating is thought to have given rise to highly virulent strains of *C. gattii*, which are the cause of an ongoing outbreak in the Pacific Northwest of the United States and Canada[35,36].

What are the benefits of unisexual reproduction? The most obvious benefit is the ability to undergo sexual reproduction even in the absence of a partner of the opposite mating type. This could be crucial for *C. neoformans*, as most natural isolates are of a single mating type[37]. Furthermore, ploidy changes can function as capacitors for evolution even in the absence of outbreeding. This was elegantly shown by a study of *Aspergillus nidulans*, which showed that diploid strains achieved greater fitness than haploid strains during long-term evolution experiments[38]. The fitter diploid strains had reverted to haploidy via parasexual recombination, and increased fitness was due to the accumulation of recessive deleterious mutations in the diploid state, which were beneficial when subsequently unmasked in haploid recombinants[38].

Same-sex mating cycles in *C. neoformans* and *C. albicans* also promote adaptation owing to the formation of chromosome aneuploidies[39–41]. Although aneuploidy often negatively affects fitness, certain aneuploid chromosomes increase resistance to antifungal drugs in both species[40,42,43]. Studies in *S. cerevisiae* have shown that aneuploidies can also drive adaptive evolution[44,45], and it is therefore probable that the karyotypic changes that are introduced by self-mating provide a diverse pool of isolates upon which selection can act[46]. Indeed, a recent study by Ni *et al.* showed that unisexual mating in *C. neoformans* resulted in phenotypic variants, most of which were due to chromosome aneuploidies[40]. Same-sex mating can provide an additional selective advantage to *C. neoformans*, as hyphal forms that are induced during mating enable nutrient foraging over an increased area[47].

We also note that mechanisms similar to fungal homothallism are encountered in protozoan parasites. For example, *Toxoplasma gondii* comprises three highly clonal lineages that have arisen via an ancestral sexual event[48]; however, this parasite can undergo selfing to generate

Aneuploidy
A change in chromosome copy number that does not parallel a change in the entire haploid or diploid genome.

Box 2 | **A phenotypic switch regulates the parasexual cycle of *C. albicans***

Candida albicans cells typically exist as white cells but can transition to an alternative 'opaque' state. The white–opaque switch was originally described in the clinical isolate WO-1 by Soll and colleagues[165]. White cells are round and generate bright, shiny colonies, whereas opaque cells are elongated and form greyer, flatter colonies[165]. The white–opaque transition is reversible and heritable. A complex between **a**1 and alpha2 proteins inhibits this switch, so that only **a** cells or α cells efficiently switch to the opaque state. Opaque cells are mating-competent and secrete and respond to sexual pheromones to undergo efficient conjugation[57,66,166]. Repression of the switch by the mating type-like (*MTL*) locus is relaxed under certain conditions, which enables a subset of natural **a**/α isolates to also undergo this transition[167].

The transcriptional circuitry that regulates the white–opaque switch has been described, and the master regulator of the opaque state is the white–opaque regulator 1 (Wor1) transcription factor. The formation of opaque cells is induced by the expression of Wor1 and is stabilized by positive feedback of Wor1 on its own promoter[168–171]. The **a**1–α2 heterodimer inhibits Wor1 expression, thus limiting the ability of **a**/α cells to undergo this phenotypic switch[172]. Wor1 forms part of an integrated circuit that contains the transcription factors Wor2, Wor3, Czf1 (zinc cluster transcription factor 1), Efg1 (enhanced filamentous growth protein 1) and Ahr1 (a zinc finger transcription factor), which act together to regulate the bistable white–opaque switch[171,173,174].

In addition to regulating mating, the white–opaque switch influences multiple aspects of *C. albicans* biology, including filamentation, metabolic regulation, biofilm formation, interactions with immune cells and virulence[59,78,81,175,176]. Furthermore, strains that overexpress Wor1 hypercolonize the mouse gastrointestinal tract and can undergo a distinct morphological transition to the gastrointestinally induced transition (GUT) phenotype[177]. It is therefore apparent that Wor1 and phenotypic switching have a central role (or roles) in regulating the *C. albicans* lifestyle.

A similar phenotypic switch regulates the mating programme of *Candida tropicalis*, and entry into this programme is also Wor1-dependent[68,178]. However, the transcriptional circuits that control the white–opaque switch have diverged among *Candida* species[178]. In addition, phenotypic switching in *C. tropicalis* is independent of *MTL* control, which indicates that the switch may have an even broader role in the biology of this species[178,179].

highly virulent isolates as well as infectious spores[49]. Similarly, the protozoan *Giardia lamblia* undergoes a novel type of selfing known as 'diplomixis', which involves nuclear fusion in an analogous manner to unisexual mating in pathogenic fungi[3,50]. Both outcrossing and selfing have also been observed in *Plasmodium* species, whereby a single protozoan can generate both male and female gametes[51]. It is therefore apparent that unisexual mating is a prevalent mechanism that is used by eukaryotic microorganisms to adapt to their environment.

Sex in successful human pathogens

More than 100,000 fungal species have been identified so far, but fewer than 200 are associated with humans and only a minority of these cause disease[52]. Members of the *Candida*, *Cryptococcus* and *Aspergillus* genera are the most important in clinical settings. In this section, we compare mating strategies in *C. albicans, C. neoformans* and *A. fumigatus*. We subsequently consider sexual cycles in other fungal pathogens that can have an impact on human health.

***Sexual reproduction in* C. albicans.** The most clinically relevant *Candida* species are not found in the environment but are commensal microorganisms of humans, suggesting co-adaptation with the host. These species are also frequent opportunistic pathogens: the most commonly isolated species is *C. albicans,* which is a cause of both mucosal infections (such as thrush and vaginitis) and life-threatening bloodstream infections[53]. Initially classified as an asexual organism, a novel parasexual programme has now been established for *C. albicans*[54–58]. Mating is regulated by a unique phenotypic switch: **a** cells and α cells mate efficiently only if they undergo a heritable and reversible transition from the typical 'white' state to the mating-competent 'opaque' state (BOX 2; FIG. 1).

Once the sexual state of *C. albicans* had been discovered, additional questions arose. What are the signals that trigger switching to the opaque state, and is this switch regulated by conditions that are encountered in the host? It is now evident that the white–opaque transition is sensitive to many environmental cues that include starvation, haemoglobin, temperature, CO_2, *N*-acetyl glucosamine (GlcNAc) and genotoxic and oxidative stress (reviewed in REFS 59,60). In the laboratory, opaque cells are unstable at 37 °C and rapidly revert to the white state. However, conditions that stabilize the opaque state have been identified, including the presence of CO_2 and GlcNAc, which suggests that opaque cells can persist in some niches in the host[61,62]. Furthermore, high rates of white–opaque switching have been observed during the passage of a clinical isolate through the mouse intestines, and this may be stimulated by anaerobic conditions in the gastrointestinal tract[63]. Mating of *C. albicans* cells has also been shown in several *in vivo* models, including systemic infection, as well as during colonization of the skin or intestines[54,59,64,65].

The white–opaque switch evolved relatively recently in the *Candida* lineage and is limited to *C. albicans*, *Candida dubliniensis* and *Candida tropicalis*[66–68]. In each of these species, the opaque forms are the mating-competent state, but the question arises as to why this switch evolved to regulate mating. It is probable that the requirement for cells to switch to the opaque state limits promiscuous sex and restricts mating to specific niches in the mammalian host[69]. In addition, the white–opaque switch may promote mating *in vivo* by regulating the formation of pheromone-induced sexual biofilms (discussed below). Sexual reproduction in non-*albicans Candida* species is discussed in BOX 3.

***Parasex but no meiosis in* C. albicans.** Despite observations of efficient mating between diploid cells, a conventional meiosis has not been observed in *C. albicans*. Instead, tetraploid mating products undergo a parasexual process of concerted chromosome loss to generate diploid and aneuploid progeny[39,56]. Genetic recombination is observed in a subset of parasexual progeny and is dependent on Spo11 — a conserved endodeoxyribonuclease that is required for meiotic recombination in diverse eukaryotes — indicating that there are parallels between the parasexual cycle and a conventional meiosis[39].

Could the parasexual cycle have specific benefits for the commensal lifestyle of *C. albicans*? A classical meiosis would be expected to generate spores, which are infectious particles that could be highly immunogenic in the

Biofilms
Complex communities of microorganisms that are commonly found attached to a prosthetic surface in the host. Cells adhere to the surface and to each other and promote the formation of extracellular matrix, which protects the biofilm community from external stress (including antifungal drugs).

Gastrointestinally induced transition
(GUT). A phenotypic transition that enables *C. albicans* cells to hypercolonize the gastrointestinal tracts of mice.

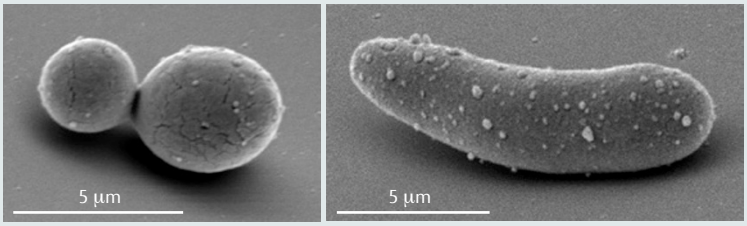
Box 3 | **Sexual reproduction in other pathogenic *Candida* species**

Genomic and experimental evidence indicate that the regulation of sexual reproduction has been rewired among *Candida* clade species[13,16]. In contrast to *Candida albicans*, several *Candida* species exhibit complete sexual cycles that culminate in meiosis and sporulation. For example, both *Candida lusitaniae* and *Candida guilliermondii* are heterothallic species that can mate and form meiotic ascospores[16], despite lacking the conserved genes that are central to meiosis in *Saccharomyces cerevisiae*[13,16]. Moreover, in these *Candida* species, meiosis is no longer under control of the **a**1–α2 complex as it is in *S. cerevisiae*, as *C. lusitaniae* lost the gene that encodes α2 and *C. guilliermondii* lost the genes that encode **a**1 and α2 during evolution[13,16]. *C. lusitaniae* was also recently shown to have coupled mating and meiosis, which enables this pathogen to have only a transitory diploid stage[180]. These results exemplify the plasticity in the regulatory mechanisms that control sexual reproduction and meiosis in fungal species.

