Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types

Sylvain Billiard[1,*], Manuela López-Villavicencio[2], Benjamin Devier[3], Michael E. Hood[4], Cécile Fairhead[5] and Tatiana Giraud[3]

[1] *Université Lille Nord de France, USTL, GEPV, CNRS, FRE 3268, F-59650 Villeneuve d'Ascq, France*
[2] *Origine, Structure, Evolution de la Biodiversité, UMR 7205 CNRS-MNHN, Muséum National d'Histoire Naturelle, CP39, 57 rue Cuvier, 75231 Paris Cedex 05, France*
[3] *Ecologie, Systématique et Evolution, Université Paris-Sud, F-91405 Orsay cedex, France; CNRS F-91405 Orsay cedex, France*
[4] *Department of Biology, McGuire Life Sciences Building, Amherst College, Amherst, MA 01002-5000, USA*
[5] *Institut de Génétique et Microbiologie, Université Paris-Sud, UMR CNRS 8621, F-91405 Orsay cedex, France*

ABSTRACT

The advantage of sex has been among the most debated issues in biology. Surprisingly, the question of why sexual reproduction generally requires the combination of distinct gamete classes, such as small and large gametes, or gametes with different mating types, has been much less investigated. Why do systems with alternative gamete classes (*i.e.* systems with either anisogamy or mating types or both) appear even though they restrict the probability of finding a compatible mating partner? Why does the number of gamete classes vary from zero to thousands, with most often only two classes? We review here the hypotheses proposed to explain the origin, maintenance, number, and loss of gamete classes. We argue that fungi represent highly suitable models to help resolve issues related to the evolution of distinct gamete classes, because the number of mating types vary from zero to thousands across taxa, anisogamy is present or not, and because there are frequent transitions between these conditions. We review the nature and number of gamete classes in fungi, and we attempt to draw inferences from these data on the evolutionary forces responsible for their appearance, loss or maintenance, and number.

Key words: sexual reproduction, gametes, syngamy, mitochondrial inheritance, cytoplasmic genetic elements, gamete classes, mating types.

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* Address for correspondence: (E-mail: sylvain.billiard@univ-lille1.fr)

I. INTRODUCTION

Sexual reproduction involves a variety of costs relative to asexual or clonal reproduction (e.g. energy, time, infection risk, and the "cost of males" in anisogamous species), and many investigations have attempted to find evolutionary benefits to sex that balance these costs (Otto, 2009). These investigations have mainly focused on the consequences of sex in terms of the genetic variation generated by recombinatorial meiosis during the transition from the diploid to the haploid phase of the sexual life cycle. Syngamy, the counterpart of meiosis that restores the diploid phase, has received much less attention, although the regulation of syngamy is central to the evolution of sexual eukaryotes. Indeed, syngamy is generally not random, occurring almost exclusively through the combination of distinct and alternative gamete classes, such as small and large gametes (anisogamy, *i.e.* a system regulating syngamy between different gamete classes that is dependent upon size) or gametes with different mating types (hereafter referring to a molecular mechanism of the gamete allowing discrimination for syngamy, independent of size dimorphism). We use the generic term "gamete classes" to refer to forms of gametes that are intercompatible but that cannot undergo syngamy within the same class, including both anisogamy and mating types as properties that may distinguish gamete classes (Fig. 1).

Many questions are raised by the existence of distinct gamete classes: how can a system that restricts the possibility of syngamy to only a subset of existing gametes in the population evolve by natural selection? In the extreme, when there are two gamete classes, each gamete can only fuse with one half of the potential gamete partners. How, then, can a system with two gamete classes arise from a system with no restrictions? And why are there thousands of gamete classes in some species while others have reverted to no size or mating type restrictions on syngamy? The most problematic case, with the existence of two gamete classes, appears paradoxically the most frequent among eukaryotes (male and female, or two mating types). Any new rare gamete class, being compatible with all other existing classes, should be selected for, leading to an increase in the number of gamete classes. Both why gamete classes exist at all and what the forces are that act upon the number of classes need to be understood from an evolutionary point of view.

Broadly satisfying answers to the questions raised above are still lacking, and groups such as fungi represent highly suitable organisms to test various hypotheses that have been put forward based upon theoretical grounds. Indeed, fungi constitute a unique kingdom where all combinations of gamete classes are represented, and with frequent transitions between different numbers of mating types and/or gamete size dimorphism. Importantly, anisogamy is decoupled from mating types in fungi, allowing separate consideration of the evolutionary forces acting on the two determinants of gamete classes. Finally, data on the number of mating types, anisogamy, and on the genetics underlying these traits are abundant for fungi.

Our aims in this review are to investigate three specific questions: (1) why did mating types and anisogamy evolve?
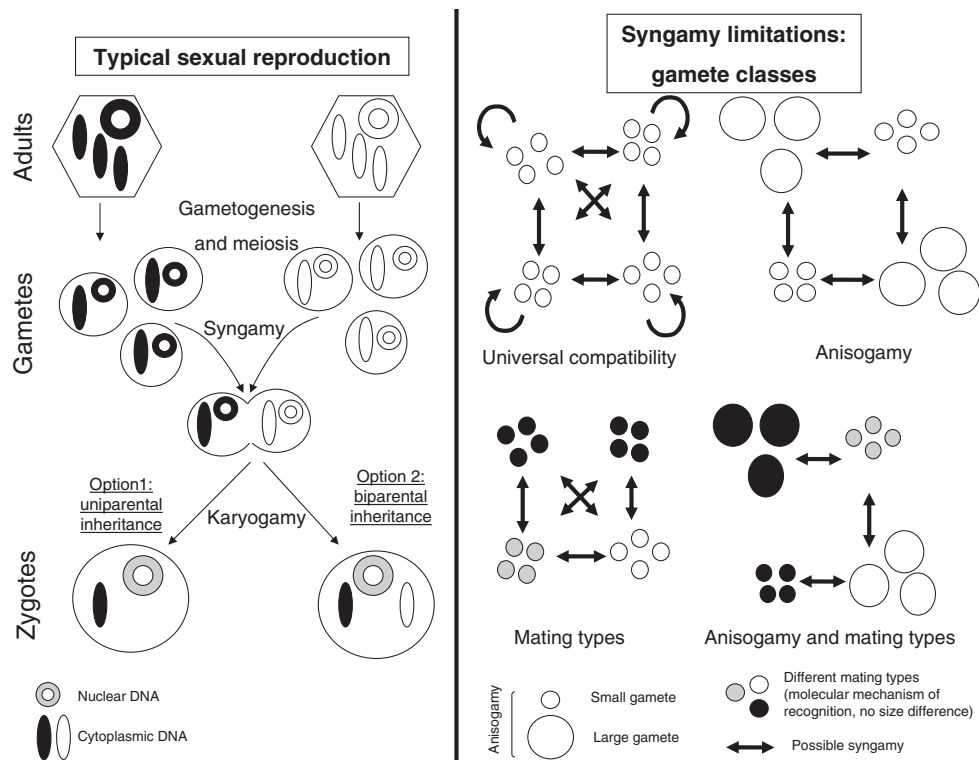
Fig. 1. Illustration of typical sexual reproduction, and its consequences on cytoplasmic inheritance and mate limitation, and of anisogamy and mating types. We have attempted to use unambiguous terminology. "Anisogamy" is defined as a system regulating syngamy between different gamete classes that is dependent upon size, *i.e.* with small and large gametes. Gamete size can be determined by either the haploid genotype (*e.g.* in mosses) or the diploid genotype (*e.g.* in other plants and animals) that produces the gamete. The term "mating types" describes a molecular mechanism allowing discrimination for syngamy at the level of the gamete, independent of size dimorphism. Mating type determination can also depend on the haploid genotype (*e.g.* in fungi, and pollen in gametophytic self-incompatibility in plants) or the diploid genotype (*e.g.* sporophytic self-incompatibility in plants) that produced the gamete. We finally use the generic term "gamete classes" as classes of gametes that are intercompatible but that cannot undergo syngamy within the same class, to include both anisogamy and mating types.

(2) What are the evolutionary or proximate forces controlling the number of gamete classes? In particular, why are systems with two mating types or two gamete sizes so prevalent, despite being *a priori* the least favourable case? (3) Why are gamete classes sometimes lost and sometimes maintained over long periods? We review the theoretical investigations performed so far, emphasizing their underlying hypotheses and predictions, as well as their limitations. We finally attempt to validate or refute these theories using data on fungi available in the literature.

II. MODELS EXPLAINING THE ORIGIN, NUMBER AND LOSS OF GAMETE CLASSES

(1) Origin of gamete classes

Numerous non-exclusive evolutionary models have been proposed to explain the emergence of distinct gamete classes. These models generally assume that all individuals were initially able to mate with any other in the population and that zygotes produced by the fusion of two gametes of different classes had the highest fitness. Below we outline different models attempting to explain the emergence of gamete classes, highlighting their central assumptions.

(a) The "by-product" model

This model assumes that mating types appeared as a by-product of a molecular system ensuring syngamy (Hoekstra, 1982, 1987; Hoekstra, Iwasa & Weissing, 1990). A bipolar system of recognition between gametes evolves in a sexual species by the reciprocal and differential loss of one of the two recognition molecules (a pheromone and pheromone receptor) initially carried by all gametes for signalling the initiation of syngamy. These losses lead to the coexistence of two gamete classes: one having lost the receptor and the other having lost the pheromone.

The central assumption of this model is that the molecular mechanism for recognition is originally symmetrical, with a pheromone receptor and a pheromone produced by every gamete, all gametes thus being undifferentiated. It is further assumed that the probability of mating between undifferentiated gametes may be lower than between differentiated

gametes, because of self-saturation of receptors by self-produced pheromones in undifferentiated gametes.

(b) The "selfish element" model

Mating types appeared together with sexual reproduction and were selected for by providing a means for selfish genetic elements to transmit horizontally (Hoekstra, 1990; Bell, 1993). In this model a cytoplasmic genetic element that can promote fusion of its host cell with another cell appears in an asexual species. This genetic element invades the population because it is transmitted both vertically and horizontally, even if it causes a fitness cost for infected cells. This genetic element can initiate fusion of its host cell with either infected or uninfected cells. A subsequent mutation allowing a variant of this genetic element to promote fusion only with uninfected cells can then invade. The population then consists of two categories of cells: uninfected cells and infected cells that can discriminate between themselves and uninfected cells.

