The evolution of mating-type switching for reproductive assurance

Bart P. S. Nieuwenhuis and Simone Immler*

Alternative ways to ensure mate compatibility, such as hermaphroditism and the breakdown of self-incompatibility, evolved repeatedly when finding a mating partner is difficult. In a variety of microorganisms where compatibility is determined by mating-types, a highly regulated form of universal compatibility system called mating-type switching has evolved several times. This sophisticated system allows for the genetic adjustment of the mating type during asexual growth, and it most likely evolved for reproductive assurance of immotile species under low densities. In this review, we compare the switching strategy to other universal compatibility systems such as ''unisexual mating'' and homothallism. We identify the costs of switching, including genome instability, and mechanistic costs, as well as the benefits, mainly the maintenance of important mating-type functions. Given the potential benefits of mating-type switching, we speculate that switching is likely to have evolved many times independently, and may be more common in groups where genetic mating types regulate mate compatibility than assumed so far.

Keywords:
Allee effect; Baker's Law; density dependence; fungi; self-incompatibility; yeast

Introduction

Sexual reproduction in eukaryotes generally involves two complementary mates, such as sexes (males and females), or mating types (indicated with for example + and − or *a* and alpha), but the seemingly simple requirement of finding a mate may not always be a trivial task. This is particularly true in species with relatively low population densities, and has led to the evolution of elaborate traits to overcome this low encounter rate. Many female insects produce costly pheromones to attract males [1] and female gametes in molluscs need to attract sperm [2]. The problem may particularly affect sessile organisms, which are dependent on external circumstances, which they can in some cases manipulate – e.g. some plants produce eye-catching flowers to attract pollinators [3, 4] – but in other cases not, e.g. the case of wind pollinators. The effect of density on mate-finding is one of the best known examples of the Allee effect (i.e. the positive relationship between a component of individual fitness and the numbers or density of conspecifics [5–7]). The "reproductive assurance" hypothesis states that in order to overcome this risk of reduced reproductive output when chance of outcrossing is low, mechanisms that allow reproduction without a partner may evolve [8–10]. In species with two separate sexes (i.e. dioecy in plants or gonochorism in animals) and a balanced sex ratio, the chance that two individuals that meet randomly are compatible is only 50%. In contrast, an individual that is compatible with any encountered conspecific individual doubles its chance of finding a suitable mate. Two well-known mechanisms serving the purpose of increasing mating probability are simultaneous (and to a lesser degree sequential) hermaphroditism and the breakdown of self-incompatibility [11–13].

Simultaneous hermaphroditism is defined as the co-existence of both sex roles in one individual at the same time, and is known to have evolved many times independently [14]. In nematodes for example, hermaphroditism has evolved from gonochorism at least three times [15], and experimental studies in *Caenorhabditis elegans* showed hermaphroditism to be beneficial when chances for outcrossing are low [16]. Similarly, bryophytes changed at least 20 times from dioecy to monoecy [17], which may be beneficial under low densities because motile sperm is limited in its dispersal [18]; and androdioecious freshwater crustaceans are thought to have evolved hermaphroditism from dioecious ancestors to assure reproduction under low densities [19]. The breakdown of self-incompatibility is a well-known form of reproductive

DOI 10.1002/bies.201600139

Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden

***Corresponding author:**
Bart P. S. Nieuwenhuis
E-mail: bart.nieuwenhuis@ebc.uu.se

assurance in plants, allowing for self-fertilization [20]. Self-incompatibility (SI) can be found in about half of all plant species, and implies that a maternal plant rejects fertilizing pollen with an identical SI allele, thereby avoiding the costs of inbreeding [21]. Under low densities, the chance of finding a compatible mate is small, and even more so in species with long-distance dispersal [22]. At the edges of a species range, and in newly colonized habitats, individuals that are self-compatible are more likely to establish, because all individuals are able to reproduce with each other – a phenomenon known as Baker's law [22–24]. Even though Baker's law is generally studied in plants, the principle also applies to hermaphrodites in other taxonomic groups including animals and fungi [12]. Preferentially outcrossing hermaphroditic *Macrostomum* sp. flatworms for example will self-fertilize in the absence of a mate [25]. Finally, in fungi, algae, and diatoms, where opposite mating partners are defined by genetic mating types rather than sexes, a breakdown of self-incompatibility has occurred many times [26, 27]. In this review, we focus on a mechanism of self-compatibility, which is commonly found in fungi [26, 28, 29] and which involves a change from one mating type to another in individuals during asexual growth, known as "mating-type switching."

The ability to self-fertilize does come at a cost, because selfing will increase the potential for inbreeding and the resulting disadvantages [11]. In addition, selfing in hermaphrodites may imply direct costs due to developmental restrictions or intra-individual sexual conflict, where the two sex roles may negatively affect each other [30]. Whereas in diploid species, selfing might be selected because it has a transmission advantage over outcrossing (known as Fisher's automatic selection hypothesis [31]), this is not the case for isogamous haploid species, and we thus suggest that the most likely cause for self-compatibility is reproductive assurance. The transitions between separate sexes and hermaphroditism, between SI and self-compatibility, and between mating types and universal compatibility, greatly depend on a balance between the need for a mate and the cost of selfing [11]. In this paper, we argue that it is the balance between these costs and reproductive assurance that drives the evolution of mating-type switching.

Sexes and mating types

While in most eukaryotic plants and animals, two sexes or two sexual functions, namely males and females, can be identified, many microorganisms lack distinct sex roles and compatibility between individuals is regulated by genetic mating types. Mating types were first described over a century ago by Blakeslee [32] in his observation that for many species of the Mucoridae, two groups of individuals could be formed that could not breed within their group, but were compatible with all individuals of the other group. Mating types have since been described for many different taxa, and are thought to have evolved independently in the amoebozoa, green and brown algae, fungi, and ciliates [27].

Mating types are genetically defined compatibility systems that act at the haploid level, and only individuals with different alleles at the mating-type locus can successfully mate, a phenomenon known as heterothallism [14, 33]. In most cases, only two mating types exist within one species, which – similar to separate sexes – reduces mate compatibility by half. Multiple mating types are expected to evolve if a novel mating type is compatible with all other individuals, thus increasing the chance of successful mating [34]. Nevertheless, only a limited number of clades with more than two different mating types exist viz. basidiomycete fungi, slime molds, ciliates, and tunicates [35]. A much more common transition is the evolution of universal compatibility, where each individual is compatible with all others including itself, which is known as homothallism [26, 29]. This transition is common in green algae [36, 37], diatoms [38], and fungi [26]. In all groups where both mating types and self-compatible species exist, self-compatibility appears to be derived from a self-sterile ancestor, though in some cases homothallism may be lost secondarily [26].

