Introduction

The process of reproduction is one of the most variable traits in the living world, with organisms giving rise to progeny either clonally or sexually, and in the latter case by selfing or outcrossing (see the Glossary for definitions). Understanding what factors shape an organism’s reproductive mode is of fundamental importance because patterns of inheritance drastically affect major evolutionary and ecological processes (adaptation, e.g. Charlesworth & Charlesworth, 1995; Otto, 2009; colonization, e.g. Busch, 2011; Richards, 2003), as well as the applied fields of biotechnology, breeding and artificial selection (Whitton et al., 2008), conservation biology (e.g. Fréville et al., 2007), species invasion (Barrett, 2011) and pathogen evolution and control (Shea et al., 2000; Zuk, 2009).

The prevalence of sexual or asexual reproduction is highly variable among eukaryotes, with the vast majority of taxa being able to perform sexual reproduction either exclusively (e.g. mammals) or alternatively with clonality (e.g. aphids, ciliates, many angiosperms and fungi). Sexual reproduction implies the succession of haploid and diploid phases, transitions occurring by meiosis, where recombination and chromosomal segregation occur, and syngamy, where two haploids fuse. The long-term persistence of eukaryotes relying exclusively on asexual reproduction is rare and is thought to include the famous bdelloid rotifers (Welch et al., 2000),
Glomeromycota fungi (Kuhn et al., 2001), some insects and some plants (Judson & Normark, 1996). Even in these textbook examples of asexuality, however, recent studies have suggested the occurrence of cryptic sex, in particular, because all the genes required for the meiotic machinery are maintained in the genomes (Schurko et al., 2009; Halary et al., 2011).

Mating systems, governing which haploids fuse at syngamy, are also highly variable: most species undergo predominantly selfing or outcrossing, whereas a few species show a mixed-mating system in both animals and plants (e.g. Jarne & Auld, 2006; Igic & Kohn, 2006). Outcrossing results from the syngamy between haploid cells produced by separate diploid individuals, whereas selfing results from the syngamy between haploid cells produced by the same diploid individual (Figs 1 and 2). Selfing occurs in plants and animals through the fusion of gametes produced from meioses of a single diploid individual, referred to as diploid selfing. In some eukaryotes, in particular those with an extended haploid life stage such as ascomycete fungi, mosses, ferns and some algae, selfing is sometimes possible through the fusion of two mitotic descendants of the same haploid cell, which is called intrahaploid mating, intragametophytic selfing (Hedrick, 1987), same-clone mating (Perrin, 2012), gametophytic selfing (Epinat & Lenormand, 2009) or haploid selfing (Billiard et al., 2011). Diploid selfing and haploid selfing can have very different consequences for genetic structure, and care should be taken in the application of different terms to describe their occurrence in eukaryotes.

The proximate mechanisms controlling mating systems are also highly diverse (sometimes called ‘breeding systems’, Neal & Anderson, 2005): obligate outcrossing can result, for instance, from the existence of (i) separate sexes (even though outcrossing may not be the main force responsible for the evolution of separate sexes), (ii) sexual morphs (e.g. heterostyly in angiosperms, where two morphs with different stigma and pistil lengths coexist in populations, mating being only possible

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**Fig. 1** Synthetic view of the different possible modes of reproduction in homothallic vs. heterothallic fungi and oomycetes. *Some diploid selfing may be possible for heterothallic oomycetes but only when in presence of a different mating partner, i.e. when also performing outcrossing.*
between individuals of different morphs), (iii) molecular recognition mechanisms (e.g. self-incompatibility systems in angiosperms), or (iv) asynchrony in the production of male and female gametes in an individual (see Barrett, 2010 for a review). The tendency for selfing can result, for instance, from nonopening hermaphroditic flowers (cleistogamy) or developmental proximity between gametes from the same individual (Giraud et al., 2008).