In addition to *C. albicans*, the white–opaque transition has been observed in *Candida dubliniensis*[67] and *Candida tropicalis*[68,179]. As in *C. albicans*, the white–opaque regulator 1 (Wor1) transcription factor controls phenotypic switching and mating in *C. tropicalis* (see the figure) but also regulates other traits that are associated with pathogenesis, including filamentous growth and biofilm formation[178]. In more distantly related ascomycetes, such as *S. cerevisiae* and *Histoplasma capsulatum*, orthologues of Wor1 function as transcriptional regulators of filamentation[181,182]. The ancestral Wor1 protein therefore seems to have been a transcriptional regulator of morphogenesis, with the white–opaque switch having evolved relatively recently in the lineage that leads to *C. albicans*, *C. dubliniensis* and *C. tropicalis*[178].

Candida parapsilosis is also a prevalent pathogen and is a member of the *Candida* clade but does not exhibit sexual activity. Isolates are exclusively *MTL***a**/**a** and the *MTL***a**1 gene is a pseudogene, which suggests that the *MTL* locus might be degenerating in this species[183]. Two species that are closely related to *C. parapsilosis* are *Candida metapsilosis* and *Candida orthopsilosis*, but only in *C. orthopsilosis* is there a mixture of mating types, suggesting the presence of an extant sexual cycle[183].

Candida glabrata is more closely related to *S. cerevisiae* than to other *Candida* clade species, but it is often the second most commonly isolated species after *C. albicans*[53]. Despite its close relationship with *S. cerevisiae*, pheromone genes are not expressed in most *C. glabrata* isolates, and neither **a** nor α mating types respond to pheromone. Mating and meiosis have not been described for this species, and the population structure is mostly clonal; thus, it remains to be seen if sexual reproduction occurs in this species[184]. Scanning electron micrograph of white (left-hand panel) and opaque (right-hand panel) *C. tropicalis* cells courtesy of M. P. Hirakawa, Rhode Island, USA.



host[11]. By contrast, generation of progeny via the parasexual cycle allows for a reduction in ploidy and recombination between chromosome homologues but avoids potentially harmful spore formation. In addition, chromosomal aneuploidies are formed at high frequency during parasexual reproduction and can generate additional phenotypic diversity, including the potential formation of drug-resistant isolates[70–72]. It remains to be seen whether the parasexual pathway is the only mechanism by which a mating cycle can be completed in *C. albicans* or whether a cryptic meiosis remains to be discovered.

In a recent development, Hickman *et al.* reported the surprising isolation of haploid strains of *C. albicans*[73]. Although *C. albicans* has long been thought to be unable to form a viable haploid state, rare haploid cells were recovered from both *in vitro* and *in vivo* experiments. Haploid cells were fully competent to undergo the white–opaque switch and could mate to regenerate diploid cells. Haploid cells were the products of parasexual chromosome loss and exhibited fitness defects that were consistent with the unmasking of recessive mutations[73]. The existence of a haploid state provides a potentially exciting new tool with which to study *C. albicans* biology and further highlights the extensive karyotypic plasticity that is exhibited by this pathogen.

***Pheromone signalling, biofilm formation and sex in* C. albicans.** *C. albicans* mating seems to be rare in nature, as the population structure is clonal and there is only limited evidence of recombination[74,75]. It is possible that stressful conditions (such as oxidative stress or antifungal drugs) stimulate the parasexual cycle, as stressors can promote loss of heterozygosity at the *MTL* locus, white–opaque switching and concerted chromosome loss[70,76]. This is also consistent with theoretical models of sexual reproduction, as organisms are predicted to undergo mating more often when stressed and stress-induced recombination can facilitate adaptation under stress[70]. In the case of *C. albicans*, expression of *MTL* genes could also be regulated by environmental factors in such a way that **a**/α cells function phenotypically as **a** cells or α cells; this could enable mating in populations that were previously thought to be unable to undergo sexual reproduction[77]. Pheromone signalling could also affect pathogenesis, as *C. albicans* white cells mount a novel response to mating pheromones that are secreted by opaque cells. Although opaque cells respond to pheromone by forming mating projections and undergoing conjugation (FIG. 1), white cells respond by upregulating genes that are involved in adhesion and biofilm formation[78–81]. Such 'sexual' biofilms enable the stable formation of pheromone gradients between mating partners, which enables opaque cells to locate one another and mate more efficiently[78,82]. As biofilms are important in establishing *Candida* spp. infections[83], the formation of sexual biofilms could directly promote infection in the host[84].

C. albicans white and opaque cells have also been shown to respond to pheromones from closely related species[85]. Pheromones from non-*albicans Candida* species were able to induce biofilm formation in *C. albicans* white cells as well as same-sex mating in *C. albicans* opaque cells[85]. The pheromone–receptor interaction therefore displays surprising plasticity and further broadens the potential conditions under which pheromone signalling, mating and/or biofilm formation might occur in nature.

Sexual reproduction in *Cryptococcus* spp.

C. neoformans is a basidiomycete yeast that is associated with soil, trees and pigeon guano. During infection, *Cryptococcus* spores or desiccated yeast cells are inhaled and lodge in lung alveoli, from where the organism is either cleared or establishes a latent infection[86]. Subsequent immunosuppression leads to reactivation of the latent fungus, followed by dissemination to the central nervous system (CNS), which results in meningoencephalitis[3,86].

Candida clade
A group of related *Candida* species; members of this group share an altered genetic code in which the CUG codon is translated as leucine instead of serine as in the universal genetic code. This group includes most pathogenic *Candida* species, except for *Candida glabrata*, which is more closely related to *Saccharomyces cerevisiae*.

Three varieties of *Cryptococcus* spp. are responsible for cryptococcosis and these have been classified on the basis of their capsular aggregation reactions and genome sequences. Most infections (95% worldwide) are due to *C. neoformans* var. *grubii* (serotype A)[87], <5% of infections are caused by *C. neoformans* var. *neoformans* (serotype D), and a hybrid serotype (AD) also exists that may be more pathogenic than serotype D alone[88]. Although these varieties predominantly infect immunocompromised individuals, *C. gattii* strains (serotypes B and C), which are mostly found in tropical regions, can infect immunocompetent, healthy individuals.

C. neoformans strains are heterothallic and both **a** and α mating types are capable of causing disease, although most clinical isolates are α strains[37]. Unlike most basidiomycetes, in which mating is regulated by a tetrapolar mating locus[86], the *MAT* locus of *C. neoformans* is bipolar and encodes homeodomain proteins, pheromones and pheromone receptors, all of which contribute to cell identity[89–91]. Mating in *C. neoformans* and *C. gattii* was reported almost 4 decades ago and involves the fusion of **a** cells and α cells to produce dikaryotic filaments[30,92] (FIG. 2). Following nuclear fusion and meiosis, long chains of sexual basidiospores are produced, which contain recombinant haploid nuclei[30,92,93].

Although heterothallic mating of *C. neoformans* strains was observed in the laboratory, how mating could occur in nature remained an open question, as most (>98%) isolates are of the α mating type. For *C. neoformans* var. *grubii* (serotype A), it became apparent that although α strains are distributed worldwide, the **a** mating type is restricted to sub-Saharan Africa, where there is evidence of recent or ongoing sexual recombination[94,95]. Studies of mating in this serotype have shown that *C. neoformans* var. *grubii* strains have mating specificity beyond **a** and α mating types, and this may further promote inbreeding of more highly adapted, pathogenic strains[96].

Hybrid AD serotypes are the result of mating between intervarietal strains and often contain both mating types (for example, **a**ADα or αAD**a**)[31,32]. These hybrids germinate poorly to produce diploid or aneuploid products, which suggests that the sexual cycle is inefficient in crosses between *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*.

Same-sex mating in* Cryptococcus *spp. *C. neoformans* strains had been known to undergo a programme of monokaryotic fruiting, during which cells formed filaments and underwent sporulation[97]. Although this was originally thought to be an asexual programme, Lin *et al.* showed that fruiting α cells underwent same-sex fusion to form α/α diploids and that these subsequently sporulated to produce recombinant haploid spores[33] (FIG. 2). Progeny formation was dependent on Spo11 and Dmc1, which are two conserved proteins that are necessary for the formation and repair of meiotic DNA double-strand breaks in yeasts and mammals[33,98].

Analysis of natural isolates of *C. neoformans* has since confirmed the existence of α/α diploids and αA/Dα hybrids, which indicates that same-sex mating occurs in nature[34,99,100]. This strategy enables inbreeding and recombination to occur even in geographical regions in which only one mating type is present. Although both **a** strains and α strains of *C. neoformans* undergo monokaryotic fruiting[101], the *MAT*α locus promotes hyphal growth, and this ability could further contribute to the prevalence of α isolates in nature[102].

Unisexual mating between two closely related α isolates of *C. gattii* is thought to have produced a highly virulent strain that is responsible for an ongoing outbreak that started on Vancouver Island, British Columbia, Canada[35,103]. The outbreak is associated with high mortality rates (>25%) in both healthy and immunosuppressed individuals as well as in domestic and wild animals[103]. Since the first cases were reported, the outbreak has spread into the Pacific Northwest of the United States and Canada[36,103,104]. Several genotypes are responsible for these infections, but the major genotype, VGIIa, is the presumed product of a homothallic mating event between closely related isolates of the VGII type[35]. The VGIIa isolates are highly virulent in animal models of infection[35,36], but how this infection emerged from Vancouver Island remains to be determined.