Self-compatibility in organisms with mating types can be obtained in three different ways (reviewed in [26, 29]). (i) Mating types can be selectively ignored (single mating-type mating or "unisexual" mating) making all cells compatible with each other. This type of self-mating was only recently discovered, and has since been described in several fungal species (reviewed in [27]); it may also be driving self-compatibility in green algae [39, 40]. (ii) The most common mechanism for self-compatibility in fungi is the presence of both mating types in the same haploid genome, i.e. an individual carries both mating types and is thus compatible with all others and itself [41]. We will refer to this form of homothallism as "true homothallism" and not the more commonly used term "primary homothallism" [33], because homothallism is almost always a derived system, and the term "primary" is thus somewhat misleading. (iii) Individuals change the genetic make-up of the mating type during asexual growth. Even though each individual is of a particular mating type, similar to heterothallic species, a single haploid individual can reproduce sexually by dividing once and mating with its clone in a second step. This last form of self-compatibility is known as mating-type switching, which is the main topic of this review.

Mating types have multiple functions

The molecular mechanisms of mating types have been studied for different species of green algae, slime molds, and ciliates, but are best understood in fungi and particularly so in the model yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (budding yeast and fission yeast, extensively reviewed in [42] and [43], respectively). Even though the mechanisms that underlie mating-type function differ greatly between the groups, they all share the following three characteristics (see Fig. 1). First, each mating type is defined at the genetic level, often by only a few tightly linked genes, sometimes displaying highly diversified alleles [44–47]. However, the mating type region can expand greatly and cover large parts of the mating-type chromosome (e.g. [46, 48, 49]). Second, the genes at the mating type locus regulate downstream genes that are involved in a compatibility reaction during mating. This can include mating-type specific

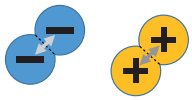
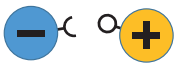
Phase	Mating-type regulated functions	
Haploid growth	• Gametogenesis • Mating-type switching	
Mating	• Extracellular recognition • Fusion • Cytoplasmic inheritance • Motility	
Diploid growth	• Sporophyte development • Suppression of mating • Virulence	
Meiosis	• Induction of meiosis	

Figure 1. Schematic overview of the multiple functions that are regulated or strongly affected by the genes located at the mating type locus.

signalling, fusion, regulation of cytoplasmic inheritance, and motility [14]. In some clades, mating types have become associated with oogamy or anisogamy, rendering the mating type locus a sex-determining locus (e.g. [40]). Third, the genes are involved in a developmental switch between the haploid and the diploid phase [14, 50, 51]. This switch can involve a different growth form, virulence, and the ability to go into meiosis. Over the course of evolution, some functions might have been lost or modified, and many others might have been added. Due to this multitude of functions, the force (or forces) that drove the initial evolution of mating types is still debated [52]. The origin of mating types has been extensively reviewed in Billiard et al. [52] and Perrin [51], with an extension by Hadjivasiliou et al. [53].

What is mating-type switching?

In mating-type-switching species, each haploid individual expresses only one mating type, even though it carries the information for both mating types. The information of the other mating type is then used during asexual growth to change the mating type identity by modification at the genetic level. In other words, mating-type switching occurs during asexual cell division and involves a reorganization of the genome. The molecular mechanisms of switching vary across taxa and can be divided into four groups: two types of reversible "inversion" systems, the "copy-paste" systems and the unidirectional switching systems.

The reversible inversion system is the simplest system of mating-type switching, as found for example, in the methylotrophic yeast *Pichia pastoris* [54], and involves a reversible inversion of part of the chromosome (Fig. 2A). The two regions containing the mating-type genes (mating-type cassettes) are located at either end of this segment, and depending on the orientation, one or the other allele is

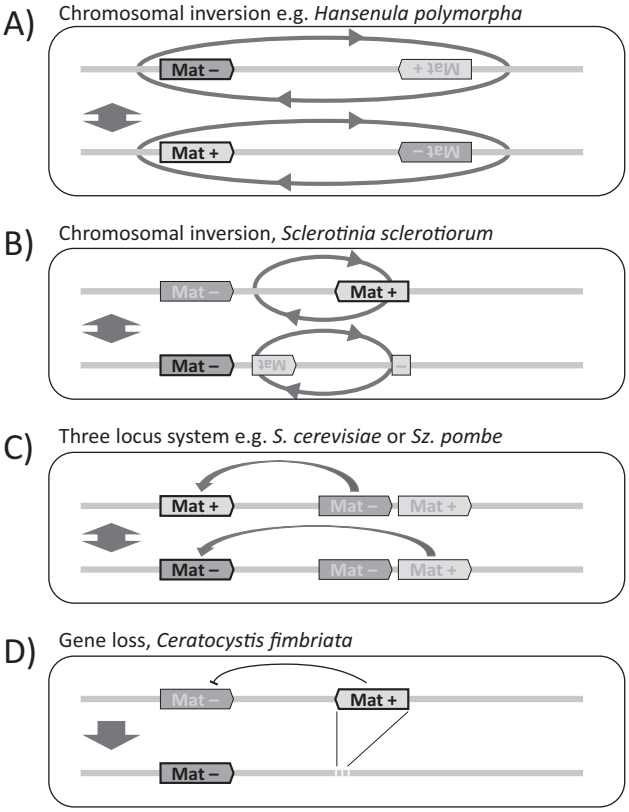


Figure 2. Diversity of mating-type switching mechanisms in fungi by **A** and **B:** chromosomal inversion flip-flop mechanisms, **C:** a three-locus copy-paste system, and **D:** by gene loss. Each line represents a genotype. The active mating-type cassette is indicated by bold lines, the silent cassette(s) with gray text.

silenced [54, 55]. This silencing occurs probably due to the localization of the suppressed mating-type cassette close to either the centromere or telomere [54]. This flip-flop system likely evolved from a homothallic species in which the two mating-type cassettes are often found in close proximity [41] due to unequal crossing over (recombination between homologous sequences at different loci) between them. In a similar system, inversions can occur within the mating type region [56], truncating an essential gene or separating it from its promoter (Fig. 2B). In both systems, the inverted region is flanked by inverted repeat sequences, which suggests that the mechanism of inversion is homologous recombination between these regions.

The best-studied switching system is the three-cassette "copy-paste" model with one active and two suppressed cassettes (Fig. 2C). During mitotic divisions, the cassette at the active locus is replaced by a copy of one of the silent cassettes. This system is found in fission yeasts of the genus *Schizosaccharomyces* [43, 57], and in the family containing budding yeast (Saccharomycetaceae), which in the latter appears to have evolved from an inversion system [54]. Mating-type switching in the three-cassette system can be very efficient, resulting in successful switching ~90% of the time in fission and budding yeast due to site-specific single or double-strand DNA break that induces directional switching [58, 59].