Biologists have struggled for more than a century to identify factors responsible for the evolution of such preference for outcrossing vs. selfing or for sexual vs. asexual reproduction. Sexual reproduction has apparently been lost independently in diverse eukaryotes (reviewed in fungi, Billiard et al., 2011; Lobuglio et al., 1993; López-Villavicencio et al., 2010; Kuhn et al., 2001; in microsporidia, Ironside, 2007; in animals, Simon et al., 2003; and in plants, Whitton et al., 2008). However, there is growing evidence that some species long thought to be asexual undergo cryptic sex (Burt et al., 1996; Schurko et al., 2009; Lee et al., 2010). Nevertheless, anciently asexual lineages undoubtedly exist in many different taxonomic groups, as revealed, for instance, by the Meselson effect, in which the divergence between alleles in the two nuclei of each cell is so great that it can only be explained by a long divergence without recombination (Kuhn et al., 2001; Enjalbert et al., 2002; Roose-Amsaleg et al., 2002; Schurko et al., 2009). Similarly, transitions between outcrossing and selfing preferences have occurred independently in the evolution of many plant groups (for instance, the gain or loss of heterostyly, e.g. Barrett & Shore, 2008; Busch, 2011; and in animals, Jarne & Auld, 2006).

Evolutionary biologists attempt to explain the derivation of particular reproductive modes in terms of fitness benefits and costs. Sexual reproduction can induce fitness costs through energetic, genetic transmission and demographic constraints compared to asexual reproduction (see Otto, 2009; Lehtonen et al., 2012, for reviews). When sex requires males that contribute only with their genes and do not directly produce offspring, a mutation allowing females to reproduce independently of males, either asexually or through various forms of self-fertilization, is expected to invade because its progeny grows at twice the rate of the ancestral lineage (the famous ‘two-fold cost of males’; Maynard Smith, 1978). Recombination can furthermore break locally adapted combinations of alleles at multiple loci, which is called the recombination load. Even in organisms without either separate sexes or outcrossing, however, meiosis itself can be costly because it takes more time and energy than mitotic cell divisions. Other costs of sex may apply in specific systems: cost of finding and courting a mate, risk of predation or contracting sexually transmitted diseases or parasitic genetic elements. On the other hand, sex is considered to provide benefits, both proximate (e.g. DNA repair) and ultimate, by limiting the accumulation of deleterious mutations and by creating novel and advantageous genetic combinations, especially in environments varying in space and time. Lineages that undergo only asexual reproduction or obligate selfing in fact tend to have high extinction rates (Beck et al., 2011; Goldberg et al., 2010; Icic et al., 2008; Simon et al., 2003).

Biological groups exhibiting a large diversity of reproductive strategies provide unique opportunities to iden-
tify the factors underlying the evolution of sex and mating systems. The majority of theories and observations have been based on vertebrate, insect and plant models, whereas other groups of eukaryotes, with even more diverse reproductive strategies, have too often been excluded from the debate (Birky, 1999). Groups such as fungi are excellent models to study these topics (Lee et al., 2010; Billiard et al., 2011; Whittle et al., 2011). Fungi include species with obligate sexual species (e.g. Microbotryum, Giraud et al., 2008), species exhibiting both sexual and asexual reproduction and others appearing strictly asexual (Taylor et al., 1999; Roose-Amsaleg et al., 2002; Schurko et al., 2009); there seem to have been multiple transitions from sexuality to asexuality (Lobuglio et al., 1993; López-Villavicencio et al., 2010; Coelho et al., 2011; Schurko et al., 2009). Fungi are also potentially informative because they present a huge diversity in the degree of haploid and diploid selfing rates (Billiard et al., 2011), and they provide significant advantages over other eukaryotic models for empirical studies (Goddard et al., 2005; Bruggeman et al., 2003, 2004). For instance, many can easily be cultivated under laboratory conditions, can be cloned, have relatively short generation times and can be revived after long-term frozen storage, which is then useful in studies of experimental evolution.