The model assumes that the selfish genetic element can be transmitted vertically during cell division and horizontally after cell fusion. Another assumption is that the selfish genetic element can be lost with some probability, which prevents its fixation. The fusion between cells is assumed to be costly, which allows the discriminating selfish genetic elements, inducing fusions with only uninfected cells, to increase in frequency when rare. The model predicts an asymmetry between mating types regarding the molecular recognition mechanism: only one type of gamete is expected to carry the genetic element inducing syngamy and allowing discrimination, and it should be transmitted to both daughter cells.

(c) The "inbreeding depression avoidance" model

Mating types were selected for by providing a means to avoid selfing and inbreeding depression (Charlesworth & Charlesworth, 1979; Uyenoyama, 1988a, b). If selfing rates and inbreeding depression are high enough, mating types that function to discriminate at the level of the diploid individual can invade a panmictic population. This is because they prevent fertilization between individuals bearing the same allele (i.e. promote disassortative mating), preventing selfing and thus inbreeding depression by reducing the proportion of the genetic load in a homozygous condition for genes required during the diploid phase of the life cycle.

These models assume inbreeding depression, i.e. that selfed or inbred offspring have a lower fitness than outbred offspring. Individuals are assumed to be diploid and inbreeding depression arises from exposure to selection of rare deleterious mutations that become homozygous in inbred offspring. These models can only be applied to species that are diploid and whose mating type determinism is controlled at the diploid level, such as plants and animals (Fig. 2).

(d) The "sex advantage enhancer" model

Mating types were selected for by maximizing the recombinatorial advantages of sex (Czárán & Hoekstra, 2004).

Several hypotheses have been invoked for the advantage of sex (DNA repair, breakdown of detrimental genetic associations and increase of genetic variance in offspring, red queen hypothesis; Agrawal, 2006). If sex is indeed advantageous for any of these reasons, syngamy must occur between gametes that are not identical haploid clones (Nauta & Hoekstra, 1992). Even DNA repair needs intact sequence fragments as templates to repair damaged genes. Czárán & Hoekstra (2004) proposed that mating types were selected for because they prevented syngamy between identical haploid clones. They showed that, if the spatial distribution of identical haploids is clumped, mating types determined at the haploid level can evolve to ensure mating between different haploid genotypes. This model is different from the "inbreeding depression avoidance" described above, which is based upon the exposure of deleterious mutations to selection when inbreeding renders them homozygous.

Within this model no deleterious mutations are assumed, the disadvantage of mating among haploid clones of the same genotype is that costs of sex are paid (loss of time and energy), while no benefit is gained by recombining between identical haploids. Sex is assumed to be advantageous. Gametes are assumed to have limited dispersal ability.

This model can only be applied to organisms like fungi or algae in which the life cycle involves replication of haploid genotypes and allows syngamy between identical haploid clones and for which the genetic determinism of mating type occurs at the haploid level (Fig. 2). The model predicts that mating types should be present in species exhibiting the following characteristics: mating types determined at the haploid level, possibility of syngamy between identical haploids, and limited gamete dispersal.

(e) The "ploidy" model

Mating types were selected for by allowing cells to respond appropriately to their ploidy status (Haag, 2007). Mating types evolved to provide a cellular signal that indicates the genomic condition of diploidy. This prevents further syngamy and indicates the potential to proceed with meiosis. Mating types are selected for that allow gametes to undergo syngamy only with another haploid cell. This model has only been articulated as a verbal argument so far.

(f) The "organelle inheritance" model

Mating types and/or anisogamy were selected for by regulating the inheritance of cytoplasmic genetic elements and avoiding the cost of intra-genomic conflict (Hurst & Hamilton, 1992; Hutson & Law, 1993; Yamauchi, 2003). One of the most important consequences of syngamy is the mixing of cytoplasmic genetic elements from different individuals. It is generally accepted that within-cell competition between different cytoplasmic elements can result in conflicting levels of selection that impair the fitness of the cell. For instance, mitochondrial mutants with an impaired contribution to the cell's performance but with more rapid within-cell replication will be able to invade. Such cases have
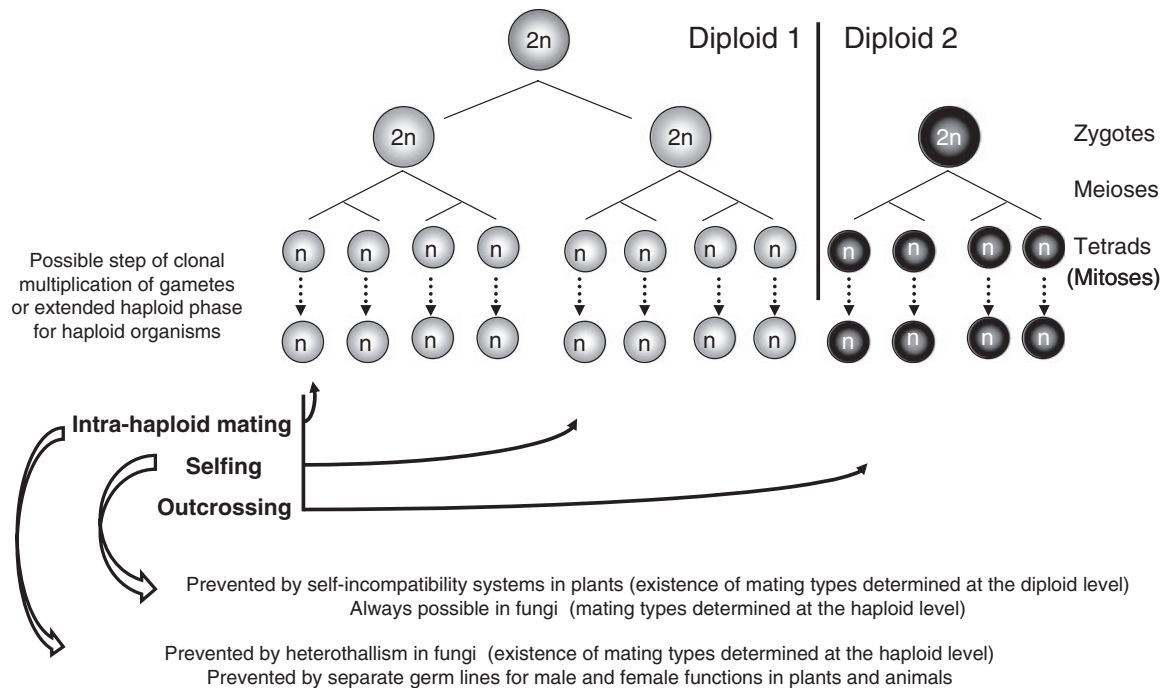
Fig. 2. Diagram showing the products of meiosis from two diploid genotypes, which are often able to proliferate mitotically in fungi and contribute eventually to mating. Various modes of mating are indicated by arrows originating from the far-left meiotic product. Homothallism does not require genetic differences for mating and allows union between any two cells, often including descendants of the same meiotic product as shown here (called intra-haploid mating) and leading to genome-wide homozygosity. Selfing is most commonly used to indicate mating between cells derived from two different meiotic tetrads of the same diploid genotype (although homothallism and intra-haploid mating can be considered subcategories of selfing). Outcrossing is the mating between cells derived from meioses in two different diploid genotypes. Intra-haploid mating is possible in homothallic fungi, but is prevented in heterothallic species by the existence of mating types determined at the haploid level. Plants and animals cannot perform intra-haploid mating because of the existence of separate germ lines for male and female functions. Mating types and heterothallism cannot prevent selfing in fungi, while self-incompatibility systems prevent selfing in plants.

in fact been reported (Hintz, Anderson & Horgen, 1988; Taylor, Zeyl & Cooke, 2002). This model considers that mating types have evolved in isogamous species because it was advantageous for the nuclear genome to limit genetic conflicts that arise through the mixing of diverse cytoplasmic elements by enforcing uniparental inheritance of cytoplasm. Anisogamy has also been proposed to have evolved to avoid such intra-genomic conflicts by enforcing uniparental inheritance of cytoplasm *via* only the larger gametes (Hurst & Hamilton 1992; Hurst, 1996).

This model predicts that whenever cytoplasmic mixing occurs at syngamy, gamete classes should exist and should be associated with uniparental control over inheritance of organelles.

These models generally assume at least three steps: appearance of a selfish cytoplasmic genetic element, appearance of a mutation allowing uniparental inheritance of the cytoplasm, and finally invasion by a mutation allowing syngamy to take place only between two different types of gametes, with one transmitting cytoplasm and the other not. A second assumption of these models is that zygotes formed by the fusion of gametes conferring uniparental inheritance of organelles are the fittest.

(g) The "gamete size" model

Anisogamy is assumed to have evolved because of fitness properties directly linked to gamete sizes (motility, zygote viability, and gamete numbers) (Parker, Baker & Smith, 1972; Maynard Smith, 1978, 1982; Bell, 1978; Charlesworth, 1978; Hoekstra, Janz & Schilstra, 1984; Hoekstra, 1984; Bulmer, 1994; Matsuda & Abrams, 1999; Randerson & Hurst, 1999; Dusenbery, 2000, 2006; Maire, Ackermann & Doebelli, 2001; Bulmer & Parker, 2002; Bonsall, 2006; Iyer & Roughgarden, 2008; Togashi *et al.*, 2009). This set of models considers three main features: (1) the relationship between gamete size and gamete number: small gametes are cheaper to produce and can thus be generated in larger numbers; (2) the relationship between zygote size and zygote fitness: a larger zygote has a higher fitness, imposing selection on gamete sizes for the ability to form a viable zygote; (3) the motility of gametes: the existence of both small motile and large immobile gametes increases the rate of encounters when fertilization is external. Most of these models assume the pre-existence of two mating types upon which the evolution of gamete size is superimposed.