Mating and virulence in* Cryptococcus *spp. Do sex and mating type have an impact on pathogenicity? Several pieces of evidence suggest that there is a close connection between mating type, sexual reproduction and virulence in *Cryptococcus* spp. First, sexual specification has been directly linked to virulence in serotype D isolates, with α cells being more virulent than **a** cells[105] (FIG. 2). Genes from outside the *MAT* locus interact with genes from within the α locus to direct pathogenesis[96,106]. The two mating types also differ in their interactions with the mammalian host: *C. neoformans* var. *grubii* α isolates are better able to disseminate through the CNS than congenic **a** isolates[107]. Moreover, sexual reproduction can generate new variants that are hypervirulent in the host, such as those that are responsible for the Vancouver Island outbreak. Sexual spores of *C. neoformans* can also function as infectious particles, leading to high mortality rates in a mouse inhalation model of infection[108,109]. Spores might be more successful at causing infection than yeast cells as they are highly resistant to various environmental stresses[110,111].

Another mechanism by which mating can affect virulence is via the pheromone-induction of specialized cell types. Pheromone signalling has been shown to promote the formation of titan cells by *C. neoformans*[112,113]. Titan cells can reach 50–100 μm in diameter, which is 5–10 times larger than a typical cryptococcal cell. These giant cells are polyploid (4N–64N), are resistant to phagocytosis by macrophages and promote the virulence of *C. neoformans* in a mammalian model of infection[114,115]. Taken together, it is therefore apparent that mating and virulence are interconnected processes in *C. neoformans* and *C. gattii*.

A sexual revolution in *Aspergillus* spp.

Most *Aspergillus* infections are associated with immunodeficiency and involve pulmonary aspergillosis, which

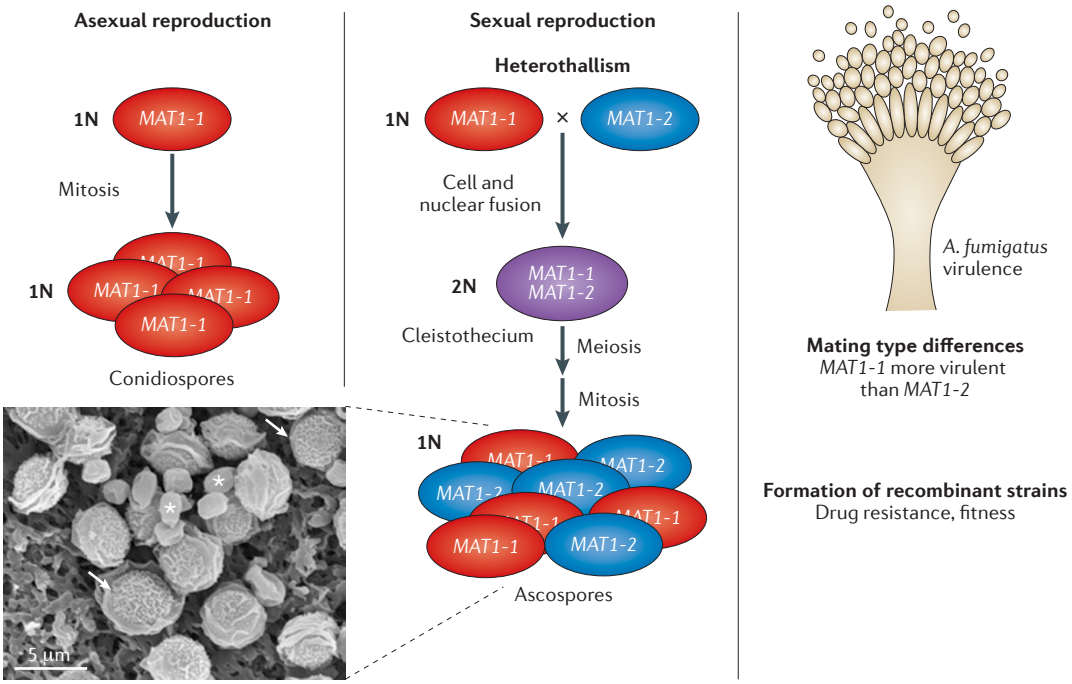


Figure 3 | **Sexual and asexual reproduction in *Aspergillus fumigatus*.** *Aspergillus fumigatus* undergoes asexual reproduction via the mitotic division of haploid (1N) cells or via the formation of asexual conidiospores. It can also undergo heterothallic mating between mating-type locus 1-1 (*MAT1-1*) and *MAT1-2* mating types. The products of mating are cleistothecia that contain multiple ascospores. Sexual reproduction and virulence are linked in *A. fumigatus*, as specified on the right-hand side of the figure. Inset, a scanning electron micrograph shows *Aspergillus lentulus* cleistothecia (white arrows) with emerging ascospores (white asterisk). Scanning electron micrograph courtesy of C. M. O'Gorman and P. Dyer, University of Nottingham, UK.

can often develop into infection of the CNS[116,117]. The most common causative agent for these infections is the ascomycete *A. fumigatus*, which is widely found in the soil and in decaying materials[118]. The primary mode of reproduction is the formation of conidia (that is, asexual spores), which are easily dispersed in the environment and can survive a wide range of conditions.

Although *A. fumigatus* was originally thought to be an asexual fungus, genome sequencing revealed the presence of multiple genes implicated in sexual reproduction[119–121]. In addition to the *MAT* locus, genes that encode putative pheromones, receptors and pheromone-signalling components were identified[120–122]. The functionality of *A. fumigatus MAT*-encoded proteins was first shown in complementation assays using *Aspergillus nidulans*, while also revealing important differences between the two species[123,124]. *A. fumigatus* mating types are present in equal ratios in the environment, and there is evidence for recombination in the population, which suggests that mating occurs in nature[125,126]. However, it was not until 2009 that O'Gorman *et al.* demonstrated the productive mating of *A. fumigatus* in the laboratory. These experiments involved extended incubation periods (>6 months) of strains on oatmeal agar medium in the dark[127] (FIG. 3). The prohibitive conditions that were required raised the question of where in the environment *A. fumigatus* might undergo sexual development and whether it might be evolving into an asexual organism[125,128]. Follow-up studies showed that *A. fumigatus* isolates from patients with invasive aspergillosis could mate in less stringent conditions (co-culture for >6 weeks at 30 °C) and a 'supermater' pair was subsequently identified[129,130]. *MAT1-1* and *MAT1-2* idiomorphs function as master regulators of *A. fumigatus* mating by controlling the expression of pheromone and pheromone-receptor genes[129].

Does *A. fumigatus* mating have an impact on pathogenesis? Similarly to *C. neoformans*, the mating types of *A. fumigatus* differ in their propensities to cause infection, and the *MAT1-1* mating type is associated with increased invasive growth and increased virulence[131,132]. Moreover, the discovery of a sexual cycle implies that recombination could yield progeny that have increased virulence and/or altered antifungal resistance (FIG. 3).

Mating in other* Aspergillus *species. Many aspects of the life cycle of *Aspergillus* species have been extensively studied using *A. nidulans*, which is a species that has a homothallic life cycle and is usually non-pathogenic[133,134]. Similarly to *A. fumigatus,* mating is regulated by two *MAT* genes, *MAT1-1* and *MAT1-2*, which encode an α-box domain protein and a high mobility group (HMG) domain protein, respectively[133]. Overexpression of these genes induced sexual development in *A. nidulans*, which suggests that the transition from a homothallic to a heterothallic life cycle (or vice versa) can occur by rewiring *MAT* regulation alone[133,134].

Idiomorphs
Distinct fungal mating type genes, which, in contrast to alleles, generally lack homology and do not seem to share an obvious ancestry.

Sexual cycles have also been uncovered in *Aspergillus flavus* and *Aspergillus parasiticus*. These species are plant pathogens as well as the primary producers of aflatoxin — a mycotoxin that is a risk factor for hepatocellular carcinoma, particularly in Africa and Asia[135]. *A. flavus* is also an important human pathogen in its own right and causes both invasive and non-invasive aspergillosis[136]. *MAT1-1* and *MAT1-2* idiomorphs are present in both *Aspergillus* species and are found in equal ratios in the environment, which supports the existence of sexual reproduction in nature[63]. *A. flavus* and *A. parasiticus* undergo heterothallic mating, which results in the development of ascospore-bearing ascocarps[137,138].

A heterothallic mating programme has also been uncovered in *Aspergillus lentulus*, which is a close sibling of *A. fumigatus*[139] (FIG. 3). *A. lentulus* is an emerging human fungal pathogen that causes invasive aspergillosis with high mortality rates in immunocompromised patients and that exhibits decreased susceptibility to antifungal drugs[140]. Thus, another supposedly asexual species undergoes a cryptic sexual cycle and hence has the potential to rapidly evolve under selective pressure.

Sex in other human fungal pathogens

Among the ascomycetes, several filamentous species are associated with human disease, including *Histoplasma capsulatum, Coccidioides immitis*, *Coccidioides posadasii*, *Blastomyces dermatitidis*, *Penicillium marneffei*, *Paraccoccidioides brasilienis* and *Sporothrix schenckii* (Supplementary information S3 (figure)). Although most of these species grow as moulds in the environment, the increase in temperature that is experienced during human infection triggers a switch to the pathogenic yeast state[141].