Here, we thus would like to argue that fungi have much to bring to questions on the evolution of sex and mating systems, being tractable experimental models for addressing the advantages and costs of the various reproductive systems, and would benefit from being studied further within an evolutionary context. Several concepts referring to the mating system of fungi, however, need clarification because the same terms (e.g. selfing and outcrossing) are used for different phenomena with completely different evolutionary consequences (Giraud et al., 2008; Neal & Anderson, 2005). Furthermore, knowledge on the advantages and costs of modes of reproduction and mating systems in fungi can have direct applications, for instance, to the numerous fungi used in industry or that threaten health and agricultural production.

We first describe the different modes of reproduction and mating systems in fungi, highlighting frequent misconceptions, and we review their respective evolutionary benefits and costs. We will also consider oomycetes, which are protists, but have long been studied by mycologists and share with fungi many morphological and genetic peculiarities. We argue that understanding the evolution of modes and systems of reproduction, especially in fungi, requires (i) assessing their frequencies in natural populations, and we will review the (scarce) evidence available in the literature, (ii) mapping these, as well as other life-history traits onto phylogenies, and (iii) measuring experimentally the advantages gained by the different modes of reproduction and mating systems. We will propose some possible experimental settings to compare the fitness of the progeny resulting from different modes and systems of reproduction.

1. The different modes of reproduction and mating systems in fungi

Fungi exhibit a huge variety of life cycles, but we tried to draw in Figs 3 and 4 some typical sexual cycles of filamentous ascomycetes and mushrooms (homobasidiomycetes), respectively. Most fungi are able to undergo both asexual reproduction and sexual reproduction (Figs 1 and 2), where, as in other sexual eukaryotes, tightly regulated mechanisms determine which haploid cells can fuse at syngamy. However, additional possibilities of syngamy exist in fungi as compared to plants and animals. In fungi considered to be ‘heterothallic’, haploid selfing is prevented because syngamy can only occur between haploid cells carrying different alleles at the mating type locus/loci (Fig. 1, Billiard et al., 2011). However, in homothallic fungi, syngamy can occur between genetically identical haploid cells, that is, clones, resulting in haploid selfing (Figs 1 and 2, Billiard et al., 2011). Several proximal mechanisms may confer homothallism in fungi: most often each haploid carries two active mating type alleles (Coppin et al., 1997), whereas in some species haploid individuals can ‘switch’ which mating type allele is expressed (e.g. in Saccharomyces cerevisiae, Haber, 1998). In other species, syngamy can simply occur between haploid cells carrying and expressing the same single mating type allele (i.e. ‘same-sex mating’, Alby et al., 2009; Fraser et al., 2005; Lin et al., 2005; Metzenberg & Glass, 1990). In homothallic fungi where mating type switching occurs, haploid selfing results from syngamy between cells genetically identical except at the expressed mating type locus.

In both heterothallic and homothallic fungi, two other types of syngamy, which are also common in plants and animals, can occur: (i) syngamy between two different meiotic products originating from a single diploid individual, that is, diploid selfing (more commonly referred to simply as selfing) in plants and animals, but the term ‘diploid selfing’ here allows the distinction from haploid selfing) and (ii) syngamy between meiotic products originating from two different diploid individuals, which is called outcrossing (Figs 1 and 2).

It should be noted that molecular and developmental systems of self-incompatibility exist in plants and animals preventing diploid selfing (such as dioecy or self-incompatibility alleles systems in angiosperms), but these are absent in true fungi where mating compatibility is determined by the genotype of haploid nuclei. Fungal species are always able to undergo both outcrossing and diploid selfing (Giraud et al., 2008); mating types in fungi can only prevent haploid selfing in heterothallic species. Nevertheless, some life-history traits may increase the probability of outcrossing in fungi. For example, many species of ascomycetes and rust fungi exhibit a long-
distance dispersal and persistence of the haploid stage. The random encounter of haploid genotypes should cause outcrossing to be more prevalent than diploid selfing, although this remains to be investigated by population genetics analyses in natural populations. Quite to the opposite extreme, cases exist where haploid dispersal is very limited or nonexistent and mating occurs among the immediate products of meiosis, a mating system called automixis (Mogie, 1986), that is, intrate-trad mating (see Fig. 2; e.g. the anther smut fungi Microbotryum, Giraud et al., 2008). The genetic consequences of automixis differ from diploid selfing, due to the restriction of the effective population of mating partners and the tendency to retain heterozygosity (Kirby, 1984; Hood & Antonovics, 2004).