All these models agree that under more or less stringent conditions two classes of gamete sizes can evolve. However,

when the assumption of the pre-existence of two mating types is relaxed (*i.e.* when the mating is not limited *a priori* to fertilization between small and large gametes), the conditions for the evolution of anisogamy are more stringent (*e.g.* Iyer & Roughgarden, 2008). Furthermore, mating can occur only between small and large gametes in all anisogamous species and not between two large gametes (Hoekstra, 1987), but it is difficult to imagine why this could be disfavoured. Differences in motility between small and large gametes should favour fusion between different-sized gametes, but why mechanisms preventing syngamy between large gametes evolved remains to be explained (see next section). Another limitation is that these models assume that mating types predate the evolution of anisogamy, and thus how gamete classes originated remains enigmatic.

(*h*) *The "anisogamy consequence" model*

It is assumed that mating types evolved as a consequence of anisogamy (Charlesworth, 1978; Parker, 1978; Hoekstra, 1984; Maire *et al.*, 2001; Iyer & Roughgarden, 2008; Togashi *et al.*, 2009). In these models, anisogamy evolved following the scenario above but syngamy could still occur between same-size gametes, and mating types thus evolved to restrict fusion between same-size gametes. They assume that syngamy between small gametes is disadvantageous because the resulting small zygote size impairs its fitness.

The problem still remains to find conditions under which the fusion between large gametes is disadvantageous. To our knowledge, only verbal models have been proposed for the evolution of a population with two mating types from a panmictic anisogamous population, each mating type associated with a given gamete size and difference in motility. Hoekstra (1984) showed that selection should favour linkage among mating type loci and loci affecting motility and size dimorphism. But this model still assumes that two mating types predated this linkage to anisogamy, and it still does not explain why syngamy between two large immobile gametes should be disfavoured.

(2) **The optimal number of gamete classes**

The number of gamete classes is highly variable among species, even between closely related taxa, ranging from only two to several thousands. We review here the factors that have been suggested to affect the number of gamete classes (see Fig. 3 for a summary).

(*a*) *The number of gamete classes depends on negative frequency-dependent selection and the effective size of the population*

The main consequence of the existence of gamete classes is that a given gamete can mate with only a fraction of the available gametes in the population. Hence, in systems where gamete classes are inter-compatible, any new gamete class appearing in a population should be favoured by selection in outcrossing species because it can mate with a larger proportion of the population than can the resident gamete

classes. This advantage remains as long as it is rarer than the other gamete classes (Iwasa & Sasaki, 1987). The dynamics of gamete classes in a population is thus governed by negative frequency-dependent selection, predicting the evolution of unlimited numbers of gamete classes. However, populations are finite and some gamete classes will be lost by drift. Finally, the number of gamete classes in a population is a balance between mutations (appearance of novel gamete classes), drift, and negative frequency-dependent selection.

In this scenario a cost to waiting for a mate is assumed to exist (Iwasa & Sasaki, 1987).

The balance between mutations (appearance of novel gamete classes), drift and negative frequency-dependent selection results in the following predictions for outcrossing species: (1) the higher the effective population size, the higher the number of gamete classes (Yokoyama & Hetherington, 1982); (2) gamete classes can be maintained during long evolutionary periods, leading to trans-specific polymorphism (*i.e.* with alleles older than species ages, being maintained across speciation events; Richman, 2000).

(*b*) *The number of gamete classes depends on their mechanism of origin*

Limitation of the number of gamete classes is inherent in the proposed mechanism for their origin for several of the models described in Section II.1, in particular the "selfish element" model, the "gamete size" model, the "by-product model" and the "anisogamy consequence" model. Indeed, these models explain the evolution of two gamete classes, differentiated either by the recognition molecule produced in a unifactorial system, by the ability to maximize horizontal transmission of selfish genetic elements, or the advantage of producing small or large gametes. In those cases, a third mating type or an intermediate gamete size cannot invade the population (Parker *et al.*, 1972; Hoekstra, 1982). Under the by-product model, the appearance of a new receptor and/or pheromone will not allow the appearance of a new mating type, because the mutant would not be recognized by any of the two extant gamete types (Hoekstra, 1987). Where gamete classes evolve to regulate cytoplasmic conflicts (the "organelle inheritance" model), it has been shown that the evolution of more than two classes is also highly unlikely (Hurst, 1996). Indeed, while it is easy to imagine mechanisms allowing uniparental inheritance of cytoplasm when there are two gamete classes (one transmitting its cytoplasm, the other not), it is much harder to imagine a mechanism by which more than two gamete classes can efficiently manage to establish rules of uniparental inheritance, although rare cases have been reported (Kawano, Kuroiwa & Anderson, 1987; Meland *et al.*, 1991; Moriyama & Kawano, 2003). The hypotheses of "inbreeding depression avoidance" and "sex advantage enhancer" by contrast do not predict *a priori* any limitation of the number of gamete classes.

(*c*) *The mating type number depends on the mating system*

Mating systems should also be able to limit the number of mating types when they are determined at the haploid
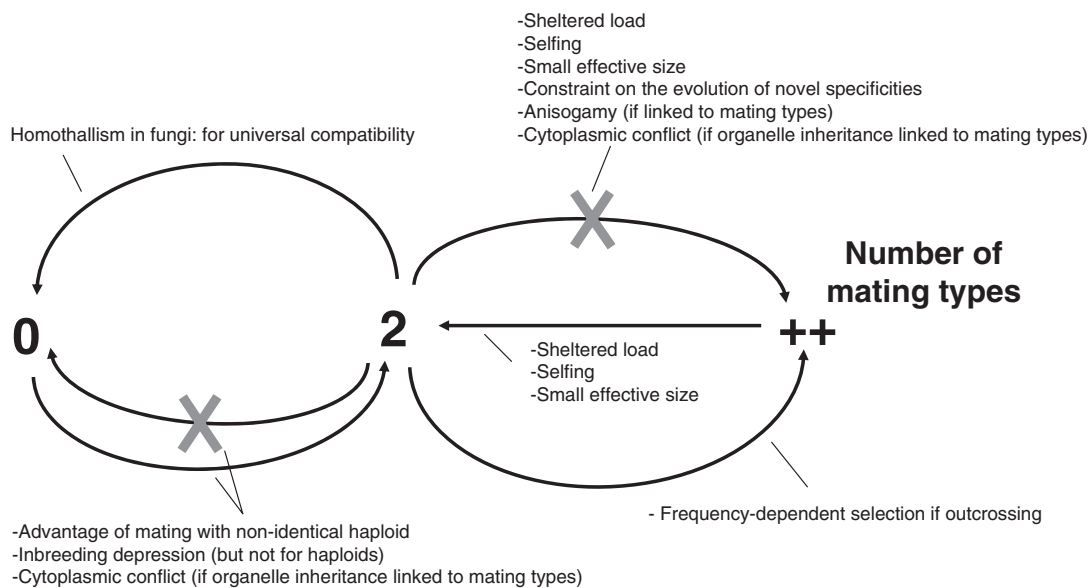
Fig. 3. Diagram summarizing the evolutionary pressures invoked to explain the appearance, maintenance and loss of mating type systems as well as the evolution of the number of mating types. "0" means no restriction at syngamy, *i.e.* no gamete classes, "2" means two gamete classes, "++" means more than two gamete classes. The text associated with each arrow lists the evolutionary pressures suggested to explain the transition in the indicated number of gamete classes. The texts associated with the blocked arrows list evolutionary pressures that can prevent the transition in the number of gamete classes indicated.

level (Giraud *et al.*, 2008). Mating types determined at the haploid level, such as in fungi, do not prevent diploid selfing, *i.e.* syngamy between gametes produced by the same diploid individual (Giraud *et al.*, 2008). Diploid individuals in such species are necessarily heterozygous at the mating type locus and thus produce inter-compatible gamete classes. Some fungi with haploid-determined mating types in fact exhibit high rates of selfing (Giraud *et al.*, 2008). Under such highly selfing mating systems and when gamete dispersal is low, the advantage for rare alleles would also be low. Low gamete dispersal reduces the chances of encountering unrelated mating partners, and because individuals self anyway, acquisition of an allele that is new to the population grants little increase in mating opportunities. This hypothesis has only been proposed verbally so far (Giraud *et al.*, 2008).

(d) The mating type number depends on mating dynamics

The temporal dynamics of searching for a mating partner is a factor capable of maintaining only two mating types: when gametes can wait for a suitable mate, a rare mating type has little advantage (Iwasa & Sasaki, 1987). This scenario assumes that the period for mate searching should be long and the cost of mate searching should be low for the advantage of being rare to decrease, limiting invasion by new mating types.

(e) The mating type number depends on genomic constraints

The molecular mechanisms underlying mating specificities may impede the emergence of new mating types. It may just be too difficult to generate a new variant that is simultaneously compatible with both pre-existing mating

types by one-step mutations: for instance, mutations may be needed simultaneously in both the pheromone and the receptor genes, or the new variant will not be able to mate. If recognition between a specific pheromone and a specific receptor is needed for syngamy, any mutants in the pheromone or receptor genes may even be incompatible with all extant mating types (Hoekstra, 1987). The number of mating types can also be greatly influenced by recombination suppression around mating type loci where recessive deleterious alleles are fixed in the permanently heterozygous state. Such 'sheltered' load can impede invasion by new mating types because of shared deleterious mutations in linkage to the ancestral and the derived mating types (Uyenoyama, 1997; Richman, 2000). In the extreme case, a whole chromosome pair evolves into highly dimorphic sex chromosomes with very little recombination between them. The larger the degree of divergence between the sex chromosomes the more likely that a new gamete class will be incompatible with half of the potential gametes because of exposing deleterious mutations that were previously sheltered in the permanently heterozygous condition, hence limiting the appearance rate of new mating types.

In species with only two mating types, it is predicted that sheltered load will be found near mating type loci, or that new mating types are difficult to evolve by simple mutations.

(3) The loss or maintenance of gamete classes

While the number of gamete classes is thus under a variety of potential evolutionary pressures, the question of why gamete classes are maintained at all remains highly enigmatic under multiple scenarios mentioned above. We define here the "loss of gamete classes" as evolution leading to a lack of

discrimination for syngamy: every haploid cell can undergo syngamy with any other haploid cell in the population, including with genetically identical haploids. We review here the factors that may be responsible for the loss or the maintenance of gamete classes (see Fig. 3 for a summary).