Coccidioidomycosis (also known as Valley Fever) is primarily caused by the inhalation of arthroconidia of the ascomycetes *C. immitis* and *C. posadasii*. These species are commonly found in the soil of arid regions of the United States, Mexico and Central and South America, and they cause progressive pulmonary and disseminated disease in otherwise healthy individuals[142]. Genomic analysis has revealed a single mating-type locus (*MAT1-2-1*) that encodes an HMG-box protein, although a complete sexual cycle has not yet been defined for either *C. immitis* or *C. posadasii*[143]. Population genetics suggests that sexual reproduction occurs in both species, but there is no interspecies flow between them[144].

H. capsulatum is closely related to *Coccidioides* and is ubiquitous in the soil, where it generates asexual microconidia that, when inhaled, cause histoplasmosis[145]. The sexual cycle of this ascomycete was defined decades ago, and mating types (which are designated + and –) are found at equal ratios in the environment, which is supportive of a sexual cycle[146,147]. In humans, only the – mating type causes infection, although the two mating types do not differ in their abilities to cause infection in a mouse model[148].

Pneumocystis jiroveci is an airborne ascomycete that can cause a lethal pulmonary infection in immunocompromised individuals[149]. *Pneumocystis* species have not been cultivated *in vitro*, and thus, observations regarding their life cycles have been made on the basis of cells in infected lung tissue. The life cycle is thought to include both a sexual phase and an asexual phase[149]. Genome sequencing of this organism revealed a cluster of genes that are potentially involved in pheromone sensing and signalling, which could represent a putative *MAT* locus in this species[150].

Recently, a *MAT* locus was identified in the ascomycete *B. dermatitidis*[151]. This dimorphic fungal pathogen is the leading cause of blastomycosis, which causes severe respiratory and disseminated disease in immunocompetent people. Distribution of mating types and population genetics studies suggest that *B. dermatitidis* might reproduce sexually, but the *MAT* locus had not been described[152,153]. The identification of the *B. dermatitidis MAT* revealed that, unlike the *MAT* loci of other dimorphic fungi, it contains transposable elements that make it unusually large and may increase sequence diversity between the two *MAT* idiomorphs, while decreasing recombination within this region[151]. However, it remains unclear whether this fungus undergoes sexual reproduction in nature and, if so, whether the resulting sexual spores are infectious.

Among the *Penicillium* species, *P. marneffei* stands out as it is thermally dimorphic and can cause lethal systemic infections that are similar to disseminated cryptococcosis. *P. marneffei* displays a pattern of extreme clonality, being genetically and spatially restricted, and it is endemically associated with AIDS in Southeast Asia[154,155]. Two mating loci have been identified in *P. marneffei*, and they seem to be widely distributed among isolates, which suggests that a heterothallic sexual cycle remains to be discovered[156]. Although there is considerable evidence to suggest that sexual recombination occurs in this organism, it seems limited to genetically similar and spatially close mating partners, and selfing may occur in this species[157].

Although most fungal species (~95%) belong to the subkingdom Dikarya (consisting of ascomycetes and basidiomycetes), other fungal phyla also include relevant human pathogens. For example, the phylum Zygomycota includes *Mucor circinelloides*, which has defined mating types that are designated as + and – . These mating types encode SexP and SexM, respectively, which are the HMG-domain sex-determining proteins[158]. *M. circinelloides* is an emerging opportunistic pathogen in immunocompromised populations. Mating types differ in spore size and virulence, and the – mating type produces larger asexual spores, which are more virulent in mouse infection and more resistant to phagocytosis by macrophages[158].

Finally, we note that the phylum Microspora is also considered to belong to true Fungi (or to be a close sister group to this kingdom). Microsporidia are obligate intracellular parasites that infect both vertebrate and invertebrate hosts and cause gastrointestinal disease in humans as well as infections of other organs[159]. In this phylum, evidence for sexual reproduction is also emerging: microsporidia contain a *MAT*-related locus that is similar to that of zygomycetes[160,161] and some species contain highly heterozygous genomes, which potentially

HMG-box
(High mobility group-box). A protein domain that is involved in DNA binding.

Dimorphic fungi
Fungi that can exist as single cells (yeast) or in a hyphal or filamentous form. Morphological transitions, such as the yeast–hyphal transition, are driven by a range of environmental conditions (for example, temperature or pH).

Zygomycetes
A phylum of fungi whose name derives from zygospores, which are resistant spherical spores that are formed during mating. Species include *Mucor circinelloides* and *Rhizopus stolonifer*, which is the black bread mould.

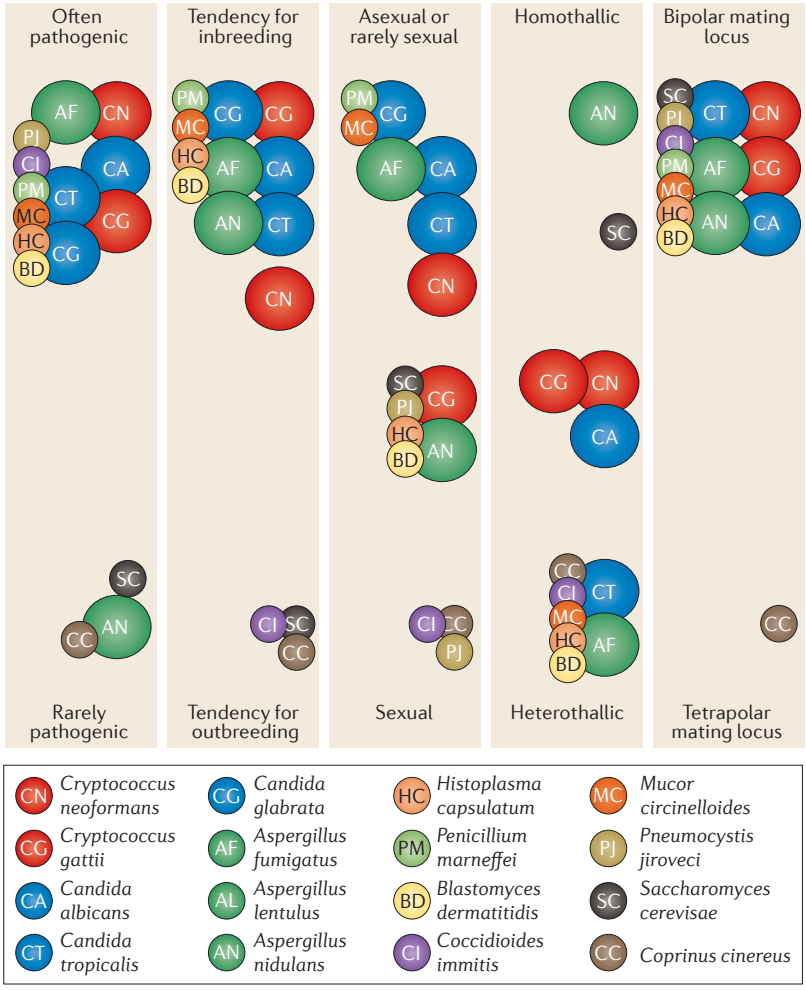


Figure 4 | **Comparative analysis of sexual reproduction and virulence leads to emerging trends among human fungal pathogens.** Some of the most prevalent pathogenic species tend to promote inbreeding, have restricted and highly specialized sexual cycles, contain bipolar mating loci and often have the potential for homothallic reproduction. *Saccharomyces cerevisiae* and *Coprinus cinereus* have been included as model organisms for ascomycetes and basidiomycetes, respectively. Members of the most commonly isolated genera — *Candida*, *Cryptococcus* and *Aspergillus* — are marked with larger symbols in blue, red and green, respectively. Where possible, we have assigned positions for the less well-studied species.

occur as a direct result of outcrossing[162]. One recent study also found homozygous isolates, which is suggestive of selfing in the population[163]. It therefore seems that diverse sexual strategies can be found across the fungal kingdom.

Fungal sex in the context of disease

What have we learned from studying sexual reproduction in fungal pathogens of humans? Many of the assumed advantages of sexual reproduction relate to the ability to increase genetic diversity, despite the associated costs and the fact that recombination can disrupt well-adapted combinations of alleles[164]. In pathogenic species, these costs could prove to be prohibitive. This perhaps explains why prevalent fungal pathogens of humans have preserved the apparatus for sexual reproduction but have restricted the use of these programmes. Thus, these species generate mostly clonal populations but still have the ability to undergo sexual reproduction if dictated by environmental conditions (FIG. 4). Furthermore, the fact that two major pathogens, *C. albicans* and *C. neoformans*, undergo both heterothallic and homothallic mating illustrates a paradigm that has emerged from studies of parasites — microbial pathogens can transition from outbreeding to inbreeding life cycles as they evolve into prevalent pathogens (FIG. 4).

Despite the fact that many of these species seem to limit sexual reproduction, we now recognize that sex ual reproduction and sex-associated programmes have important consequences for pathogenesis. Mating types can differ in virulence as well as in their ability to evade the host immune responses; sexual spores can function as infectious particles or can promote persistence by resisting host stresses, recombinant progeny can exhibit increased resistance to antifungal drugs, and pheromone signalling can promote filamentous growth and the formation of biofilms. Moreover, the genetic variation that results from sexual reproduction can mediate the formation of hypervirulent strains. Therefore, even when rare, recombination events can dictate shifts in the lifestyles of these species, and their effects can persist in subsequent clonal lineages. Indeed, as genetic and genomic tools become increasingly available, sexual cycles are likely to be uncovered in many other 'asexual' fungi.

Important questions concerning the sex lives of human fungal pathogens remain. How do these species regulate the balance between inbreeding and outbreeding? How often do fungal pathogens mate in nature, and which niches support their sexual cycles? How are sexual cycles regulated by host cues? For those species that are human commensal microorganisms, does their sexual behaviour modulate the balance between commensalism and virulence? What are the mechanisms that underlie the differences in virulence between cell mating types, and how do altered ploidy states influence virulence?