Even though these systems show many similarities, the mechanisms of switching and suppression of the silent cassettes differ greatly between them [42, 43].

In some species, mating-type switching is not reversible, and always occurs in one direction (e.g. *Hypocrea spinulosa*, *Botrytinia fuckeliana* [28]; Fig. 2D). A self-fertile strain carries both mating-type cassettes in its genome, but only one is active, probably by suppressing expression of the other [60, 61]. During switching, the active mating-type genes are lost, releasing suppression of the other mating type. As a result, the switched clone is compatible with the sister, but it cannot switch itself anymore.

In the protozoan ciliates, a system of mating-type determination exists that can be considered switching. In these organisms, each cell contains a diploid micronucleus (the germline) and a polyploid macronucleus (the soma) and the latter contains a rearranged subsample of genes from the first [62]. During construction of the macronuclear genome, the cell apparently randomly assigns one of seven possible mating types, after which mating is only possible between cells with different mating types [47]. Similar to fungi, mating between identical cells – in ciliates identical at the level of the diploid micronucleus – is not prevented [51].

Mating-type switching evolved multiple times

Mating-type switching evolved at least six times independently within the fungi: five times in the Ascomycetes and once in the Basidiomycetes. The largest group of known switching species is in the Saccharomycetaceae, containing both species with a three-cassette system or a flip-flop system. In this group, a simple switching system progressively evolved increased efficiency, incorporating more complicated systems such as the transposon derived HO endonuclease, which produces the double strand break that induces switching [42, 63]. Other species within this clade are heterothallic (e.g. *Lachancea kluyveri*), probably due to loss of the switching mechanism [64]. Three other origins of switching in the Ascomycetes are in filamentous fungi (see "*Switching in multicellular fungi*" below) and the fifth is the *Schizosaccharomyces* genus [57]. The only basidiomycete fungus (the group of fungi to which the mushroom forming fungi belong) for which switching has been described is the multicellular *Agrocybe aegerita* [65]. Many Basidiomycota have more than two mating types, which increases the chance that two individuals that meet are compatible [35, 66], and thus should reduce selection for switching.

Most unicellular algae are homothallic species [36, 67], but observations in a laboratory mutant strain of *Closterium ehrenbergii* suggest the occurrence of unidirectional switching [68]. Unfortunately, the molecular mechanisms underlying homothallism in algae have not been studied explicitly, and both true homothallism – with expression of both mating types in one individual – as well as switching are possible explanations. Even though mating-type switching has only been shown to exist in a limited number of clades, its occurrence might be more common than currently thought, because switching species are easily classified as true homothallic on account of their highly similar mating systems.

Why switch?

An obvious benefit of mating-type switching in microorganisms is reproductive assurance under ecological situations where population density is low and mate encounters rare. This is particularly true for small, sessile organisms, because these will only meet by *growing* into each other. This idea is known as the "lonely spore hypothesis" for the evolution of mating-type switching [54, 69]. Effectively, it is a microscopic version of Baker's law: "With self-compatible individuals, a single propagule is sufficient to start a sexually-reproducing colony [. . .]" [13 p. 348]. Having said that, switching is certainly an efficient solution to overcome low densities also for multicellular and/or more motile organisms (see section "Multicellular fungi also switch").

In addition, to overcome the problem of low densities, switching may be beneficial in sessile cells even under high cell densities. The reason for this is that in such sessile organisms, asexual growth by cell duplication yields spatial clustering of same-mating-type cells, removing the possibility of mating for cells in the center (see Fig. 3). Efficient switching during each mitosis will strongly reduce the number of unmated cells in a patch [54]. Some yeast species only switch right before mating, which reduces mating to only those cells that are still able to divide [54, 55]. In these species, we expect switching to be induced by crowding.

A recent theoretical paper by Hadjivasiliou et al. [70] suggests an interesting alternative hypothesis for switching. In a predominantly asexually reproducing population, the mating type ratio might become highly skewed due to drift or beneficial traits linked to one of the mating types (e.g. [71]). An individual that can switch mating type will have higher population level compatibility and thus a switcher can invade a population of non-switchers. The importance of this principle might be more applicable to motile organisms.

Why haploid selfing?

The main benefit of sexual reproduction is the efficient removal of deleterious mutations and the association of beneficial alleles by recombination [72]. Haploid selfing is apparently useless, because recombination will be between identical chromosomes [52, 73]. However, sex and recombination have secondary consequences that can be beneficial, such as a switch in ploidy level and different physiology [51]. An important consequence that is often seen in facultatively asexual organisms such as fungi is the association of sexual reproduction with the production of survival structures, which are favorable under harsh circumstances. This association might be developmentally constrained, and hence sexual reproduction is needed for survival even without generating the above-mentioned genetic benefits [74, 75]. Examples for such secondary benefits of sexual reproduction

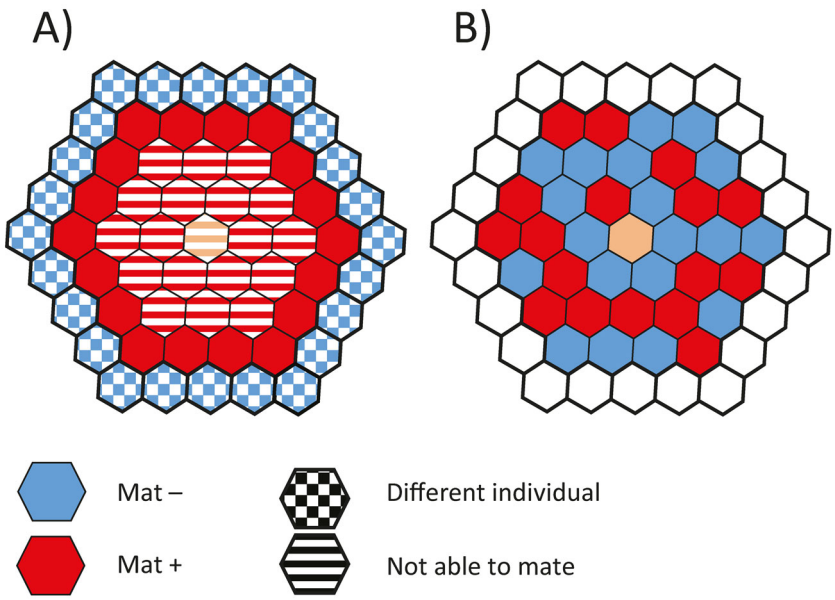


Figure 3. Schematic of mating in a single cell colony, in which each hexagon represents one cell. The central cell (light) initiates a colony of asexually reproducing cells without switching (**A**), or with switching (**B**). In (**A**), only the cells at the edge of the colony have the potential to mate, and then still only when another individual of the opposite mating type is neighboring (chequered cells). No cells in the centre can mate (hashed cells). In the switching colony, each cell has the potential to mate within the colony while retaining the ability to outcross, similar to the cells in the non-switching colony.

are meiotic spores that exhibit increased tolerance to digestion, heat, drought, or frost [54, 76–78].