(a) Gamete classes can be lost when population or ecological factors render universal compatibility advantageous

When there are gamete classes, a given gamete cannot mate with a fraction of the population. If there is a cost to waiting for a mate, a universally compatible gamete could invade the population because of the advantage of being able to cross with all individuals, including itself. The conditions allowing for loss of gamete classes have been extensively investigated in the case of self-incompatibility systems in plants (Charlesworth & Charlesworth, 1979; Porcher & Lande, 2005). If, for instance there is a limited probability of encountering mates and thus a risk of not finding a compatible mate at all, being able to self may represent reproductive insurance. This is similar to self-compatibility evolving in plants due to pollen limitation, where female reproductive output is decreased because all ovules cannot be fertilized (Porcher & Lande, 2005). The advantage of finding compatible mates increases when the number of gamete classes is small (Charlesworth & Charlesworth, 1979). If there are two gamete classes in the population, a universally compatible gamete, when rare (after its appearance by mutation for instance), may mate with twice as many individuals as either of the two existing gamete classes. By contrast, if there is an infinitely large number of gamete classes, a universally compatible gamete has no outcrossing advantage relative to other gamete types. So, if the advantage of being universally compatible is so high, why are gamete classes maintained, sometimes over long evolutionary periods?

(b) Gamete classes can be maintained for the same reasons they appeared

Anisogamy is certainly maintained because gametes of an intermediate size would be disfavoured (Parker *et al.*, 1972). By contrast, the only theoretical models investigating the maintenance of mating type systems focused on self-incompatibility systems in plants and invoked genetic load as the main force (Porcher & Lande, 2005). We have found no models that investigate the importance of inbreeding depression or the advantage of sex when the life cycle is mainly haploid, but it seems reasonable to suggest that if these forces can promote the emergence of gamete classes, then they can also contribute to their maintenance.

III. MATING TYPES, ANISOGAMY AND SEX IN FUNGI

The different models outlined above regarding the origin, loss or maintenance, and number of gamete classes make different assumptions and predictions, which we aim here to assess using available data for fungi. Fungi are of particular interest regarding the rules of compatibility between gametes, and it has been a long-standing challenge to integrate concepts of sex in fungi with those developed for plants and animals (Link, 1929). We summarize below the most prominent features of the regulation of syngamy in fungi, which necessarily leads to some over-simplification. Most terms are defined and illustrated in Figs 1 and 4. We compiled literature data regarding systems of mating type determination, mode of reproduction, and number of mating types in the two main groups of fungi, the ascomycetes (many moulds and yeasts) and the basidiomycetes (smut, rusts and mushrooms), and we present these traits in a phylogenetic context (Fig. 5; supporting information available online, see Section VII).

(1) Anisogamy and mitochondrial inheritance in fungi

Filamentous ascomycetes are most often anisogamous (Debuchy, Berteaux-Lecellier & Silar, 2010), while yeasts and basidiomycetes are frequently isogamous, although exceptions exist in both groups. Many filamentous ascomycetes produce small "spermatia" as dispersing gametes (also called microconidia) and more complex and sessile "female" structures (e.g. ascogonia), functioning as large gametes (Debuchy *et al.*, 2010). Mitochondria are then inherited *via* the female-like mating partner (*i.e.* analogous to maternal inheritance), although some paternal leakage can occur (Barroso & Labarère, 1995; Barr, Neiman & Taylor, 2005; Xu, 2005). Importantly, anisogamous ascomycetes are almost never dioecious; haploid individuals of each mating type produce both large and small gametes, except for a very few cases with "female-sterile strains" (Leslie & Raju, 1985; Tredway, Stevenson & Burpee, 2003).

In isogamous yeasts, mitochondria are inherited from both cells involved in syngamy ("biparentally" hereafter) and then actively segregate during subsequent divisions (Berger & Yaffe, 2000). In basidiomycetes, sexual reproduction is most often achieved by the fusion of two haploid mycelia or similar-sized cells. In this group isogamy is the rule and most often there are no differentiated cells playing the role of gametes (Casselton, 2002). Mitochondria can be inherited either uni- or biparentally in the fused cells (Barr *et al.*, 2005; Xu, 2005; Barroso & Labarère, 1995). When inheritance is uniparental at plasmogamy, it can be controlled by mating type. When inheritance is biparental at plasmogamy, they nevertheless usually segregate rapidly during the subsequent mitoses, most probably by an active mechanism (Jin & Horgen, 1994). The "Buller phenomenon" in basidiomycetes describes the case where a heterokaryotic mycelium (*i.e.* with two different haploid nuclei per cell) can fertilize a homokaryotic mycelium (Buller, 1931; Callac *et al.*, 2006) and organelle uniparental inheritance is ensured without mating type playing a role. Mitochondria in the resulting heterokaryotic mycelium are then those of the recipient homokaryotic mycelium. There are some cases of anisogamy in basidiomycetes, such as in some rusts (Bruckart *et al.*, 2010), but the rules of mitochondrial inheritance are unknown.
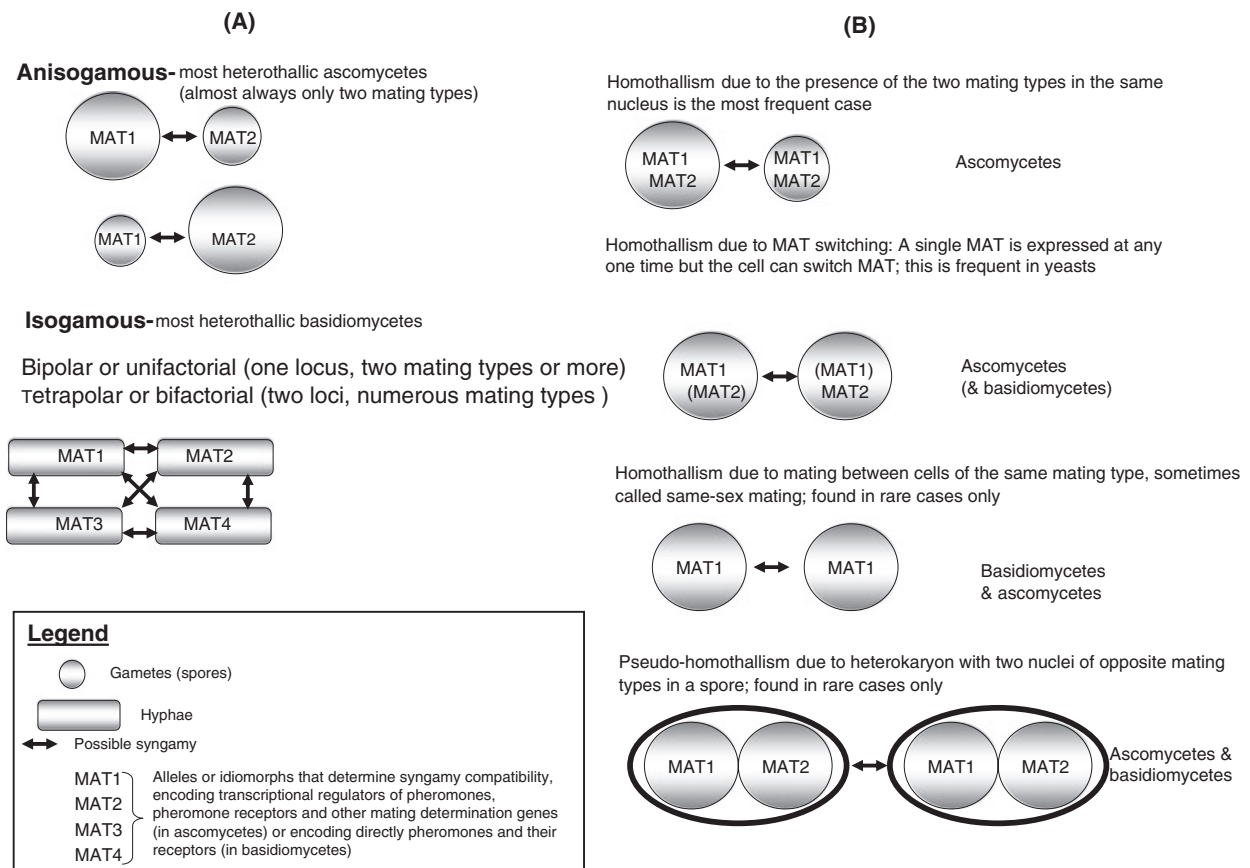
Fig. 4. Summary of modes of syngamy in fungi. (A) Heterothallism; (B) different forms of homothallism.

(2) Mating types in fungi, homothallism and heterothallism

In addition to anisogamy when it is present, strict molecular mechanisms exist in most fungi governing which haploid cells can fuse, controlled by mating type (*MAT*) loci that present two or many alternative forms. Mating types are not associated with anisogamy (Fig. 4) and all genotypes in anisogamous fungi are able to produce large and small gametes.

Mating type determinism is regulated strictly in the haploid stage in fungi, in contrast to most well-studied eukaryotes, in which syngamy compatibility is determined by developmental differences between the sexes or male and female organs in the diploid stage. In fungal species where syngamy is restricted by the *MAT* locus, successful fusion of gametes can occur between haploids carrying different mating type alleles; this is called heterothallism. Compatibility can be determined by alleles at a single locus, a condition called unifactorial (or bipolar) heterothallism, or by alleles at two unlinked loci, called bifactorial (or tetrapolar) heterothallism. Most heterothallic ascomycete fungi present a unifactorial system, regulated by highly dissimilar mating type genes present at the same locus, referred to as "idiomorphs" rather than alleles due to the uncertainty of their origin by common descent (Debuchy *et al.*, 2010; Butler, 2007). These genes code for transcription factors that induce, in particular, the production of pheromones and pheromone receptors. Although most of the ascomycete species present only two alternative idiomorphs, rare cases of a single *MAT* locus with more than two mating types have been reported in this group (Fig. 5A).