Given the diversity of mechanisms that have been found to regulate sexual reproduction, it is probable that new paradigms, similar to the white–opaque switch in *C. albicans* and same-sex mating (that is, monokaryotic fruiting) in *C. neoformans*, remain to be discovered. It is therefore apparent that studies of fungal sexual reproduction will continue to improve our understanding of these important pathogens and will uncover novel biological mechanisms that have evolved to regulate entry into, and passage through, the sexual cycle.

Commensalism
The close relationship between two organisms, in which one organism (the commensal organism) benefits without affecting its host. The term is derived from the Latin '*commensalis*', which means 'sharing a table'.

1. Van Valen, L. A new evolutionary law. *Evol. Theory* **1**, 1–30 (1973).
This paper proposes the Red Queen hypothesis.
2. Heitman, J. Evolution of eukaryotic microbial pathogens via covert sexual reproduction. *Cell Host Microbe* **8**, 86–99 (2010).
3. Heitman, J., Sun, S. & James, T. Y. Evolution of fungal sexual reproduction. *Mycologia* **105**, 1–27 (2013).
4. Lively, C. M. A review of Red Queen models for the persistence of obligate sexual reproduction. *J. Hered.* **101** S13–S20 (2010).
5. Jokela, J., Dybdahl, M. F. & Lively, C. M. The maintenance of sex, clonal dynamics, and host–parasite coevolution in a mixed population of sexual and asexual snails. *Am. Nat.* **174** S43–S53 (2009).
6. Morran, L. T. *et al.* Running with the Red Queen: host–parasite coevolution selects for biparental sex. *Science* **333**, 216–218 (2011).

7. Paterson, S. *et al.* Antagonistic coevolution accelerates molecular evolution. *Nature* **464**, 275–278 (2010).
8. Schulte, R. D., Makus, C. & Schulenburg, H. Host–parasite coevolution favours parasite genetic diversity and horizontal gene transfer. *J. Evol. Biol.* **26**, 1836–1840 (2013).
9. Nyabuga, F. N., Loxdale, H. D., Heckel, D. G. & Weisser, W. W. Coevolutionary fine-tuning: evidence for genetic tracking between a specialist wasp parasitoid and its aphid host in a dual metapopulation interaction. *Bull. Entomol. Res.* **102**, 149–155 (2012).
10. Zhan, J., Mundt, C. C. & McDonald, B. A. Sexual reproduction facilitates the adaptation of parasites to antagonistic host environments: evidence from empirical study in the wheat–*Mycosphaerella graminicola* system. *Int. J. Parasitol.* **37**, 861–870 (2007).
This study explores the Red Queen hypothesis from the 'point of view' of the pathogen.
11. Heitman, J. Sexual reproduction and the evolution of microbial pathogens. *Curr. Biol.* **16**, R711–R725 (2006).
12. Nielsen, K. & Heitman, J. Sex and virulence of human pathogenic fungi. *Adv. Genet.* **57**, 143–173 (2007).
13. Butler, G. *et al.* Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature* **459**, 657–662 (2009).
This study provides genome analysis of multiple species in the *Candida* clade and reveals extensive rewiring of the regulation of mating and meiosis in these species.
14. Lee, S. C., Ni, M., Li, W., Shertz, C. & Heitman, J. The evolution of sex: a perspective from the fungal kingdom. *Microbiol. Mol. Biol. Rev.* **74**, 298–340 (2010).
15. Schurko, A. M. & Logsdon, J. M.,Jr. Using a meiosis detection toolkit to investigate ancient asexual "scandals" and the evolution of sex. *Bioessays* **30**, 579–589 (2008).
16. Reedy, J. L., Floyd, A. M. & Heitman, J. Mechanistic plasticity of sexual reproduction and meiosis in the *Candida* pathogenic species complex. *Curr. Biol.* **19**, 891–899 (2009).
This study establishes that a complete sexual cycle occurs in *C. lusitaniae,* although it lacks the conserved genes that are often considered to be essential for meiosis.
17. Fraser, J. A. & Heitman, J. Chromosomal sex-determining regions in animals, plants and fungi. *Curr. Opin. Genet. Dev.* **15**, 645–651 (2005).
18. Jones, S. K.,Jr & Bennett, R. J. Fungal mating pheromones: choreographing the dating game. *Fungal Genet. Biol.* **48**, 668–676 (2011).
19. Lengeler, K. B. *et al.* Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. *Eukaryot. Cell* **1**, 704–718 (2002).
20. Fraser, J. A. *et al.* Chromosomal translocation and segmental duplication in *Cryptococcus neoformans*. *Eukaryot. Cell* **4**, 401–406 (2005).
21. Hsueh, Y. P., Fraser, J. A. & Heitman, J. Transitions in sexuality: recapitulation of an ancestral tri- and tetrapolar mating system in *Cryptococcus neoformans*. *Eukaryot. Cell* **7**, 1847–1855 (2008).
22. Fraser, J. A. & Heitman, J. Evolution of fungal sex chromosomes. *Mol. Microbiol.* **51**, 299–306 (2004).
23. Hsueh, Y. P., Idnurm, A. & Heitman, J. Recombination hotspots flank the *Cryptococcus* mating-type locus: implications for the evolution of a fungal sex chromosome. *PLoS Genet.* **2**, e184 (2006).
24. Sun, S., Hsueh, Y. P. & Heitman, J. Gene conversion occurs within the mating-type locus of *Cryptococcus neoformans* during sexual reproduction. *PLoS Genet.* **8**, e1002810 (2012).
25. Srikantha, T. *et al.* Nonsex genes in the mating type locus of *Candida albicans* play roles in a/α biofilm formation, including impermeability and fluconazole resistance. *PLoS Pathog.* **8**, e1002476 (2012).
26. Findley, K. *et al.* Discovery of a modified tetrapolar sexual cycle in *Cryptococcus amylolentus* and the evolution of *MAT* in the *Cryptococcus* species complex. *PLoS Genet.* **8**, e1002528 (2012).
27. Tibayrenc, M., Kjellberg, F. & Ayala, F. J. 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 (1990).
28. Magwene, P. M. *et al.* Outcrossing, mitotic recombination, and life-history trade-offs shape genome evolution in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **108**, 1987–1992 (2011).
29. Alby, K., Schaefer, D. & Bennett, R. J. Homothallic and heterothallic mating in the opportunistic pathogen *Candida albicans*. *Nature* **460**, 890–893 (2009).
This paper provides the first demonstration that *C. albicans* can undergo same-sex homothallic mating as well as opposite-sex heterothallic mating.
30. Kwon-Chung, K. J. Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. *Mycologia* **68**, 821–833 (1976).
31. Lengeler, K. B., Cox, G. M. & Heitman, J. Serotype AD strains of *Cryptococcus neoformans* are diploid or aneuploid and are heterozygous at the mating-type locus. *Infect. Immun.* **69**, 115–122 (2001).
32. Cogliati, M., Esposto, M. C., Clarke, D. L., Wickes, B. L. & Viviani, M. A. Origin of *Cryptococcus neoformans* var. *neoformans* diploid strains. *J. Clin. Microbiol.* **39**, 3889–3894 (2001).
33. Lin, X., Hull, C. M. & Heitman, J. Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. *Nature* **434**, 1017–1021 (2005).
This paper establishes that monokaryotic fruiting in *C. neoformans* actually represents a novel form of unisexual α–α mating in this species.
34. Bui, T., Lin, X., Malik, R., Heitman, J. & Carter, D. Isolates of *Cryptococcus neoformans* from infected animals reveal genetic exchange in unisexual, α mating type populations. *Eukaryot. Cell* **7**, 1771–1780 (2008).
35. Fraser, J. A. *et al.* Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature* **437**, 1360–1364 (2005).
This paper shows that the major genotype responsible for an outbreak of *C. gattii* that initiated on Vancouver Island was due to the formation of a hypervirulent strain produced by same-sex mating.
36. Byrnes, E. J.,3rd *et al.* Emergence and pathogenicity of highly virulent *Cryptococcus gattii* genotypes in the Northwest United States. *PLoS Pathog.* **6**, e1000850 (2010).
37. Kwon-Chung, K. J. & Bennett, J. E. Distribution of α and a mating types of *Cryptococcus neoformans* among natural and clinical isolates. *Am. J. Epidemiol.* **108**, 337–340 (1978).
38. Schoustra, S. E., Debets, A. J., Slakhorst, M. & Hoekstra, R. F. Mitotic recombination accelerates adaptation in the fungus *Aspergillus nidulans*. *PLoS Genet.* **3**, e68 (2007).
39. Forche, A. *et al.* The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biol.* **6**, e110 (2008).
This study shows that the sexual cycle of *C. albicans* results in the generation of recombinant progeny that have diverse phenotypes.
40. Ni, M. *et al.* Unisexual and heterosexual meiotic reproduction generate aneuploidy and phenotypic diversity *de novo* in the yeast *Cryptococcus neoformans*. *PLoS Biol.* **11**, e1001653 (2013).
This study shows that the sexual cycle of *C. neoformans* results in the generation of recombinant progeny that have diverse phenotypes.
41. Morrow, C. A. & Fraser, J. A. Ploidy variation as an adaptive mechanism in human pathogenic fungi. *Semin. Cell Dev. Biol.* **24**, 339–346 (2013).
42. Selmecki, A., Bergmann, S. & Berman, J. Comparative genome hybridization reveals widespread aneuploidy in *Candida albicans* laboratory strains. *Mol. Microbiol.* **55**, 1553–1565 (2005).
43. Sionov, E., Lee, H., Chang, Y. C. & Kwon-Chung, K. J. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog.* **6**, e1000848 (2010).
44. Torres, E. M. *et al.* Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* **317**, 916–924 (2007).
45. Rancati, G. *et al.* Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. *Cell* **135**, 879–893 (2008).
46. Ni, M., Feretzaki, M., Sun, S., Wang, X. & Heitman, J. Sex in fungi. *Annu. Rev. Genet.* **45**, 405–430 (2011).
47. Phadke, S. S., Feretzaki, M. & Heitman, J. Unisexual reproduction enhances fungal competitiveness by promoting habitat exploration via hyphal growth and sporulation. *Eukaryot. Cell* **12**, 1155–1159 (2013).
48. Howe, D. K. & Sibley, L. D. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J. Infect. Dis.* **172**, 1561–1566 (1995).
49. Wendte, J. M. *et al.* Self-mating in the definitive host potentiates clonal outbreaks of the apicomplexan parasites *Sarcocystis neurona* and *Toxoplasma gondii*. *PLoS Genet.* **6**, e1001261 (2010).
50. Poxleitner, M. K. *et al.* Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. *Science* **319**, 1530–1533 (2008).
51. Dixon, M. W., Thompson, J., Gardiner, D. L. & Trenholme, K. R. Sex in *Plasmodium*: a sign of commitment. *Trends Parasitol.* **24**, 168–175 (2008).
52. Xu, J. in *Sex in fungi* (eds Heitman, J., Kronstad, J. W., Taylor, J. W. & Casselton, L. A.) 461–475 (ASM Press, 2007).
53. Pfaller, M. A. & Diekema, D. J. Epidemiology of invasive mycoses in North America. *Crit. Rev. Microbiol.* **36**, 1–53 (2010).
54. Hull, C. M., Raisner, R. M. & Johnson, A. D. Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. *Science* **289**, 307–310 (2000).
This study provides the first demonstration that *C. albicans* can undergo sexual mating, in this case using strains that were inoculated into a mouse model of systemic infection.
55. Magee, B. B. & Magee, P. T. Induction of mating in *Candida albicans* by construction of *MTL*a and *MTL*α strains. *Science* **289**, 310–313 (2000).
This study shows that *C. albicans* strains can mate, in this case using strains that were co-incubated on laboratory agar.
56. Bennett, R. J. & Johnson, A. D. Completion of a parasexual cycle in *Candida albican*s by induced chromosome loss in tetraploid strains. *EMBO J.* **22**, 2505–2515 (2003).
This paper shows that *C. albicans* tetraploid cells can undergo a parasexual programme of chromosome loss instead of meiosis to return to the diploid state.
57. Bennett, R. J. & Johnson, A. D. Mating in *Candida albicans* and the search for a sexual cycle. *Annu. Rev. Microbiol.* **59**, 233–255 (2005).
58. Hull, C. M. & Johnson, A. D. Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans*. *Science* **285**, 1271–1275 (1999).
This study provides the first clue towards the discovery of a *C. albicans* sexual cycle by identifying a mating type-like locus.
59. Morschhauser, J. Regulation of white–opaque switching in *Candida albicans*. *Med. Microbiol. Immunol.* **199**, 165–172 (2010).
60. Huang, G. Regulation of phenotypic transitions in the fungal pathogen *Candida albicans*. *Virulence* **3**, 251–261 (2012).
61. Huang, G., Srikantha, T., Sahni, N., Yi, S. & Soll, D. R. CO_2 regulates white-to-opaque switching in *Candida albicans*. *Curr. Biol.* **19**, 330–334 (2009).
62. Huang, G. *et al. N*-acetylglucosamine induces white to opaque switching, a mating prerequisite in *Candida albicans*. *PLoS Pathog.* **6**, e1000806 (2010).
63. Ramirez-Zavala, B., Reuss, O., Park, Y. N., Ohlsen, K. & Morschhauser, J. Environmental induction of white–opaque switching in *Candida albicans*. *PLoS Pathog.* **4**, e1000089 (2008).
64. Lachke, S. A., Lockhart, S. R., Daniels, K. J. & Soll, D. R. Skin facilitates *Candida albicans* mating. *Infect. Immun.* **71**, 4970–4976 (2003).
65. Dumitru, R. *et al. In vivo* and *in vitro* anaerobic mating in *Candida albicans*. *Eukaryot. Cell* **6**, 465–472 (2007).
66. Miller, M. G. & Johnson, A. D. White–opaque switching in *Candida albicans* is controlled by mating-type locus homeodomain proteins and allows efficient mating. *Cell* **110**, 293–302 (2002).
This paper shows that the mating type-like locus controls the white–opaque phenotypic switch in *C. albicans* and that opaque cells are the mating-competent form of the species.
67. Pujol, C. *et al.* The closely related species *Candida albicans* and *Candida dubliniensis* can mate. *Eukaryot. Cell* **3**, 1015–1027 (2004).
68. Porman, A. M., Alby, K., Hirakawa, M. P. & Bennett, R. J. Discovery of a phenotypic switch regulating sexual mating in the opportunistic fungal pathogen *Candida tropicalis*. *Proc. Natl Acad. Sci. USA* **108**, 21158–21163 (2011).
69. Johnson, A. The biology of mating in *Candida albicans*. *Nature Rev. Microbiol.* **1**, 106–116 (2003).
70. Berman, J. & Hadany, L. Does stress induce (para)sex? Implications for *Candida albicans* evolution. *Trends Genet.* **28**, 197–203 (2012).