Another benefit of haploid mating is that it facilitates the shift to diploidy, which by some species is favored over haploidy during asexual growth. In *Saccharomyces paradoxus* for example, the preferred ploidy level is environment-dependent [79] but propagule dispersal in nature is probably as a haploid spore [80]. Other species require a diploid phase for filamentous growth or virulence [27, 73]. Switching and fusion might thus ensure diploidization. When the sexual phase is essential, selfing can be preferred over outcrossing, especially in pathogenic species in which gene combinations can be essential in fending off a host's defence mechanisms [81]. Recent experiments showed that haploid selfing can generate de novo mutations and beneficial phenotypes [82].

Switching is beneficial over true homothallism

From a population genetic point of view, homothallism and switching are very similar: both lead to self-compatibility, while maintaining the potential to outcross. However, many of the benefits of mating types are lost in true homothallics, where both mating types are expressed in the same individual. Individuals of switching species that express only one mating type retain those benefits.

There are three main benefits of mating-type switching over true homothallism. First, mating types are of importance in regulating expression of ploidy specific genes, known as the "developmental switch" model [50, 51]. Experiments using mutants or constructed strains that express both mating types in the same individual show highly reduced fitness (e.g. in fission yeast and *Schizophyllum commune* [83, 84]). Second, mating types are often also involved in regulation of cytoplasmic inheritance, probably to avoid conflict between nuclear and organellar genomes (e.g. from mitochondria and chloroplasts [85, 86]). The cytoplasmic elements of one mating type are consistently destroyed (e.g. [87]), a feature that might be hampered in true homothallism, where both parents carry both genetic elements. This conflict probably plays a limited role in haploid selfing, where all individuals share cytoplasm, but will still be costly during outcrossing events. Third, compounds involved in pre-mating recognition are mating-type specific in green algae [88], diatoms [89], fungi [90], and ciliates [91]. These compounds are of importance for recognition of conspecifics [92, 93] and for sexual selection [94, 95]. Hoekstra [96, 97] proposed that gamete recognition is necessary for mate finding, and that this can only arise when cells express different compounds because expressing both would lead to self-activation. Hadjivasiliou et al. [53] show in an explicit model that asymmetric signalling in motile organisms greatly increases mating efficiency, and that simultaneous signalling-and-receiving reduces cell–cell recognition. Recent experiments in non-motile fission yeast cells seem to confirm these predictions [98]. Maintaining different mating types might thus be beneficial in mate finding, which can increase the chance of outcrossing.

Many species, especially multicellular ones, are truly homothallic [99]. In these species, the costs of self-activation might be reduced by physiological restriction of mating type expression. In some filamentous fungi, for instance, two different mate-recognition compounds are produced by different structures [100]. Alternatively, one of the mating-type genes might have lost functionality as seen in many homothallic *Neurospora* species [101], which probably reduces costs of self-activation. Other systems have likely taken over the haploid-diploid switch function, as suggested by conservation of the pheromone-receptor system in these species [102].

Mating-type switching is likely to be costly

Mating-type switchers do carry both mating-type cassettes in the same genome, which are strictly regulated, hence avoiding deleterious effects, as described above for homothallic species. To avoid expressing both mating types, many species

have evolved a variety of gene-silencing mechanisms, through epigenetic control, that act together for high efficiency. Silenced regions can, for instance, incorporate centromere-specific heterochromatin by localization close to a centromere, or incorporating silenced features such as centromeres or transposable elements [57]. In budding yeast, both trans and cis acting elements produce 3 kb regions of heterochromatin around the mating-type cassette [42]. In fission yeast, silencing is further mediated by RNA interference (RNAi) and acts both trans and cis [43]. An unintended consequence of silencing might be that silencing of one cassette extends beyond the intended region and affects other genes. This might constitute a cost, especially at the early stages of evolution when switching has just appeared, but extra layers of regulation have not evolved yet and suppression is, therefore, leaky.

The mechanism of switching itself is also likely to incur costs, because it generally actively changes the genome, which may result in coding errors. Switching often involves repetitive sequences that are the target of site-specific recombination. During normal meiosis, this can result in crossing-over events between non-homologous regions, resulting in costly unwanted reorganization of the genome [103, 104]. Additionally, maintenance and expression of the switching and suppression mechanisms is likely to have some energetic costs [105]. For instance, genome replication around the mating-type region in fission yeast is blocked from the centromere-proximal site [106], which might prolong the S-phase. However, the actual cost of switching over non-switching has, to our knowledge, not been measured yet.

If switching incurs a substantial cost, the ability to switch might be lost whenever its benefits are small, for instance, when sexual reproduction is very rare, and only asexual propagation is of importance. Similarly, switching is less important under constant mixing, because it breaks up the spatial clustering of clones with the same mating type (but see [70]). Many species of domesticated fungi show polymorphism in the ability to switch (e.g. in budding yeast where loss of *HO* endonuclease occurs regularly [107, 108] and fission yeast by rearrangements in the region around the mating-type locus [109]). These organisms are often maintained asexually for many generations, grown in liquid culture, and kept under high density, which are all factors that may favor heterothallism over switching if the latter has a cost. Many pathogenic yeast strains show a near clonal population structure, e.g. [110], possibly to avoid outbreeding and maintain well adapted virulent strains. Some of these species lost essential switching elements and reverted to self-sterile heterothallism-(e.g. *L. kluyveri* [64]), others have the machinery but do not switch (e.g. *Candida nivariensis* and *Candida bracarensis* [111]) or hardly switch at all (e.g. *Candida glabrata* in which *HO* is not expressed; [112]). It is not clear whether this transition is driven by the cost of switching, or that of outcrossing [64].

Multicellular fungi also switch

Above, we argued that multicellular individuals can limit mating-type-specific expression to specific structures to avoid the cost associated with true homothallism. Nevertheless, this regulation may be leaky, and thus it may have the same cost as it would have in unicellular organisms. Mating-type switching allows avoiding this potential cost in multicellular organisms and is found mainly in ascomycete fungi. The most common form of mating-type switching observed is unidirectional switching (as described for example in *Sc. trifoliorum*, *Ceratocystis fimbriata*, *Cochliobolus heterostrophus*, *Hypocrea spinulosa*, *Botrytinia fuckeliana* [28, 60, 61]). For multiple *Ceratocystis* spp. [60, 113] and for *H. spinulosa* [61] it has been shown that mating occurs by fusion of two nuclei from the same individual, in which one nucleus contains both mating-type cassettes and the other lost one cassette. The result of selfing is that half of the offspring – those that inherited both cassettes – can reproduce by themselves, but that the other half needs a self-fertile individual. The same pattern is seen in the other species, which suggests that a similar mechanism is acting here [28, 60, 61]. Only one mushroom-forming fungus shows mating-type switching: *Agrocybe aegerita* produces meiotic haploid spores, which are self-fertile [65]. Like most mushroom forming fungi, *A. aegerita* has many mating type alleles, and each type switches to a specific alternative type. The molecular mechanism underlying this switching has not yet been investigated.