By contrast, mating types in basidiomycetes correspond to different alleles of the same genes (*i.e.* they are not idiomorphs; Devier *et al.*, 2009), coding for pheromones, pheromone receptors, and homeodomain proteins. Most basidiomycete fungi present bifactorial systems, with the pheromone-related and homeodomain genes at unlinked loci. The *MAT* loci in bifactorial basidiomycetes are often highly polymorphic, yielding species with many allelic forms that combine to produce thousands of mating types (Casselton & Kues, 2007). Roughly a quarter of basidiomycete species present a unifactorial heterothallism, which is either due to the tight linkage of the two loci containing pheromone-related genes and homeodomain genes, or the loss of function of one of the two loci contributing to mating type specificity (Fraser *et al.*, 2007). Unifactorial basidiomycetes show either two or more mating types, while bifactorial basidiomycetes always show more than two (Fig. 5C).

In contrast to heterothallism described above, in which syngamy can only occur between haploid cells carrying different mating type alleles, many fungi are homothallic, especially among ascomycetes, meaning in the strict historical (experimental) sense that a haploid individual is able to mate

with its own mitotic descendants. Homothallism is often called "self-compatibility" in the fungal literature, but we avoid this term here. "Self-compatibility" indeed can be confused with selfing ability in diploid plants and animals, which involves syngamy between the products of separate meioses and has different genetic consequences than the genome-wide homozygosity resulting from "intra-haploid mating" by homothallic fungi (Giraud *et al.*, 2008) (Fig. 2). Furthermore, the term "self-compatible" may suggest that the evolutionary cause responsible for homothallism was to allow for intra-haploid mating, which is not necessarily true. Homothallism can indeed be viewed also as a lack of discrimination at syngamy: each haploid is compatible with all other haploids in the population in homothallic species, including genetically identical haploids.

Three different proximal mechanisms can cause homothallism, which occurs mostly in ascomycetes (Fig. 5A). The most frequent proximal cause is that each haploid possesses the two idiomorphs in its genome (Yun *et al.*, 1999; Lin & Heitman, 2007). It has never been investigated however whether both mating type idiomorphs are expressed in all cells or if they are expressed alternately. Recent data on mutants however suggest that both pheromones and pheromone receptors would be constitutively expressed (Alby, Schaefer & Bennet, 2009). In some fungi, mainly yeasts, homothallism may arise from mating-type switching: in some yeasts the expressed *MAT* locus can be replaced during mitotic divisions by translocation involving either one of the two flanking silent *MAT* idiomorphs (Strathern *et al.*, 1982; Butler, 2007). Another, rarer, type of homothallism is to allow for mating between cells carrying a single and identical *MAT* allele, which is called "same-sex mating" (Lin & Heitman, 2007). Phylogenies of ascomycetes and basidiomycetes show that homothallism has evolved at least a dozen times independently. Some other fungal species are referred to as "pseudohomothallic", which involves cytological processes that package nuclei from a single meiosis, but with different mating types, into a single spore (Lin & Heitman, 2007; Raju & Perkins, 1994; Rizet, 1943). Thus pseudohomothallism is more analogous to diploid selfing, and even intra-tetrad mating, and does not lead to the same genetic consequences as intra-haploid mating (Lin & Heitman, 2007; Raju & Perkins, 1994; Rizet, 1943).

IV. ORIGIN, NUMBER AND LOSS OF GAMETE CLASSES IN FUNGI

(1) The origin and maintenance of gamete classes in fungi

Fungi are relevant organisms to the understanding of why systems might evolve that impose limitations at syngamy, *i.e.* to study the emergence and maintenance of gamete classes, particularly since anisogamy and mating types in fungi are decoupled (Fig. 4) and variable (Fig. 5). Among all the models

Fig. 5. Phylogenies indicating mating type systems in fungi and the number of mating types. For further details and references, see supplementary material available online (see Section VIII for details). (A) Phylogeny of ascomycetes based on previously published phylogenies showing the diversity of reproductive systems. In all ascomycetes, compatibility is determined by a single mating-type locus with two different idiomorphs/alleles, except in *Glomerella cingulata* where multiple alternative alleles exist. In heterothallic species only one of the two *MAT* idiomorphs is present in each haploid. Pseudohomothallic species produce spores with two nuclei of opposite mating types. Homothallic species bear two *MAT* genes in a single haploid either linked in the same chromosome or in different chromosomes. Same-sex-mating species are homothallic species where only one *MAT* idiomorph has been found in the genome. We use the term "same-sex mating" both for species undergoing mating between identical haploids carrying a single mating type, such as in some homothallic *Neurospora* species, and for species where genetically different cells carrying the same mating type idiomorph/allele are able to fuse, such as in *Cryptococcus* (see C). Marginal systems occur in some species where low proportions of strains present a different system. We also indicate species where regions of suppressed recombination around the mating locus have been described. Anisogamy is the rule in ascomycetes, is independent of mating type, and mitochondria are maternally inherited in most cases. Where data were not available for a particular character, no symbol is shown. (B) Phylogeny of yeast ascomycetes (Saccharomycotina) based on previously published phylogenies showing the diversity of reproductive systems. The two *MAT* alleles are idiomorphs coding for regulators of transcription. Homothallic species bear three *MAT* genes in a single haploid, one copy being expressed, the other two being silent and able to replace the expressed copy. In heterothallic species only one of two *MAT* idiomorphs is present in each haploid, except in the species with the symbol "3" that bear three copies but without being able to switch mating types. Same-sex mating species are homothallic species where only one *MAT* idiomorph has been found in the genome. Yeasts are isogamous, mitochondria are inherited biparentally and are then actively segregated. Where data were not available for a particular character, no symbol is represented. (C) Phylogeny of basidiomycetes based on previously published phylogenies showing the diversity of reproductive systems. In heterothallic species the *MAT* locus includes genes coding for pheromone receptors and pheromones, as well as homeodomain proteins, either at a single locus (unifactorial species) or at two distinct loci (bifactorial species). Pseudohomothallic species produce spores with two nuclei of opposite mating type. Same-sex mating species are homothallic species with a single *MAT* allele, but able to mate with cells carrying identical *MAT* alleles. The numbers of *MAT* alleles are indicated (two or multiple alleles). We also indicate species where regions of suppressed recombination around the mating locus have been described. Most basidiomycetes are isogamous, plasmogamy occurring by fusion between hyphae, without differentiation of particular cells into gametes. Mitochondria can be inherited either uni- or biparentally at plasmogamy (indicated by different arrow types) in the fused cells. When inheritance is biparental, mitochondria from the two parents are however usually segregated rapidly during the subsequent mitoses, probably by an active mechanism. Where data were not available for a particular character, no symbol is represented.
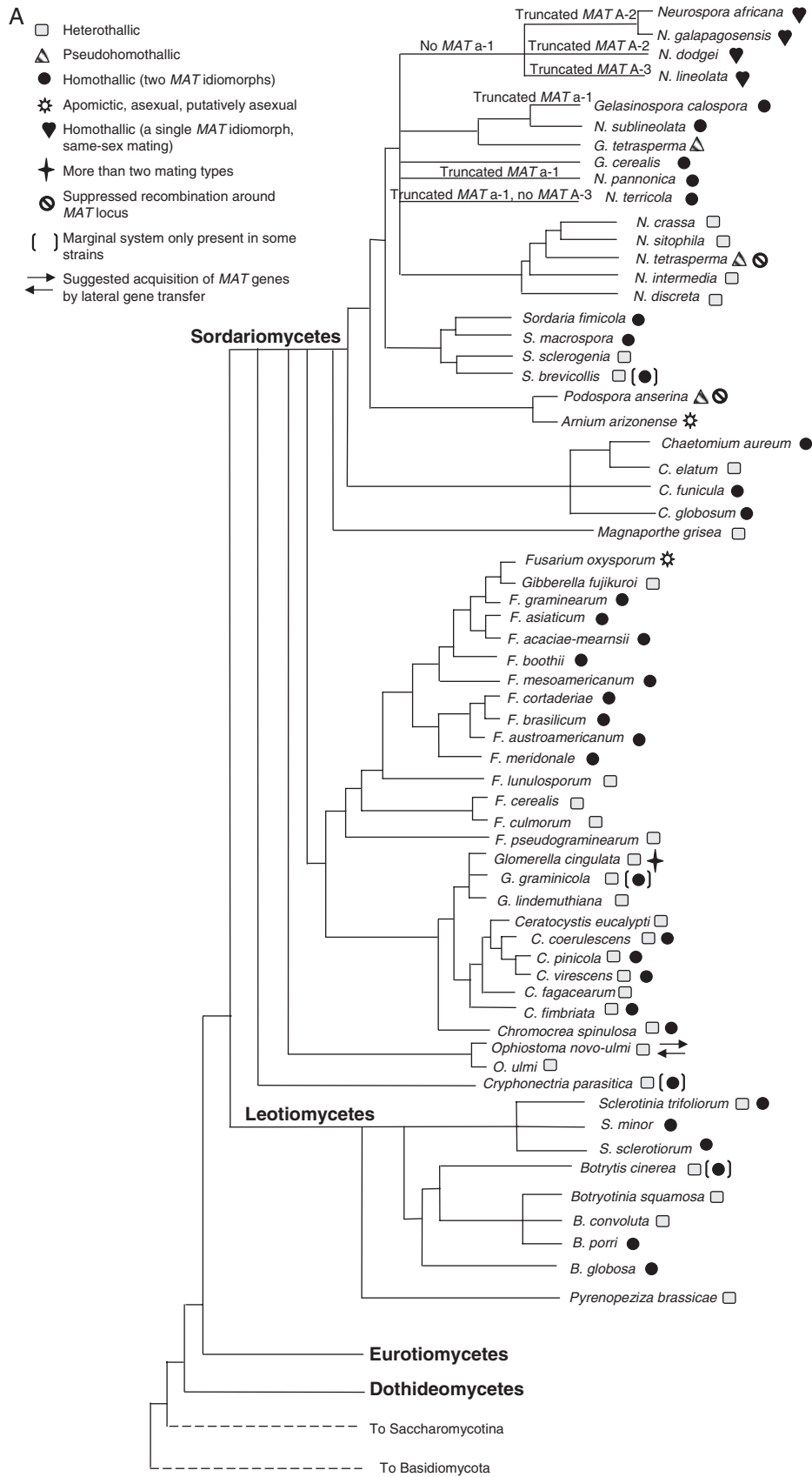
Fig. 5. Legend on previous page.