71. Selmecki, A., Forche, A. & Berman, J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science* **313**, 367–370 (2006).
This paper reveals that chromosome aneuploidy drives increased drug resistance in clinical isolates of *C. albicans*.
72. Selmecki, A., Gerami-Nejad, M., Paulson, C., Forche, A. & Berman, J. An isochromosome confers drug resistance *in vivo* by amplification of two genes, *ERG11* and *TAC1*. *Mol. Microbiol.* **68**, 624–641 (2008).
73. Hickman, M. A. *et al.* The 'obligate diploid' *Candida albicans* forms mating-competent haploids. *Nature* **494**, 55–59 (2013).
This paper provides the first demonstration of a viable haploid state for *C. albicans*.
74. Graser, Y. *et al.* Molecular markers reveal that population structure of the human pathogen *Candida albicans* exhibits both clonality and recombination. *Proc. Natl Acad. Sci. USA* **93**, 12473–12477 (1996).
75. Tibayrenc, M. Are *Candida albicans* natural populations subdivided? *Trends Microbiol.* **5**, 253–254 (1997).
76. Forche, A. *et al.* Stress alters rates and types of loss of heterozygosity in *Candida albicans*. *mBio* **2**, e00129-11 (2011).
77. Pendrak, M. L., Yan, S. S. & Roberts, D. D. Hemoglobin regulates expression of an activator of mating-type locus α genes in *Candida albicans*. *Eukaryot. Cell* **3**, 764–775 (2004).
78. Daniels, K. J. Srikantha, T., Lockhart, S. R., Pujol, C. & Soll, D. R. Opaque cells signal white cells to form biofilms in *Candida albicans*. *EMBO J.* **25**, 2240–2252 (2006).
This study demonstrates the role of white and opaque cells in the formation of 'sexual biofilms'.
79. Chen, J., Chen, J., Lane, S. & Liu, H. A conserved mitogen-activated protein kinase pathway is required for mating in *Candida albicans*. *Mol. Microbiol.* **46**, 1335–1344 (2002).
80. Soll, D. R. *Candida* biofilms: is adhesion sexy? *Curr. Biol.* **18**, R717–R720 (2008).
81. Lin, C. H. *et al.* Genetic control of conventional and pheromone-stimulated biofilm formation in *Candida albicans*. *PLoS Pathog.* **9**, e1003305 (2013).
82. Park, Y. N., Daniels, K. J., Pujol, C. Srikantha, T. & Soll, D. R. *Candida albicans* forms a specialized "sexual" as well as "pathogenic" biofilm. *Eukaryot. Cell* **12**, 1120–1131 (2013).
83. Finkel, J. S. & Mitchell, A. P. Genetic control of *Candida albicans* biofilm development. *Nature Rev. Microbiol.* **9**, 109–118 (2011).
84. Soll, D. R. Why does *Candida albicans* switch? *FEMS Yeast Res.* **9**, 973–989 (2009).
85. Alby, K. & Bennett, R. J. Interspecies pheromone signaling promotes biofilm formation and same-sex mating in *Candida albicans*. *Proc. Natl Acad. Sci. USA* **108**, 2510–2515 (2011).
86. Hull, C. M. & Heitman, J. Genetics of *Cryptococcus neoformans*. *Annu. Rev. Genet.* **36**, 557–615 (2002).
87. Perfect, J. R. & Casadevall, A. Cryptococcosis. *Infect. Dis. Clin. North Am.* **16**, 837–874 (2002).
88. Barchiesi, F. *et al.* Comparative analysis of pathogenicity of *Cryptococcus neoformans* serotypes A, D and AD in murine cryptococcosis. *J. Infect.* **51**, 10–16 (2005).
89. Hull, C. M., Davidson, R. C. & Heitman, J. Cell identity and sexual development in *Cryptococcus neoformans* are controlled by the mating-type-specific homeodomain protein Sxi1α. *Genes Dev.* **16**, 3046–3060 (2002).
90. Hull, C. M., Boily, M. J. & Heitman, J. Sex-specific homeodomain proteins Sxi1α and Sxi2a coordinately regulate sexual development in *Cryptococcus neoformans*. *Eukaryot. Cell* **4**, 526–535 (2005).
91. Stanton, B. C., Giles, S. S., Staudt, M. W., Kruzel, E. K. & Hull, C. M. Allelic exchange of pheromones and their receptors reprograms sexual identity in *Cryptococcus neoformans*. *PLoS Genet.* **6**, e1000860 (2010).
92. Kwon-Chung, K. J. A new genus, *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. *Mycologia* **67**, 1197–1200 (1975).
References 30 and 92 provide the initial description of the *C. neoformans* sexual cycle.
93. Kruzel, E. K. & Hull, C. M. Establishing an unusual cell type: how to make a dikaryon. *Curr. Opin. Microbiol.* **13**, 706–711 (2010).
94. Litvintseva, A. P. *et al.* Evidence of sexual recombination among *Cryptococcus neoformans* serotype A isolates in sub-Saharan Africa. *Eukaryot. Cell* **2**, 1162–1168 (2003).
95. Litvintseva, A. P. & Mitchell, T. G. Population genetic analyses reveal the African origin and strain variation of *Cryptococcus neoformans* var. *grubii*. *PLoS Pathog.* **8**, e1002495 (2012).
96. Nielsen, K. *et al.* Sexual cycle of *Cryptococcus neoformans* var. *grubii* and virulence of congenic a and α isolates. *Infect. Immun.* **71**, 4831–4841 (2003).
97. Wickes, B. L., Mayorga, M. E., Edman, U. & Edman, J. C. Dimorphism and haploid fruiting in *Cryptococcus neoformans*: association with the α-mating type. *Proc. Natl Acad. Sci. USA* **93**, 7327–7331 (1996).
98. Feretzaki, M. & Heitman, J. Genetic circuits that govern bisexual and unisexual reproduction in *Cryptococcus neoformans*. *PLoS Genet.* **9**, e1003688 (2013).
99. Lin, X. *et al.* αADα hybrids of *Cryptococcus neoformans*: evidence of same-sex mating in nature and hybrid fitness. *PLoS Genet.* **3**, 1975–1990 (2007).
100. Lin, X. *et al.* Diploids in the *Cryptococcus neoformans* serotype A population homozygous for the α mating type originate via unisexual mating. *PLoS Pathog.* **5**, e1000283 (2009).
101. Tscharke, R. L., Lazera, M., Chang, Y. C., Wickes, B. L. & Kwon-Chung, K. J. Haploid fruiting in *Cryptococcus neoformans* is not mating type α-specific. *Fungal Genet. Biol.* **39**, 230–237 (2003).
102. Lin, X., Huang, J. C., Mitchell, T. G. & Heitman, J. Virulence attributes and hyphal growth of *C. neoformans* are quantitative traits and the *MAT*α allele enhances filamentation. *PLoS Genet.* **2**, e187 (2006).
103. Byrnes, E. J.,3rd & Marr, K. A. The outbreak of *Cryptococcus gattii* in western North America: epidemiology and clinical issues. *Curr. Infect. Dis. Rep.* **13**, 256–261 (2011).
104. Byrnes, E. J.,3rd *et al.* Molecular evidence that the range of the Vancouver Island outbreak of *Cryptococcus gattii* infection has expanded into the Pacific Northwest in the United States. *J. Infect. Dis.* **199**, 1081–1086 (2009).
105. Kwon-Chung, K. J., Edman, J. C. & Wickes, B. L. Genetic association of mating types and virulence in *Cryptococcus neoformans*. *Infect. Immun.* **60**, 602–605 (1992).