In *Sclerotinia trifoliorum*, *H. spinulosa*, *Co. heterostrophus*, and *Ceratocystis* spp., self-fertility is associated with spore size dimorphism [113–115]. Each meiosis produces eight spores: four self-fertile and four switched self-sterile, of which the first are considerably larger than the latter. The self-fertile spores obtain a larger part of the resources, which increases their ability to germinate [116] or grow right after germination [113, 115], thus increasing the chance of successfully establishing an infection. Strains isolated in nature are always self-fertile, which suggests that switching is the optimal strategy, but that lack of bidirectional mating leads to evolution of resource allocation toward the self-fertile strain. This could be an intermediate phase between heterothallism and bidirectional switching. Alternatively, if evolution continues and the self-sterile spores are completely aborted, such a strain will probably not be recognizable as a mating-type switcher, but as a true homothallic.

In contrast to the above-mentioned species, switching in *Sclerotinia sclerotiorum* occurs by a bidirectional flip-flop system (Fig. 2B). In this species, switching occurs by the inversion of 3.6 kb of the mating type locus, and the respective orientation determines the mating type expression [56]. In the fruiting body – where mating takes place in outcrossing species – some of the nuclei probably change orientation, which results in the opposite mating type and creates compatibility between nuclei of the new and old mating type. Half of the offspring will be of the original type, and half of the inverted type. Furthermore, because switching is bidirectional, each spore is self-fertile again. Switching in these species might have evolved to maintain the ability to outcross, but additionally maintains reproductive assurance [117].

The above-described filamentous fungi often occur under high population densities, and generally show a clonal but polymorphic distribution (e.g. [118]) which would also be expected when haploid selfing is the main mode of sexual

reproduction. Assuming that sexual reproduction occurs, the clonal structure suggests that switching in these species did not evolve for reproductive assurance, but more likely to facilitate haploid selfing. Beneficial gene combinations will thus be maintained, and recombination load be reduced [72], as is predicted for haploid pathogens [81]. Nevertheless, the benefits of switching over true homothallism as described above remain the same.

Conclusions and outlook

Mating-type switching provides reproductive assurance by selfing without losing the likely benefits of having separate mating types, nor increasing the potential costs of homothallism. It appears that switching evolved in free-living unicellular organisms to assure mating under low density, while retaining the ability to outcross. Additionally, mating-type switching evolved in multicellular fungi where switching might not be expected. However, these multicellular fungi are all pathogenic, which suggests that switching evolved to avoid the break up of beneficial virulence traits during outcrossing, which can be retained via selfing [119].

Switching – especially when bidirectional – is difficult to distinguish from other forms of self-fertility, and might be more common than currently thought – especially in yeast forms that live on solid substrates relevant to the lonely spore hypothesis. New genome sequencing projects should provide further insight into how widespread switching really is, but even at the genome level, switching can seem very similar to true homothallism. Inversion systems (Fig. 2A and B) are common, which have two mating type cassettes, just like true homothallics. In the switching *S. sclerotiorum* for example, the genes from both mating types were initially thought to induce true homothallism, but are actually part of a flip-flop system [120]. Future genome studies on homothallic species should, therefore, take into account that the patterns frequently observed in mating-type switching involve genes from both mating types in close vicinity with a repeated and often inverted motif flanking the switching regions.

The multiple independent origins of mating-type switching in the fungi suggest that in species with mating types, switching can be a stable mechanism to assure reproduction, while retaining the important functions of the mating types. Future studies in other groups where compatibility is determined by mating types, such as the green or brown algae, might show that mating-type switching is a universal solution for reproductive assurance.

Acknowledgments

The authors thank Sergio Tusso and Jochen Wolf for discussions, Aga Lipinska for information on the brown algae, and Sergio Tusso, James Rodger, Duur Aanen, Andrew Moore, and two anonymous reviewers for comments on an earlier version of the manuscript. This study was funded by the Carl Tryggers Foundation to BN and a grant from the European Research Council to SI.

The authors have declared no conflict of interest.

References

1. **Harari AR, Steinitz H.** 2013. The evolution of female sex pheromones. *Curr Zool* **59**: 569–78.
2. **Levitan DR.** 2004. Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. *Am Nat* **164**: 298–309.
3. **Mustajärvi K, Siikamäki P, Rytkönen S, Lammi A.** 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. *J Ecol* **89**: 80–7.
4. **Grindeland JM, Sletvold N, Ims RA.** 2005. Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Funct Ecol* **19**: 383–90.
5. **Allee WC.** 1931. *Animal Aggregations: A Study in General Sociology*. Chicago, IL: University of Chicago Press.
6. **Gascoigne J, Berec L, Gregory S, Courchamp F.** 2009. Dangerously few liaisons: a review of mate-finding Allee effects. *Popul Ecol* **51**: 355–72.
7. **Stephens PA, Sutherland WJ, Freckleton RP.** 1999. What is the Allee effect? *Oikos* **87**: 185–90.
8. **Darwin C.** 1876. *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. London: John Murray.
9. **Lloyd DG.** 1992. Self- and cross-fertilization in plants. II. The selection of self- fertilization. *Int J Plant Sci* **153**: 370–80.
10. **Busch JW, Delph LF.** 2012. The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Ann Bot* **109**: 553–62.
11. **Jarne P, Charlesworth D.** 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu Rev Ecol Syst* **24**: 441–66.
12. **Jarne P, Auld JR.** 2006. Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. *Evol Int J Org Evol* **60**: 1816–24.
13. **Leonard JL.** 2006. Sexual selection: lessons from hermaphrodite mating systems. *Integr Comp Biol* **46**: 349–67.
14. **Beukeboom L, Perrin N.** 2014. *The Evolution of Sex Determination*. Oxford: Oxford University Press.
15. **Kiontke K, Gavin NP, Raynes Y, Roehrig C**, et al. 2004. *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. *Proc Natl Acad Sci USA* **101**: 9003–8.
16. **Theologidis I, Chelo IM, Goy C, Teotónio H.** 2014. Reproductive assurance drives transitions to self-fertilization in experimental *Caenorhabditis elegans*. *BMC Biol* **12**: 93.
17. **McDaniel SF, Atwood J, Burleigh JG.** 2013. Recurrent evolution of dioecy in bryophytes. *Evolution* **67**: 567–72.
18. **Glime JM.** 2013. Sexual strategies chpt 3–1. In Glime JM. ed; *Bryophyte Ecology*. Houghton, MI: Michigan Technological University.
19. **Weeks SC, Benvenuto C, Reed SK.** 2006. When males and hermaphrodites coexist: a review of androdioecy in animals. *Integr Comp Biol* **46**: 449–64.
20. **Igic B, Lande R, Kohn JR.** 2008. Loss of self-incompatibility and its evolutionary consequences. *Int J Plant Sci* **169**: 93–104.
21. **de Nettancourt D.** 1997. Incompatibility in angiosperms. *Sex Plant Reprod* **10**: 185–99.
22. **Baker HG.** 1955. Self-compatibility and establishment after "long-distance" dispersal. *Evolution* **9**: 347–9.
23. **Barrett SCH.** 2014. Evolution of mating systems: outcrossing versus selfing. In Losos JB, ed; *The Princeton Guide to Evolution*. Princeton, NJ: Princeton University Press. p. 356–62.
24. **Pannell JR.** 2015. Evolution of the mating system in colonizing plants. *Mol Ecol* **24**: 2018–37.
25. **Ramm SA, Schlatter A, Poirier M, Schärer L.** 2015. Hypodermic self-insemination as a reproductive assurance strategy. *Proc R Soc B* **282**: 20150660.
26. **Lin X, Heitman J.** 2007. Mechanisms of homothallism in fungi and transitions between heterothallism and homothallism. In Taylor JW, Kronstad JW, Heitman J, Casselton LA, ed; *Sex in Fungi*. Washington, DC: ASM Press. p 35–57.
27. **Heitman J.** 2015. Evolution of sexual reproduction: a view from the fungal kingdom supports an evolutionary epoch with sex before sexes. *Fungal Biol Rev* **29**: 108–17.