Fig. 5. *(Cont.)*

B ☐ Heterothallic
▲ Homothallic by *MAT* switching (silent cassettes are present)
♥ Homothallic without switching by same-sex mating
☼ Putatively asexual
3 Three *MAT* loci are present despite lack of *MAT* switching

Saccharomyces cerevisiae ▲
Saccharomyces paradoxus ▲
Candida glabrata ☼
Kluyveromyces delphensis ▲
Candida castellii ☼
Zygosaccharomyces rouxii ▲
Kluyveromyces thermotolerans ☐ 3
Saccharomyces kluyveri ☐
Kluyveromyces lactis var. *lactis* ☐ 3
Eremothecium (Ashbya) gossypii ☐ 3
Candida albicans ☼
Candida tropicalis ☼
Candida parapsilosis ☼
Lodderomyces elongisporus ♥
Candida (Pichia) guilliermondii ☐
Debaryomyces hansenii ♥
Candida (Clavispora) lusitaniae ☐
Yarrowia lipolytica ☐
Schizosaccharomyces pombe ▲

C ☐ Heterothallic
△ Pseudohomothallic (spores including nuclei with different mating types)
▲ Homothallic by mating type switching
♥ Homothallic by same sex mating

⊘ Tetrapolar
○ Bipolar

✚ Multiple mating types

2 Two mating types

🚫 Region of supressed recombination

→ Uniparental mitochondrial inheritance at plasmogamy

➡ Uniparental mitochondrial inheritance at plasmogamy controlled by mating type

↔ Biparental mitochondrial inheritance at plasmogamy

[ ] Marginal system only present in some strains

Agrocybe aegerita ⊘ ✚ → ▲
Agaricus bisporus ○ ✚ → △ (♥)
Agaricus bitorquis ○ ✚ → ☐
Coprinus cinereus ⊘ ✚ ↔ ☐
Coprinellus disseminatus ○ ✚ ☐
Armillaria bulbosa ⊘ ✚ ↔ ☐
Pleurotus djamor ○ ✚ ☐
Schizophyllum commune ⊘ ✚ → ☐
Lentinula edodes ⊘ ✚ ↔ ☐
Stropharia rugosoannulata ⊘ ✚ ☐
Pholiotoa nameko ○ 2 ☐
Laccaria bicolor ⊘ ✚ ☐
Amylostereum areolatum ⊘ ✚ ☐
Heterobasidion insulare ○ ✚ ☐
Heterobasidion annosum ○ ✚ ☐
Ganoderma boninense ⊘ ✚ ☐
Thyphula ishikariensis ⊘ ✚ ☐
Thanatephorus cucumeris ○ 2 ☐
Cryptococcus neoformans ○ 2 🚫 ➡ ☐ ♥
Sporisorium reilianum ⊘ ✚ ☐
Ustilago maydis ⊘ ✚ ☐
Ustilago hordei ○ 2 ☐
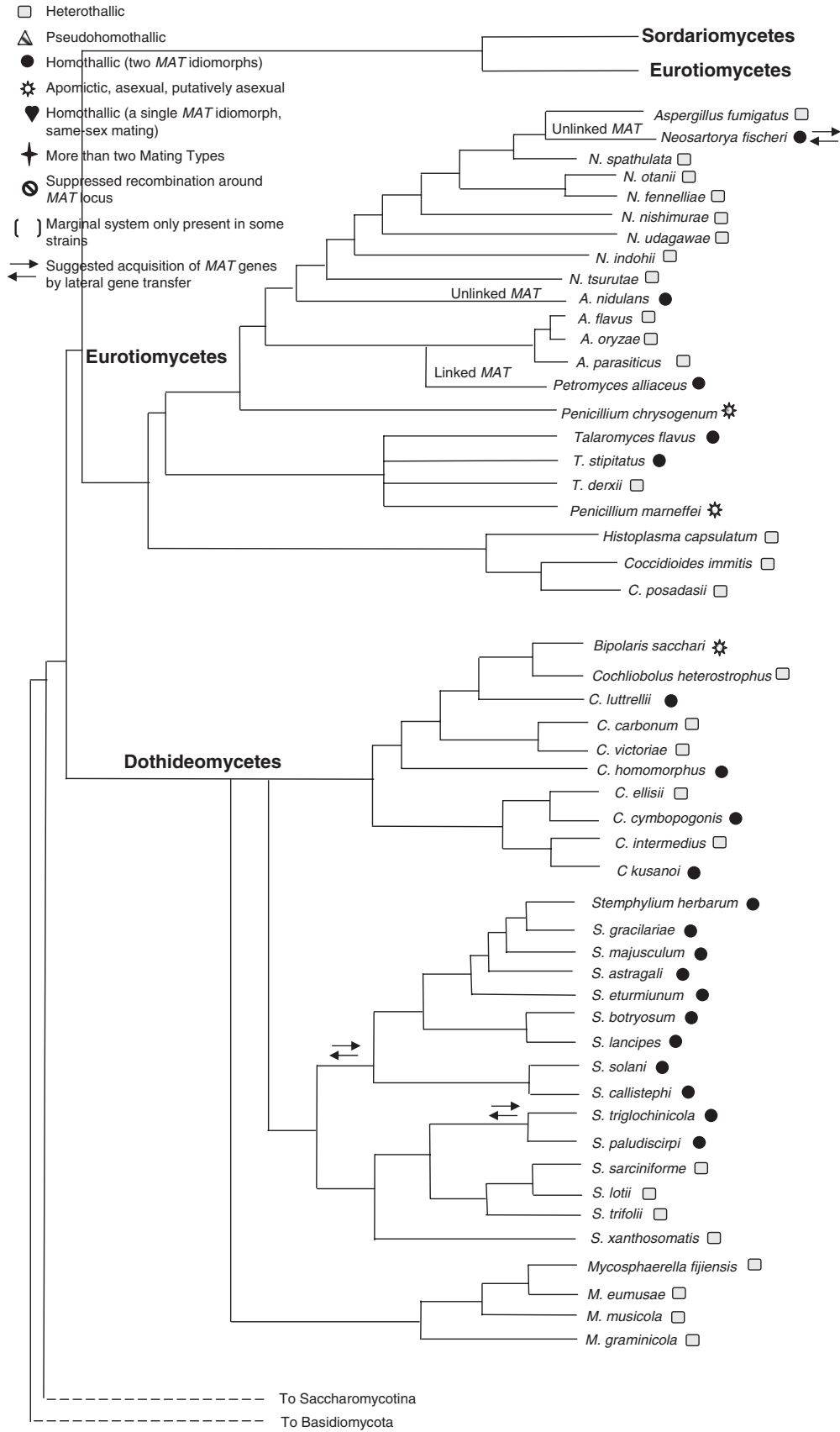*Microbotryum violaceum* ○ 2 🚫 ➡ ☐
Rhodosporium turoloides ○ 2 ☐

Fig. 5. *(Cont.)*

proposed to explain the evolution of gamete classes, several do not seem consistent with observations in fungi. We first argue that one of the most widely accepted hypotheses, the "organelle inheritance" model, certainly does not hold true for fungi. We then evaluate the other models, particularly the "inbreeding depression avoidance" and "sex advantage enhancer" models. Finally, we propose an evolutionary scenario for the emergence of gamete classes in fungi.

(a) The "organelle inheritance" model

The "organelle inheritance" model is one of the most generally accepted hypotheses to explain the emergence of gamete classes. This model predicts an association between gamete classes and the control of inheritance of organelles in species where plasmogamy occurs at syngamy. Most ascomycetes are anisogamous, with inheritance of mitochondria through the larger gamete, even if some paternal leakage of mitochondrial inheritance through the smaller gamete does occur (Barr *et al.*, 2005; Xu, 2005; Milgroom & Lipari, 1993; Lee & Taylor, 1993). This case could therefore appear consistent with the "organelle inheritance" model (Hurst & Hamilton, 1992). It is however not simply the size difference between the gametes that allows maternal inheritance in ascomycetes because there is an active molecular mechanism ensuring that mitochondria are inherited from only one gamete even when isogamy is forced (Lee & Taylor, 1993). Anisogamy therefore does not seem necessary to ensure uniparental inheritance and appears only to be associated with molecular mechanisms controlling organelle transmission.

In fact, fungi show numerous cases of mitochondrial uniparental inheritance that are completely independent of any gamete class (either anisogamy or mating type), such as in yeasts and in basidiomycetes. In yeasts, syngamy is isogamous and mitochondria actively segregate during the first few rounds of cell division (Berger & Yaffe, 2000). Regarding basidiomycetes, Hurst & Hamilton (1992) argued that they did not undergo fusional sex, *i.e.* that there was no cytoplasmic mixing and therefore no need to control selfish genetic elements. This may be true for some mushrooms in which plasmogamy is immediately followed by reciprocal backwards nuclear migration (but not mitochondrial migration) along the mycelia, but not for many other basidiomycete species (Barroso & Labarere, 1995; Alexopoulos, Mims & Blackwell, 1996). In many basidiomycetes, mating occurs between yeast-like cells and cytoplasmic fusion occurs (Wilch, Ward & Castle, 1992). Furthermore, even when mating occurs between mycelia, there are many cases where a filamentous heterokaryon grows after the syngamy of haploid cells, containing the two parental haploid nuclei and possibly the two parental cytoplasms, without any nuclear migration (Hintz *et al.*, 1988). The mode of inheritance of cytoplasmic elements is therefore highly relevant in most species of basidiomycetes. In several species the mating type loci in fact control mitochondrial inheritance (Barr *et al.*, 2005; Wilch *et al.*, 1992; Yan & Xu, 2003) (Fig. 5C). There are nevertheless a number of basidiomycete species with biparental mitochondrial inheritance, at least immediately

following syngamy (Barr *et al.*, 2005; Wilch *et al.*, 1992; Aanen *et al.*, 2004) (Fig. 5C). Mitochondrial heteroplasmy usually resolves after a number of mitotic divisions in the hyphae, more rapidly than expected by simple stochastic processes, suggesting that another active mechanism exists to regulate cytoplasmic conflicts, here again unrelated to gamete classes (Hintz *et al.*, 1988; Barroso & Labarere, 1995, 1997). In cases where a germinating spore or a heterokaryotic mycelium fertilizes a haploid mycelium (Buller, 1931; Callac *et al.*, 2006), there also appear to be rules controlling mitochondrial inheritance independent of the size of the cells or their mating type. Basidiomycetes therefore also seem to stand in contradiction to the "organelle inheritance" model, because gamete classes do not appear to be needed to regulate cytoplasmic conflicts.