106. Nielsen, K. *et al.* Interaction between genetic background and the mating-type locus in *Cryptococcus neoformans* virulence potential. *Genetics* **171**, 975–983 (2005).
107. Nielsen, K. *et al. Cryptococcus neoformans* α strains preferentially disseminate to the central nervous system during coinfection. *Infect. Immun.* **73**, 4922–4933 (2005).
108. Giles, S. S., Dagenais, T. R., Botts, M. R., Keller, N. P. & Hull, C. M. Elucidating the pathogenesis of spores from the human fungal pathogen *Cryptococcus neoformans*. *Infect. Immun.* **77**, 3491–3500 (2009).
109. Velagapudi, R., Hsueh, Y. P., Geunes-Boyer, S., Wright, J. R. & Heitman, J. Spores as infectious propagules of *Cryptococcus neoformans*. *Infect. Immun.* **77**, 4345–4355 (2009).
110. Botts, M. R., Giles, S. S., Gates, M. A., Kozel, T. R. & Hull, C. M. Isolation and characterization of *Cryptococcus neoformans* spores reveal a critical role for capsule biosynthesis genes in spore biogenesis. *Eukaryot. Cell* **8**, 595–605 (2009).
111. Botts, M. R. & Hull, C. M. Dueling in the lung: how *Cryptococcus* spores race the host for survival. *Curr. Opin. Microbiol.* **13**, 437–442 (2010).
112. Zaragoza, O. *et al.* Fungal cell gigantism during mammalian infection. *PLoS Pathog.* **6**, e1000945 (2010).
113. Okagaki, L. H. *et al.* Cryptococcal titan cell formation is regulated by G-protein signaling in response to multiple stimuli. *Eukaryot. Cell* **10**, 1306–1316 (2011).
114. Okagaki, L. H. *et al.* Cryptococcal cell morphology affects host cell interactions and pathogenicity. *PLoS Pathog.* **6**, e1000953 (2010).
115. Crabtree, J. N. *et al.* Titan cell production enhances the virulence of *Cryptococcus neoformans*. *Infect. Immun.* **80**, 3776–3785 (2012).
116. Fraser, R. S. Pulmonary aspergillosis: pathologic and pathogenetic features. *Pathol. Annu.* **28**, 231–277 (1993).
117. Hohl, T. M. & Feldmesser, M. *Aspergillus fumigatus*: principles of pathogenesis and host defense. *Eukaryot. Cell* **6**, 1953–1963 (2007).
118. Latge, J. P. *Aspergillus fumigatus* and aspergillosis. *Clin. Microbiol. Rev.* **12**, 310–350 (1999).
119. Poggeler, S. Genomic evidence for mating abilities in the asexual pathogen *Aspergillus fumigatus*. *Curr. Genet.* **42**, 153–160 (2002).
This study is the first to suggest the existence of a sexual cycle in *A. fumigatus*.
120. Nierman, W. C. *et al.* Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* **438**, 1151–1156 (2005).
121. Paoletti, M. *et al.* Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Curr. Biol.* **15**, 1242–1248 (2005).
122. Ronning, C. M. *et al.* Genomics of *Aspergillus fumigatus*. *Rev. Iberoam. Micol.* **22**, 223–228 (2005).
123. Grosse, V. & Krappmann, S. The asexual pathogen *Aspergillus fumigatus* expresses functional determinants of *Aspergillus nidulans* sexual development. *Eukaryot. Cell* **7**, 1724–1732 (2008).
124. Pyrzak, W., Miller, K. Y. & Miller, B. L. Mating type protein Mat1-2 from asexual *Aspergillus fumigatus* drives sexual reproduction in fertile *Aspergillus nidulans*. *Eukaryot. Cell* **7**, 1029–1040 (2008).
125. Dyer, P. S. & Paoletti, M. Reproduction in *Aspergillus fumigatus*: sexuality in a supposedly asexual species? *Med. Mycol.* **43**, S7–S14 (2005).
126. Bain, J. M. *et al.* Multilocus sequence typing of the pathogenic fungus *Aspergillus fumigatus*. *J. Clin. Microbiol.* **45**, 1469–1477 (2007).
127. O'Gorman, C. M., Fuller, H. & Dyer, P. S. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* **457**, 471–474 (2009).
This paper is the first demonstration of a sexual cycle in *A. fumigatus*, which required the extended incubation of cells for 6 months in the laboratory.
128. Kwon-Chung, K. J. & Sugui, J. A. Sexual reproduction in *Aspergillus* species of medical or economical importance: why so fastidious? *Trends Microbiol.* **17**, 481–487 (2009).
129. Szewczyk, E. & Krappmann, S. Conserved regulators of mating are essential for *Aspergillus fumigatus* cleistothecium formation. *Eukaryot. Cell* **9**, 774–783 (2010).
130. Sugui, J. A. *et al.* Identification and characterization of an *Aspergillus fumigatus* "supermater" pair. *mBio* **2**, e00234-11 (2011).
131. Alvarez-Perez, S., Blanco, J. L., Alba, P. & Garcia, M. E. Mating type and invasiveness are significantly associated in *Aspergillus fumigatus*. *Med. Mycol.* **48**, 273–277 (2010).
132. Cheema, M. S. & Christians, J. K. Virulence in an insect model differs between mating types in *Aspergillus fumigatus*. *Med. Mycol.* **49**, 202–207 (2011).
133. Paoletti, M. *et al.* Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. *Curr. Biol.* **17**, 1384–1389 (2007).
134. Dyer, P. S. & O'Gorman, C. M. Sexual development and cryptic sexuality in fungi: insights from *Aspergillus* species. *FEMS Microbiol. Rev.* **36**, 165–192 (2012).
135. Groopman, J. D. & Kensler, T. W. Role of metabolism and viruses in aflatoxin-induced liver cancer. *Toxicol. Appl. Pharmacol.* **206**, 131–137 (2005).
136. Morgan, J. *et al.* Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Med. Mycol.* **43**, S49–S58 (2005).
137. Horn, B. W., Moore, G. G. & Carbone, I. Sexual reproduction in *Aspergillus flavus*. *Mycologia* **101**, 423–429 (2009).
138. Horn, B. W., Ramirez-Prado, J. H. & Carbone, I. The sexual state of *Aspergillus parasiticus*. *Mycologia* **101**, 275–280 (2009).
139. Swilaiman, S. S., O'Gorman, C. M., Balajee, S. A. & Dyer, P. S. Discovery of a sexual cycle in *Aspergillus lentulus*, a close relative of *A. fumigatus*. *Eukaryot. Cell* **12**, 962–969 (2013).
140. Balajee, S. A., Weaver, M., Imhof, A., Gribskov, J. & Marr, K. A. *Aspergillus fumigatus* variant with decreased susceptibility to multiple antifungals. *Antimicrob. Agents Chemother.* **48**, 1197–1203 (2004).
141. Klein, B. S. & Tebbets, B. Dimorphism and virulence in fungi. *Curr. Opin. Microbiol.* **10**, 314–319 (2007).
142. Chiller, T. M., Galgiani, J. N. & Stevens, D. A. Coccidioidomycosis. *Infect. Dis. Clin. North Am.* **17**, 41–57 (2003).
143. Fraser, J. A. *et al.* Evolution of the mating type locus: insights gained from the dimorphic primary fungal pathogens *Histoplasma capsulatum, Coccidioides immitis*, and *Coccidioides posadasii*. *Eukaryot. Cell* **6**, 622–629 (2007).
144. Koufopanou, V., Burt, A., Szaro, T. & Taylor, J. W. Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). *Mol. Biol. Evol.* **18**, 1246–1258 (2001).