28. **Perkins DD.** 1987. Mating-type switching in filamentous ascomycetes. *Genetics* **115**: 215–6.
29. **Wilson AM, Wilken PM, van der Nest MA, Steenkamp ET**, et al. 2015. Homothallism: an umbrella term for describing diverse sexual behaviours. *IMA Fungus* **6**: 207–14.
30. **Abbott JK.** 2011. Intra-locus sexual conflict and sexually antagonistic genetic variation in hermaphroditic animals. *Proc R Soc B Biol Sci* **278**: 161–9.
31. **Holsinger KE.** 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am Nat* **138**: 606–22.
32. **Blakeslee AF.** 1904. Zygospore formation a sexual process. *Science* **19**: 864–6.
33. **Whitehouse HLK.** 1949. Heterothallism and sex in the fungi. *Biol Rev* **24**: 411–47.
34. **May G, Shaw F, Badrane H, Vekemans X.** 1999. The signature of balancing selection: fungal mating compatibility gene evolution. *Proc Natl Acad Sci USA* **96**: 9172–7.
35. **Nieuwenhuis BPS, Billiard S, Vuilleumier S, Petit Elsa E**, et al. 2013. Evolution of uni- and bifactorial sexual compatibility systems in fungi. *Heredity* **111**: 445–55.
36. **Lewin RA.** 1976. *The Genetics of Algae*. Berkeley and Los Angeles: University of California Press.
37. **Coleman AW.** 2012. A comparative analysis of the Volvocaceae (Chlorophyta). *J Phycol* **48**: 491–513.
38. **Poulíčková A, Sato S, Evans KM, Chepurnov VA**, et al. 2015. Repeated evolution of uniparental reproduction in *Sellaphora* (Bacillariophyceae). *Eur J Phycol* **50**: 62–79.
39. **Hamaji T, Ferris PJ, Nishii I, Nishimura Y**, et al. 2013. Distribution of the sex-determining gene MID and molecular correspondence of mating types within the isogamous genus Gonium (Volvocales, Chlorophyta). *PLoS ONE* **8**: e64385.
40. **Geng S, De Hoff P, Umen JG.** 2014. Evolution of sexes from an ancestral mating-type specification pathway. *PLoS Biol* **12**: e1001904.
41. **Paoletti M, Seymour FA, Alcocer MJC, Kaur N**, et al. 2007. Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. *Curr Biol* **17**: 1384–9.
42. **Haber JE.** 2012. Mating-type genes and *MAT* switching in *Saccharomyces cerevisiae*. *Genetics* **191**: 33–64.
43. **Klar AJS.** 2007. Lessons learned from studies of fission yeast mating-type switching and silencing. *Annu Rev Genet* **41**: 213–36.
44. **Glass NL, Grotelueschen J, Metzenberg RL.** 1990. *Neurospora crassa A* mating-type region. *Proc Natl Acad Sci USA* **87**: 4912–6.
45. **Bloomfield G, Skelton J, Ivens A, Tanaka Y**, et al. 2010. Sex determination in the social amoeba *Dictyostelium discoideum*. *Science* **330**: 1533–6.
46. **Ferris P, Olson BJSC, De Hoff PL, Douglass S**, et al. 2010. Evolution of an expanded sex-determining locus in *Volvox*. *Science* **328**: 351–4.
47. **Cervantes MD, Hamilton EP, Xiong J, Lawson MJ**, et al. 2013. Selecting one of several mating types through gene segment joining and deletion in *Tetrahymena thermophila*. *PLoS Biol* **11**: e1001518.
48. **Hood ME.** 2002. Dimorphic mating-type chromosomes in the fungus *Microbotryum violaceum*. *Genetics* **160**: 457–61.
49. **Immler S, Otto SP.** 2015. The evolution of sex chromosomes in organisms with separate haploid sexes. *Evolution* **69**: 694–708.
50. **Haag ES.** 2007. Why two sexes? Sex determination in multicellular organisms and protistan mating types. *Semin Cell Dev Biol* **18**: 348–9.
51. **Perrin N.** 2012. What uses are mating types? The "developmental switch" model. *Evolution* **66**: 947–56.
52. **Billiard S, López-Villavicencio M, Devier B, Hood ME**, et al. 2011. Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. *Biol Rev* **86**: 421–42.
53. **Hadjivasiliou Z, Iwasa Y, Pomiankowski A.** 2015. Cell-cell signalling in sexual chemotaxis: a basis for gametic differentiation, mating types and sexes. *J R Soc Interface* **12**: 20150342.
54. **Hanson SJ, Byrne KP, Wolfe KH.** 2014. Mating-type switching by chromosomal inversion in methylotrophic yeasts suggests an origin for the three-locus *Saccharomyces cerevisiae* system. *Proc Natl Acad Sci USA* **111**: E4851–8.
55. **Maekawa H, Kaneko Y.** 2014. Inversion of the chromosomal region between two mating type loci switches the mating type in *Hansenula polymorpha PLoS Genet* **10**: e1004796.
56. **Chitrampalam P, Inderbitzin P, Maruthachalam K, Wu B-M**, et al. 2013. The *Sclerotinia sclerotiorum* mating type locus (MAT) contains a 3.6-kb region that is inverted in every meiotic generation. *PLoS ONE* **8**: e56895.
57. **Rhind N, Chen Z, Yassour M, Thompson DA**, et al. 2011. Comparative functional genomics of the fission yeasts. *Science* **332**: 930–6.