(b) The "inbreeding depression avoidance" model

The "inbreeding depression avoidance" models were originally developed to explain the appearance of self-incompatibility systems in angiosperms. Some investigations have shown that inbreeding depression exists in fungi (Leslie & Raju, 1985; Xu, 1995). Ascomycete fungi, however, are haploid for most of their life cycle, which should purge deleterious mutations and thus prevent an inbreeding depression effect. Furthermore, these models cannot fully be applied to fungi because unifactorial, and to a lesser extent bifactorial, haploid mating types cannot prevent diploid selfing (Giraud *et al.*, 2008; Fig. 2). Indeed, because each diploid genotype is necessarily heterozygous at the *MAT* locus in heterothallic species, it will produce haploid gametes with different mating types that are thus able to mate (*i.e.* equivalent to diploid selfing). In fact several fungi are known to undergo high rates of diploid selfing while having mating types (Giraud *et al.*, 2008) (Fig. 2). Inbreeding depression therefore cannot be a force acting on the origin or maintenance of mating types determined at the haploid level, such as in fungi.

(c) The "sex advantage enhancer" model

The "sex advantage enhancer" model, where mating between unlike gametes facilitates a variety of fitness benefits afforded by sexual reproduction, seems more relevant to fungi because many identical haploid clones of a given gamete can in fact be produced and may not necessarily disperse (Day & Garber, 1988). Mating types may well then have evolved in fungi to prevent intra-haploid mating, *i.e.* syngamy between strictly identical haploid clones, which would have no advantage over asexual reproduction. Recombination between genetically identical haploid yeasts indeed seems to be costly (Birdsell & Willis, 1996). Homothallic fungi are capable of intra-haploid mating, but they may not constitute an invalidation of this hypothesis because they may still outcross most often in nature if gametes disperse before mating. In fact, it has been shown for some homothallic species that mating is preferentially disassortative (*i.e.* between genetically different individuals); this is called "relative heterothallism" (Pontecorvo *et al.*, 1953). Also, in some species considered as homothallic in the

laboratory the great majority of syngamy in nature occurs between cells that are distinct meiotic products rather than mitotic descendants of a common clone. The yeast *Saccharomyces cerevisae* for instance, capable of *MAT* switching in the laboratory, undergoes mostly intra-tetrad mating in some natural populations, *i.e.* syngamy between cells that are distinct meiotic products rather than mitotic descendants of a common clone (Katz Ezov *et al.*, 2010). However, some homothallic fungi, such as the homothallic *Neurospora* species, rely mainly on intra-haploid mating for reproduction. These species do not produce asexual conidia and they do not seem to outcross in nature (Glass, Jacobson & Shiu, 2000). Sexual reproduction by intra-haploid mating could provide some advantages. This is the case for instance when other traits, like resistance, become linked to sexual reproduction. Sexual spores are indeed often more resistant and can survive more adverse conditions than asexual conidia (Aanen & Hoekstra, 2007). Sex is also associated with cell rejuvenation, avoiding senescence (Sinclair, Mills & Guarente, 1998; Haedens, Malagnac & Silar, 2005). For some species, it has been found that slightly deleterious mutations accumulate at a lower rate when individuals reproduce by intra-haploid mating compared with asexual reproduction (Bruggeman *et al.*, 2003; Aanen & Hoekstra, 2007). Finally, in some ascomycetes infected by RNA viruses, the transmission of the virus will be vertical only *via* the asexual conidiospores while no transmission is observed to the sexual progeny (Rogers, Buck & Brasier, 1986; Coenen, Kevei, & Hoekstra, 1997).

(d) Other models

The assumptions or predictions of the other hypotheses invoked to explain the origin of mating types do not seem to hold in fungi.

The "by-product" model predicts that a given mating type should produce either pheromones or receptors, but not both. All fungal species in fact can produce both pheromones and receptors. Furthermore, the existence of fungal species capable of "same-sex mating" (Fig. 5), *i.e.* having a single *MAT* allele in their genome and able to mate with cells carrying the same *MAT* allele, indicates that there is no absolute requirement to harbour different molecules to undergo syngamy.

The "selfish element" model also appears inconsistent with symmetry in the signalling for mating compatibility in most fungal systems, where members of all gamete classes carry both pheromones and pheromone receptors. For the "selfish element" model, only infected cells should be expected actively to provide signalling mechanisms that promote syngamy. Also, for this model to operate, the selfish genetic element must itself induce syngamy and be transmitted to all daughter cells, or at least to more than half of the daughter cells. However, in all fungal mating type systems, the transmission of mating types is Mendelian. The *MAT* genes thus do not benefit from syngamy more than the rest of the nuclear genome.

The "ploidy" model is consistent with the fact that one of the current roles of mating types in fungi is to induce changes in the physiology and gene expression patterns of the organism (Stanton & Hull, 2007). However, this model does not seem compatible with observations, as the numerous homothallic fungi manage to respond developmentally in an appropriate manner to when they are haploid or diploid, in the absence of any differences at their mating type locus.

The "gamete size" model and the "anisogamy consequence" model are difficult to test in fungi because there are no available data on the relationship between zygote fitness and its size and there have not been multiple transitions between anisogamy and isogamy.

(e) An evolutionary scenario for the origin of gamete classes in fungi and in other taxa

Support for the "organelle inheritance" model was claimed from various taxa: in fact, a correlation between uniparental inheritance of organelles and anisogamy, or with mating types in isogamous species, seems to exist (Hurst & Hamilton, 1992). However, such a correlation is not indicative of cause and effect, and an alternative hypothesis is that the mechanisms controlling organelle inheritance evolved by being superimposed upon pre-existing gamete classes that evolved for other reasons. This evolutionary sequence was suggested by Maynard Smith & Szathmary (1995, p. 162). In fact, there are several examples in fungi where mitochondrial inheritance is uniparental although apparently not controlled by gamete classes, either anisogamy or mating type. The hypothesis that gamete classes pre-date control over organelle inheritance is also consistent with paternal inheritance or biparental inheritance of mitochondria and chloroplasts in gymnosperms (Reboud & Zeyl, 1994) and some chytrid fungi (Borkhardt & Olson, 1983), because then it is not the gamete class (in this case, gamete size) that forces uniparental inheritance. In the myxomycete *Didymium iridis*, uniparental inheritance is frequent while not being associated with any known gamete class (Silliker, Liles & Monroe, 2002; Scheer & Silliker, 2006).

Under such an evolutionary sequence, it is still important to explain the emergence of gamete classes before the evolution of uniparental inheritance of organelles. We argue that two evolutionary models appear the best candidates for explaining the emergence of mating types: the "inbreeding depression avoidance" model and "sex advantage enhancer" model. Which of these two models is the most relevant may depend on the organism being considered and whether mating compatibility is determined at the haploid or diploid level in particular. The "inbreeding depression avoidance" model has indeed received much attention in the literature as an explanation for the evolution of self-incompatibility in angiosperms, but it may not be generally applicable as the haploid determinism of mating compatibility in fungi does not prevent diploid selfing. Indeed, deleterious mutations can still be exposed to selection by selfing in heterothallic fungal species: the requirement for mating between haploid cells carrying different mating types does not prevent inbreeding depression. The "sex advantage enhancer" model has received much less attention, but we expect that it could be of fundamental importance for organisms like fungi or

some algae having a mating type determined at the haploid stage. For such organisms, mating types would be needed to benefit from recombination, by preventing syngamy between identical haploids. In organisms such as plants or animals, intra-haploid mating is impossible, rendering the "sex advantage enhancer" model irrelevant to explain the maintenance of mating types. For such organisms, the "inbreeding depression avoidance" model appears the most valid. Regarding the evolution of anisogamy, we suggest that the best candidate would be the "gamete size" model: large and small gametes evolved by disruptive selection on gamete size.

(2) The optimal number of gamete classes in fungi

Beyond the evolutionary forces responsible for the emergence of gamete classes, it is interesting to investigate those acting on their numbers, and in particular why some species retain only two gamete classes even though it appears *a priori* to be the least favourable case. The existence of only two gamete sizes is understandable as it has been shown that selection will not then favour the evolution of a third class of intermediate size. The most puzzling problem is therefore the abundance of species with only two mating types. It is striking on Fig. 5 that almost all hemiascomycetes and ascomycetes have only two mating types or are homothallic (except *Glomerella cingulata*, Fig. 5A, B), while the number of mating types varies from two to thousands in basidiomycetes, with frequent transitions between two and many (Fig. 5C). Fungi thus are highly suitable to investigate the factors responsible for variability in mating type number.

Frequency-dependent selection should act to increase the number of mating types because any new rare allele could potentially mate with any other gamete in the population. Several of the evolutionary forces that may explain the emergence of gamete classes would also limit their number to two, in particular the "by-product" model, the "selfish element" model, the "gamete size" model, and the "anisogamy consequence" model. However, as we have seen above, these models are probably not the best explanations for the emergence of mating types in fungi. Under the "organelle inheritance" model, it also seems unlikely that more than two gamete classes can evolve. However, this model cannot explain the maintenance of only two mating types in ascomycetes, hemiascomycetes, and some basidiomycetes because inheritance of mitochondria in these groups is not controlled by mating type. Hence, it appears that the factors limiting the number of mating types in many fungi to only two are not generally also the evolutionary forces responsible for their origin. It may nevertheless be the case that once uniparental inheritance is associated with mating type, as in some basidiomycetes (Fig. 5), the emergence of a third mating type would be selected against (Hurst, 1996).