145. Edwards, J. A. & Rappleye, C. A. *Histoplasma* mechanisms of pathogenesis — one portfolio doesn't fit all. *FEMS Microbiol. Lett.* **324**, 1–9 (2011).
146. Kwon-Chung, K. J. Sexual stage of *Histoplasma capsulatum*. *Science* **175**, 326 (1972).
This paper describes the existence of a sexual cycle in *H. capsulatum*.
147. Kwon-Chung, K. J., Weeks, R. J. & Larsh, H. W. Studies on *Emmonsiella capsulata* (*Histoplasma capsulatum*). II. Distribution of the two mating types in 13 endemic states of the United States. *Am. J. Epidemiol.* **99**, 44–49 (1974).
148. Kwon-Chung, K. J., Hill, W. B. & Bennett, J. E. New, special stain for histopathological diagnosis of cryptococcosis. *J. Clin. Microbiol.* **13**, 383–387 (1981).
149. Cushion, M. T. *Pneumocystis*: unraveling the cloak of obscurity. *Trends Microbiol.* **12**, 243–249 (2004).
150. Smulian, A. G., Sesterhenn, T., Tanaka, R. & Cushion, M. T. The *STE3* pheromone receptor gene of *Pneumocystis carinii* is surrounded by a cluster of signal transduction genes. *Genetics* **157**, 991–1002 (2001).
151. Li, W. *et al.* Identification of the mating-type (*MAT*) locus that controls sexual reproduction of *Blastomyces dermatitidis*. *Eukaryot. Cell* **12**, 109–117 (2013).
152. McDonough, E. S. & Lewis, A. L. *Blastomyces dermatitidis*: production of the sexual stage. *Science* **156**, 528–529 (1967).
This study is the first to identify a sexual cycle in *B. dermatitidis*.
153. Kwon-Chung, K. J. Genetic analysis on the incompatibility system of *Ajellomyces dermatitidis*. *Sabouraudia* **9**, 231–238 (1971).
154. Fisher, M. C., Aanensen, D., de Hoog, S. & Vanittanakom, N. Multilocus microsatellite typing system for *Penicillium marneffei* reveals spatially structured populations. *J. Clin. Microbiol.* **42**, 5065–5069 (2004).
155. Fisher, M. C. *et al.* Low effective dispersal of asexual genotypes in heterogeneous landscapes by the endemic pathogen *Penicillium marneffei*. *PLoS Pathog.* **1**, e20 (2005).
156. Woo, P. C. *et al.* Genomic and experimental evidence for a potential sexual cycle in the pathogenic thermal dimorphic fungus *Penicillium marneffei*. *FEBS Lett.* **580**, 3409–3416 (2006).
157. Henk, D. A. *et al.* Clonality despite sex: the evolution of host-associated sexual neighborhoods in the pathogenic fungus *Penicillium marneffei*. *PLoS Pathog.* **8**, e1002851 (2012).
158. Li, C. H. *et al.* Sporangiospore size dimorphism is linked to virulence of *Mucor circinelloides*. *PLoS Pathog.* **7**, e1002086 (2011).
159. Didier, E. S. & Weiss, L. M. Microsporidiosis: current status. *Curr. Opin. Infect. Dis.* **19**, 485–492 (2006).
160. Lee, S. C. *et al.* Microsporidia evolved from ancestral sexual fungi. *Curr. Biol.* **18**, 1675–1679 (2008).
161. Lee, S. C., Weiss, L. M. & Heitman, J. Generation of genetic diversity in microsporidia via sexual reproduction and horizontal gene transfer. *Commun. Integr. Biol.* **2**, 414–417 (2009).
162. Cuomo, C. A. *et al.* Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res.* **22**, 2478–2488 (2012).
163. Selman, M. *et al.* Extremely reduced levels of heterozygosity in the vertebrate pathogen *Encephalitozoon cuniculi*. *Eukaryot. Cell* **12**, 496–502 (2013).
164. Tibayrenc, M. & Ayala, F. J. Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proc. Natl Acad. Sci. USA* **109**, E3305–E3313 (2012).
165. Slutsky, B. *et al.* "White–opaque transition": a second high-frequency switching system in *Candida albicans*. *J. Bacteriol.* **169**, 189–197 (1987).
This paper provides the first demonstration that *C. albicans* cells undergo the white–opaque switch.
166. Zhao, R. *et al.* Unique aspects of gene expression during *Candida albicans* mating and possible G_1 dependency. *Eukaryot. Cell* **4**, 1175–1190 (2005).
167. Xie, J. *et al.* White-opaque switching in natural *MTL*a/α isolates of *Candida albicans*: evolutionary implications for roles in host adaptation, pathogenesis, and sex. *PLoS Biol.* **11**, e1001525 (2013).
168. Huang, G. *et al.* Bistable expression of *WOR1*, a master regulator of white–opaque switching in *Candida albicans*. *Proc. Natl Acad. Sci. USA* **103**, 12813–12818 (2006).
169. Srikantha, T. *et al. TOS9* regulates white–opaque switching in *Candida albicans*. *Eukaryot. Cell* **5**, 1674–1687 (2006).
170. Zordan, R. E., Galgoczy, D. J. & Johnson, A. D. Epigenetic properties of white–opaque switching in *Candida albicans* are based on a self-sustaining transcriptional feedback loop. *Proc. Natl Acad. Sci. USA* **103**, 12807–12812 (2006).
References 168, 169 and 170 identify *WOR1* (*TOS9*) as the master transcription factor that regulates the opaque state in *C. albicans*.
171. Zordan, R. E., Miller, M. G., Galgoczy, D. J., Tuch, B. B. & Johnson, A. D. Interlocking transcriptional feedback loops control white–opaque switching in *Candida albicans*. *PLoS Biol.* **5**, e256 (2007).
172. Lockhart, S. R. *et al.* In *Candida albicans*, white–opaque switchers are homozygous for mating type. *Genetics* **162**, 737–745 (2002).
173. Wang, H. *et al. Candida albicans* Zcf37, a zinc finger protein, is required for stabilization of the white state. *FEBS Lett.* **585**, 797–802 (2011).
174. Hernday, A. D. *et al.* Structure of the transcriptional network controlling white–opaque switching in *Candida albicans*. *Mol. Microbiol.* **90**, 22–35 (2013).
175. Lan, C. Y. *et al.* Metabolic specialization associated with phenotypic switching in *Candida albicans*. *Proc. Natl Acad. Sci. USA* **99**, 14907–14912 (2002).
176. Lohse, M. B. & Johnson, A. D. Differential phagocytosis of white versus opaque *Candida albicans* by *Drosophila* and mouse phagocytes. *PLoS ONE* **3**, e1473 (2008).
177. Pande, K., Chen, C. & Noble, S. M. Passage through the mammalian gut triggers a phenotypic switch that promotes *Candida albicans* commensalism. *Nature Genet.* **45**, 1088–1091 (2013).
178. Porman, A. M., Hirakawa, M. P., Jones, S. K., Wang, N. & Bennett, R. J. *MTL*-independent phenotypic switching in *Candida tropicalis* and a dual role for Wor1 in regulating switching and filamentation. *PLoS Genet.* **9**, e1003369 (2013).
179. Xie, J. *et al. N*-acetylglucosamine induces white-to-opaque switching and mating in *Candida tropicalis*, providing new insights into adaptation and fungal sexual evolution. *Eukaryot. Cell* **11**, 773–782 (2012).
180. Sherwood, R. K., Scaduto, C. M., Torres, S. E. & Bennett, J. E. Convergent evolution of a fused sexual cycle promotes the haploid lifestyle. *Nature* http://dx.doi.org/10.1038/nature12891 (2014).
181. Nguyen, V. Q. & Sil, A. Temperature-induced switch to the pathogenic yeast form of *Histoplasma capsulatum* requires Ryp1, a conserved transcriptional regulator. *Proc. Natl Acad. Sci. USA* **105**, 4880–4885 (2008).
182. Cain, C. W., Lohse, M. B., Homann, O. R., Sil, A. & Johnson, A. D. A conserved transcriptional regulator governs fungal morphology in widely diverged species. *Genetics* **190**, 511–521 (2012).
183. Sai, S., Holland, L. M., McGee, C. F., Lynch, D. B. & Butler, G. Evolution of mating within the *Candida parapsilosis* species group. *Eukaryot. Cell* **10**, 578–587 (2011).
184. Muller, H., Hennequin, C., Gallaud, J., Dujon, B. & Fairhead, C. The asexual yeast *Candida glabrata* maintains distinct a and α haploid mating types. *Eukaryot. Cell* **7**, 848–858 (2008).
185. Byrnes, E. J.,3rd *et al.* A diverse population of *Cryptococcus gattii* molecular type VGIII in southern Californian HIV/AIDS patients. *PLoS Pathog.* **7**, e1002205 (2011).

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Competing interests statement
The authors declare no competing interests.

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