58. **Butler G, Kenny C, Fagan A, Kurischko C**, et al. 2004. Evolution of the *MAT* locus and its Ho endonuclease in yeast species. *Proc Natl Acad Sci USA* **101**: 1632–7.
59. **Jakočiūnas T, Holm LR, Verhein-Hansen J, Trusina A**, et al. 2013. Two portable recombination enhancers direct donor choice in fission yeast heterochromatin *PLoS Genet* **9**: e1003762.
60. **Wilken PM, Steenkamp ET, Wingfield MJ, de Beer ZW**, et al. 2014. DNA loss at the *Ceratocystis fimbriata* mating locus results in self-sterility *PLoS ONE* **9**: e92180.
61. **Yun S-H**. 2015. Molecular analysis of unidirectional mating type switching in *Chromocrea spinulosa*. *Fungal Genet Rep* **61S**: Abstract 178.
62. **Prescott DM.** 2000. Genome gymnastics: unique modes of dna evolution and processing in ciliates. *Nat Rev Genet* **1**: 191–8.
63. **Barsoum E, Martinez P, Aström SU.** 2010. α3, a transposable element that promotes host sexual reproduction. *Genes Dev* **24**: 33–44.
64. **Payen C, Fischer G, Marck C, Proux C**, et al. 2009. Unusual composition of a yeast chromosome arm is associated with its delayed replication. *Genome Res* **19**: 1710–21.
65. **Labarere J, Noel T.** 1992. Mating type switching in the tetrapolar basidiomycete *Agrocybe aegerita*. *Genetics* **131**: 307–319.
66. **Kües U, James TY, Heitman J.** 2011. Mating type in basidiomycetes: unipolar, bipolar, and tetrapolar patterns of sexuality. In Pöggeler S, Wöstemeyer J. ed; *Evolution of Fungi and Fungal-Like Organisms*. Berlin, Heidelberg: Springer-Verlag. p. 97–160.
67. **Tsuchikane Y, Tsuchiya M, Hindák F, Nozaki H**, et al. 2011. Zygospore formation between homothallic and heterothallic strains of *Closterium*. *Sex Plant Reprod* **25**: 1–9.
68. **Ichimura T, Kasai F.** 1995. Dissection of conjugants and mating type plus and minus cells in selfing clones of the isogamous green alga *Closterium ehrenbergii*. *Sex Plant Reprod* **8**: 44–8.
69. **Herskowitz I.** 1988. Life cycle of the budding yeast *Saccharomyces cerevisiae*. *Microbiol Rev* **52**: 536–53.
70. **Zena H, Pomiankowski A, Kuijper B.** 2016. The evolution of mating type switching. *Evolution* **70**: 1569–81.
71. **Bell G.** 2005. Experimental sexual selection in *Chlamydomonas*. *J Evol Biol* **18**: 722–34.
72. **Otto SP.** 2009. The evolutionary enigma of sex. *Am Nat* **174**: S1–14.
73. **Giraud T, Yockteng R, Lopez-Villavicencio M, Refregier G**, et al. 2008. Mating system of the anther smut fungus *Microbotryum violaceum*: selfing under heterothallism. *Eukaryot Cell* **7**: 765–75.
74. **Aanen DK, Hoekstra RF.** 2007. Why sex is good: on fungi and beyond. In Heitman J, Kronstad JW, Taylor JW, et al. ed; *Sex in Fungi: Molecular Determination and Evolutionary Implications*. Washington, D.C.: ASM Press. p. 527–34.
75. **Phadke SS, Feretzaki M, Heitman J.** 2013. Unisexual reproduction enhances fungal competitiveness by promoting habitat exploration via hyphal growth and sporulation. *Eukaryot Cell* **12**: 1155–9.
76. **Clarkson JP, Staveley J, Phelps K, Young CS**, et al. 2003. Ascospore release and survival in *Sclerotinia sclerotiorum*. *Mycol Res* **107**: 213–22.
77. **Coluccio AE, Rodriguez RK, Kernan MJ, Neiman AM.** 2008. The yeast spore wall enables spores to survive passage through the digestive tract of *Drosophila*. *PLoS ONE* **3**: e2873.
78. **Wyatt TT, Wösten HAB, Dijksterhuis J.** 2013. Fungal spores for dispersion in space and time. In Gadd GM. ed; *Advances in Applied Microbiology*. San Diego, CA: Academic Press. p. 43–91.
79. **Zörgö E, Chwialkowska K, Gjuvsland AB, Garré E**, et al. 2013. Ancient evolutionary trade-offs between yeast ploidy states. *PLoS Genet* **9**: e1003388.
80. **Reuter M, Bell G, Greig D.** 2007. Increased outbreeding in yeast in response to dispersal by an insect vector. *Curr Biol* **17**: R81–3.
81. **Gladieux P, GuéRin F, Giraud T, Caffier V**, et al. 2011. Emergence of novel fungal pathogens by ecological speciation: importance of the reduced viability of immigrants. *Mol Ecol* **20**: 4521–32.
82. **Ni M, Feretzaki M, Li W, Floyd-Averette A**, et al. 2013. Unisexual and heterosexual meiotic reproduction generate aneuploidy and phenotypic diversity de novo in the yeast *Cryptococcus neoformans*. *PLoS Biol* **11**: e1001653.
83. **Kothe E, Gola S, Wendland J.** 2003. Evolution of multispecific mating-type alleles for pheromone perception in the homobasidiomycete fungi. *Curr Genet* **42**: 268–75.
84. **Nielsen O, Egel R.** 2007. The *mat* genes of *Schizosaccharomyces pombe*: expression, homothallic switch, and silencing. In Heitman J, Kronstad JW, Taylor JW, Casselton LA, ed; *Sex in Fungi: Molecular Determination and Evolutionary Implications*. Washington, D.C.: ASM Press.