How can we explain the prevalence of only two mating types in hemiascomycetes and ascomycetes whereas their number varies between two and thousands in basidiomycetes? The main difference between ascomycetes and basidiomycetes lies in the genetic determinism of mating types. In basidiomycetes, it can be imagined that different

specificities can easily evolve because haploid cells need only to carry different alleles to be compatible with extant mating types (Kohte, Gola & Wendland, 2003). In ascomycetes, mating types are idiomorphs that encode transcription factors inducing pheromone and receptor gene expression. The emergence of a third mating type in ascomycetes depends on the mechanism of recognition at syngamy. If syngamy does not occur unless a specific pheromone has activated its specific corresponding receptor, a third mating type cannot evolve, because mutants will not be able to mate with any extant mating type (Hoekstra, 1987). The maintenance of only two mating types in ascomycetes may thus only be due to proximal constraints, although more experimental work is required. There is a single example in ascomycetes (*G. cingulata*) where they have overcome this constraint (Fig. 5A), although the underlying molecular basis is not known.

Within basidiomycetes, the number of mating types is highly variable, ranging from two to thousands (Fig. 5C). Frequency-dependent selection is likely to be responsible for increasing the number of mating types, but several forces could act to prevent an increase. The genetic basis of mating type determinism is relevant: for a given number of alleles at each of the two loci involved in mating type determination, unifactorial systems will generate less mating type phenotypes in the population than bifactorial systems, which have recombination between the loci. Some unifactorial species however have more than a dozen mating types and linkage between the two *MAT* loci has evolved several times independently (Fig. 5C), suggesting that genetic determinism (*i.e.* unifactorial *versus* bifactorial) does not represent a strong constraint, and may even be selected for promoting a high or a low number of mating types.

Restriction of the number of mating types to only two in some basidiomycetes may be due to mating systems that are highly selfing, where the existence of mating types prevents fusion between identical haploids but a third mating type would have little advantage because each diploid individual is heterozygous for mating type and can self (Giraud *et al.*, 2008). Unfortunately, data to link the number of mating types with the selfing rate in natural populations of basidiomycetes are still too scarce to test this hypothesis. In basidiomycetes, hyphae can grow short distances to contact mates and thus some mate-finding effort is possible which could limit the advantage of rare mating types. However, data are not available on the costs associated with waiting for a mate. Finally, regions of suppressed recombination are known to exist around mating type loci in several fungal species (Fraser & Heitman, 2005; Hood, 2002) (Fig. 5). Deleterious alleles linked to mating type have been reported, particularly in species with only two mating types (Oudemans *et al.*, 1998; Thomas *et al.*, 2003; Callac *et al.*, 2006). Phylogenies of mating type alleles in the basidiomycete *Coprinus cinereus* have longer terminal branches than expected (May *et al.*, 1999), which could be a signature of a decreasing rate of mating type appearance because of deleterious mutations associated with the mating type loci. Such sheltered load

may be a major force limiting the number of mating types (Uyenoyama, 1997), perhaps even to two. Several forces may therefore act on the number of mating types in fungi, but more data are needed.

(3) The loss and maintenance of gamete classes in fungi

The loss of gamete classes is evolution leading to a lack of discrimination for syngamy and this should be favoured when there is a low probability or a cost to waiting to find a compatible mate. Fungi appear to be unusual in that multiple independent losses of gamete classes have occurred. Same-sex mating in fungi indeed conforms to our definition of loss of gamete class (Figs 4 and 5). The most frequent form of homothallism, resulting from the presence of two inter-compatible *MAT* idiomorphs in the haploid genome is one mechanism by which this is achieved (Figs 4 and 5). However it is not known whether differential temporal expression occurs in these cases. By contrast, hemiascomycete species that exhibit mating type switching are not compatible with all other cells in the population but only with those expressing the alternative mating type. The phylogenies of ascomycetes and basidiomycetes show a large number of independent origins of homothallism (Fig. 5), and rare reverse transitions from homothallism to heterothallism have also been proposed in ascomycetes (Galagan *et al.*, 2005).

Because of the potential advantages of universal compatibility during mating, homothallism can easily be selected for in fungi (Nauta & Hoekstra, 1992). In homothallic species, there is no restriction at syngamy except anisogamy, which is not genetically determined to distinguish haploid genotypes: every haploid has the potential to mate with any other haploid in the population, including itself. Homothallism is often presented as an adaptation in fungi to allow a haploid-selfing mating, but the reverse interpretation is also interesting to consider: homothallism may have evolved in outcrossing species to allow universal compatibility among gametes (Giraud *et al.*, 2008). An experimental study has shown that, under experimental conditions mimicking natural conditions, pseudo-homothallic spores of the basidiomycete *Agaricus bisporus* do not self but instead fertilize homokaryons (Callac *et al.*, 2006). In fact, little evidence of pseudoho-mothallic reproduction was found in a spatial-temporal analysis of natural populations (Xu, Desmerger, & Callac, 2002). These results are consistent with homothallism or pseudo-homothallism having evolved to achieve universal compatibility and not to allow selfing or intra-haploid mating. Other observations however are more consistent with homothallism having evolved, or at least being maintained, to allow intra-haploid mating and to benefit from the advantages of sex not related to recombination. For instance, homothallic *Neurospora* species do not produce any trichogynes or conidia (Glass & Kuldau, 1992), *i.e.* the male and female organs allowing cross-fertilization. Mating between different mycelia is therefore prevented; intra-haploid mating is the only possible sexual reproduction in these species, and occurs regularly.

Again, when comparing the phylogenies of basidiomycetes and ascomycetes, it is striking that the evolution of homothallism differs between the two groups (Fig. 5). In basidiomycetes, all species are heterothallic with a few exceptions, while in ascomycetes homothallism evolved several times independently, different proximal causes likely being responsible (linkage of idiomorphs, same-sex mating, and mating type switching). Reverse transition from homothallism to heterothallism has also been proposed in ascomycetes (Galagan *et al.*, 2005). In fact, it seems that ascomycetes and basidiomycetes use different strategies to increase the probability of compatibility at syngamy: many basidiomycetes have high numbers of mating types, facilitating the finding of compatible mates but preventing intra-haploid mating. By contrast, in ascomycetes molecular constraints linked to the molecular mechanisms of gamete recognition may mean that homothallism is the only way to achieve universal compatibility. Homothallism however allows intra-haploid mating, precluding many of the advantages of sex. If gametes are dispersed and do not remain clumped together, this may not be a significant problem. Evolution towards homothallism or the maintenance of heterothallism may therefore depend on gamete dispersal in ascomycetes. Unfortunately, no data are available to test this suggestion. Inbreeding depression could also play a role in the persistence of mating type systems in fungi. Ascomycetes are haploid for most of their life cycle while basidiomycetes are dikaryotic, which could allow the accumulation of more deleterious alleles in the latter, preventing the emergence of homothallism in the latter group. Again, however, not enough data are available yet to disentangle these hypotheses.

V. CONCLUSIONS

(1) Hurst & Hamilton (1992) defined gamete classes based on their evolutionary origin: "sexes" when gamete classes determine the uniparental inheritance of organelles, and "mating types" when gamete classes allow the avoidance of inbreeding depression. We suggest that these definitions are not adequate for several reasons. First, there remain doubts about the sequence of events leading to the evolution of gamete classes and uniparental inheritance. Second, at least in some organisms, uniparental inheritance of organelles is not associated with a particular gamete class. Hence, even when uniparental inheritance is observed, it is not possible to define different sexes on the basis of this observation alone. Third, in some taxa it can be difficult to determine whether organelle inheritance is uniparental, and whether it is associated with a gamete class, such that this definition of "sex" is not appropriate. Finally, the definition of "mating types" as a means for avoiding inbreeding depression can neglect other evolutionary forces acting at the origin of gamete classes, for instance in the "sex advantage enhancer" model. In conclusion, it is not appropriate

or useful to define "sexes" and "mating types" on the basis of the evolutionary causes at their origin. Instead, we propose the definition of "sexes" as gamete classes based on morphological differences, such as size, and "mating types" as cases where molecular mechanisms restrain gamete compatibilities independent of size dimorphism. These definitions are more relevant to empiricists and are in more general agreement with how these terms are used across other branches of biology. Moreover, such a definition for "sexes" makes simple the relationships between gamete classes and gender evolution.

(2) Regarding the forces at the origin of gamete classes, we have attempted to show that Fungi represents a unique kingdom to test various hypotheses. We conclude that the "organelle inheritance" model may not be as general as previously considered to explain either the emergence of mating types or anisogamy. Uniparental inheritance of organelles may have acted on pre-existing gamete classes. The most plausible explanations for the evolution of mating types are the "inbreeding depression avoidance" model and "sex advantage enhancer" model, and the most acceptable model for the evolution of anisogamy is the "gamete size" model.

(3) Regarding the forces responsible for the number of mating types, we argue that Fungi also allow us to disentangle various hypotheses. High numbers of mating types may have evolved in basidiomycetes in response to selection for increased compatibility at syngamy. However, some basidiomycete species retain two mating types, possibly due to constraints applied by their mating system, genetic load around *MAT* loci, or organelle inheritance being controlled by mating type. Ascomycetes may not be able to evolve multiple mating types due to proximal constraints, but have increased their compatibility at syngamy by homothallism (*i.e.* reversion to zero functional mating types). Heterothallism is maintained in other species, probably by the same forces that promoted the emergence of mating types, in particular the prevention of intra-haploid mating.

(4) Further work in fungi clearly are needed, for example on selfing rates in natural populations, the natural frequency of intra-haploid matings in homothallic species, the cost of waiting for a mate, the dispersal range of gametes, the existence and strength of inbreeding depression, and the shape of functions linking zygote size and fitness.

VI. ACKNOWLEDGEMENTS

VII. REFERENCES

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VIII. SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1. Supplemental data for Fig. 5A: ascomycete phylogeny.

Appendix S2. Supplemental data for Fig. 5B: hemiascomycete phylogeny.

Appendix S3. Supplemental data for Fig. 5C: basidomycete phylogeny.

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