85. **Barr CM, Neiman M, Taylor DR.** 2005. Inheritance and recombination of mitochondrial genomes in plants, fungi and animals. *New Phytol* **168**: 39–50.
86. **Murlas Cosmides L, Tooby J.** 1981. Cytoplasmic inheritance and intragenomic conflict. *J Theor Biol* **89**: 83–129.
87. **Yan Z, Hull CM, Heitman J, Sun S**, et al. 2004. *SXI1α* controls uniparental mitochondrial inheritance in *Cryptococcus neoformans*. *Curr Biol* **14**: R743–44.
88. **Sekimoto H, Abe J, Tsuchikane Y.** 2012. Chapter nine – new insights into the regulation of sexual reproduction in *Closterium*. In Jeon KW, ed; *International Review of Cell and Molecular Biology*. San Diego, CA: Academic Press. p. 309–38.
89. **Frenkel J, Vyverman W, Pohnert G.** 2014. Pheromone signaling during sexual reproduction in algae. *Plant J* **79**: 632–44.
90. **Kothe E.** 2008. Sexual attraction: on the role of fungal pheromone/receptor systems (a review). *Acta Microbiol Immunol Hung* **55**: 125–43.
91. **Luporini P, Vallesi A, Alimenti C, Ortenzi C.** 2006. The cell type-specific signal proteins (pheromones) of protozoan ciliates. *Curr Pharm Des* **12**: 3015–24.
92. **Murphy HA, Kuehne HA, Francis CA, Sniegowski PD.** 2006. Mate choice assays and mating propensity differences in natural yeast populations. *Biol Lett* **2**: 553–6.
93. **MacLean CJ, Greig D.** 2008. Prezygotic reproductive isolation between *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. *BMC Evol Biol* **8**: 1.
94. **Jackson CL, Hartwell LH.** 1990. Courtship in *Saccharomyces cerevisiae*: an early cell-cell interaction during mating. *Mol Cell Biol* **10**: 2202–2213.
95. **Rogers DW, Greig D.** 2009. Experimental evolution of a sexually selected display in yeast. *Proc R Soc B Biol Sci* **276**: 543–9.
96. **Hoekstra RF.** 1987. The evolution of sexes. In Stearns SC, ed; *The Evolution of Sex and Its Consequences*. Basel: Birkhauser Verlag. p. 59–91.
97. **Hoekstra RF.** 1982. On the asymmetry of sex: evolution of mating types in isogamous populations. *J Theor Biol* **98**: 427–51.
98. **Merlini L, Khalili B, Bendezú FO, Hurwitz D**, et al. 2016. Local pheromone release from dynamic polarity sites underlies cell-cell pairing during yeast mating. *Curr Biol* **26**: 1117–25.
99. **Dyer PS.** 2008. Evolutionary biology: genomic clues to original sex in fungi. *Curr Biol* **18**: R207–9.
100. **Kim H, Wright SJ, Park G, Ouyang S**, et al. 2012. Roles for receptors, pheromones, G-proteins, and mating type genes during sexual reproduction in *Neurospora crassa*. *Genetics* **190**: 1389–404.
101. **Wik L, Karlsson M, Johannesson H.** 2008. The evolutionary trajectory of the mating-type (*mat*) genes in *Neurospora* relates to reproductive behavior of taxa. *BMC Evol Biol* **8**: 1–12.
102. **Nygren K, Strandberg R, Gioti A, Karlsson M**, et al. 2012. Deciphering the relationship between mating system and the molecular evolution of the pheromone and receptor genes in *Neurospora*. *Mol Biol Evol* **29**: 3827–42.
103. **Hicks WM, Kim M, Haber JE.** 2010. Increased mutagenesis and unique mutation signature associated with mitotic gene conversion. *Science* **329**: 82–5.
104. **Gordon JL, Armisén D, Proux-Wéra E, ÓhÉigeartaigh SS**, et al. 2011. Evolutionary erosion of yeast sex chromosomes by mating-type switching accidents. *Proc Natl Acad Sci USA* **108**: 20024–9.
105. **Connolly B, White CI, Haber JE.** 1988. Physical monitoring of mating type switching in *Saccharomyces cerevisiae*. *Mol Cell Biol* **8**: 2342–9.
106. **Dalgaard JZ, Klar AJS.** 2001. A DNA replication-arrest site *RTS1* regulates imprinting by determining the direction of replication at *mat1* in *S. pombe*. *Genes Dev* **15**: 2060–8.
107. **Louis EJ.** 2011. Population genomics and speciation in yeasts. *Fungal Biol Rev* **25**: 136–42.
108. **Katz Ezov T, Chang S-L, Frenkel Z, Segrè AV**, et al. 2010. Heterothallism in *Saccharomyces cerevisiae* isolates from nature: effect of *HO* locus on the mode of reproduction. *Mol Ecol* **19**: 121–31.
109. **Jeffares DC, Rallis C, Rieux A, Speed D**, et al. 2015. The genomic and phenotypic diversity of *Schizosaccharomyces pombe*. *Nat Genet* **47**: 235–41.
110. **Lott TJ, Frade JP, Lockhart SR.** 2010. Multilocus sequence type analysis reveals both clonality and recombination in populations of *Candida glabrata* bloodstream isolates from U.S. surveillance studies. *Eukaryot Cell* **9**: 619–25.
111. **Gabaldón T, Martin T, Marcet-Houben M, Durrens P**, et al. 2013. Comparative genomics of emerging pathogens in the Candida glabrata clade. *BMC Genomics* **14**: 623.
112. **Boisnard S, Li YZ, Arnaise S, Sequeira G**, et al. 2015. Efficient mating-type switching in *Candida glabrata* induces cell death. *PLoS ONE* **10**: e0140990.
113. **Witthuhn RC, Harrington TC, Wingfield BD, Steimel JP**, et al. 2000. Deletion of the *MAT-2* mating-type gene during uni-directional mating-type switching in *Ceratocystis*. *Curr Genet* **38**: 48–52.
114. **Mathieson MJ.** 1952. Ascospore dimorphism and mating type in *Chromocrea spinulosa* (Fuckel) Petch n. Comb. *Ann Bot* **16**: 449–68.
115. **Uhm JY, Fujii H.** 1983. Ascospore dimorphism in *Sclerotinia trifoliorum* and cultural characters of strains from different-sized spores. *Phytopathology* **73**: 565.
116. **Lee DH, Roux J, Wingfield BD, Wingfield MJ.** 2015. Variation in growth rates and aggressiveness of naturally occurring self-fertile and self-sterile isolates of the wilt pathogen *Ceratocystis albifundus*. *Plant Pathol* **64**: 1103–9.
117. **Coppin E, Debuchy R, Arnaise S, Picard M.** 1997. Mating types and sexual development in filamentous ascomycetes. *Microbiol Mol Biol Rev* **61**: 411–428.
118. **Clarkson JP, Coventry E, Kitchen J, Carter HE**, et al. 2013. Population structure of *Sclerotinia sclerotiorum* in crop and wild hosts in the UK. *Plant Pathol* **62**: 309–24.
119. **de Vienne DM, Refrégier G, López-Villavicencio M, Tellier A**, et al. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytol* **198**: 347–85.
120. **Amselem J, Cuomo CA, van Kan JAL, Viaud M**, et al. 2011. Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genet* **7**: e1002230.