Several ascomycete and basiomycete fungi have been characterized as being ‘pseudo-homothallic’, due to their apparent ability to complete the sexual cycle without the
need for a mate that is achieved by the presence of two haploid nuclei of opposite mating types and from a single meiosis in the dispersed spore (e.g. Neurospora tetrasperma, Merino et al., 1996; Raju & Perkins, 1994; Agaricus bisporus, Callac et al., 2006; Saccharomyces ludwigii, Zakharov, 2005). Here, a developmental process allows, and even seems to promote, the automic combination of post-meiotic nuclei. However, here again one can wonder whether the cosegregation of two haploid nuclei of opposite mating types has been selected to favour automixis or to allow universal compatibility under outcrossing. The presence of nuclei of the two mating types within dispersing spores can indeed ensure that the spore will be able to mate with any first encountered haploid. Interestingly, recent studies have suggested that pseudo-homothallic fungi may exhibit a mechanism favouring outcrossing over automixis. In A. bisporus, a choice experiment in seminatural conditions has shown that outcrossing was indeed more frequent than automixis in this pseudo-homothallic mushroom (Callac et al., 2006). In this case, the cosegregation of two meiotic products of opposite mating types in a spore may have actually evolved for allowing universal mating compatibility that achieves outcrossing rather than to favour automixis (Billiard et al., 2011). It should be noted, however, that the choice experiment was performed in conditions where spore inoculum was maybe higher than in natural conditions (Callac et al., 2006) and pseudo-homothallism may also allow intertetrad mating when no other mating is available. In contrast, a few basidiomycetes do undergo preferentially automixis, even when gametes from other diploid individuals are available, via a developmental specificity promoting automixis (e.g. the anther smut fungi Microbotryum, Giraud et al., 2005).

The terms ‘homothallism’ and ‘heterothallism’ are also used to describe the phenomena in other groups as fungal-like oomycetes, which have been studied for long by mycologists. Nevertheless, the genetic basis and the evolutionary consequences are strikingly different from true fungi (Fig. 1). In oomycetes, which are phylogenetically closer to brown algae and diatoms, ‘heterothallism’ and ‘homothallism’ are used to describe how sexual reproduction is initiated: heterothallic oomycetes cannot undergo gamete production and sexual reproduction unless an individual of the opposite mating type is present, while homothallic oomycetes can (Fig. 1, see also Judelson, 2007). Once gametes are produced, diploid selfing and outcrossing are possible in both, homothallic and heterothallic oomycetes. Haploid selfing (a kind of syngamy possible in homothallic fungi) is prevented in oomycetes due to the lack of mitotic multiplication of gametes, as in plants and animals. The terms ‘homothallism’ and ‘heterothallism’ thus correspond to different phenomena in different organisms. This is probably because of the potential to isolate haploid cell lineages (therefore considered as the ‘thallus’) in fungi, but this is not possible in oomycetes, where the diploid stage was considered the ‘thallus’. This situation is confusing, especially as the terms ‘homothallism’ and ‘heterothallism’ are also used interchangeably with ‘selfing’ and ‘outcrossing’ in both oomycetes and fungi. It is essential to distinguish the types of syngamy illustrated in Figs 1 and 2 with the consistent use of terminology, because there are different consequences in evolutionary terms, as explained in the section 2 below.

More generally, the confusion over the different forms of the term ‘selfing’ as described above stems from the fact that ascomycetes have a long-lasting haploid stage, so that the ‘individual’ is considered as the haploid mycelium or cells. Thus, haploid selfing is called selfing in ascomycetes, and it remains essential to distinguish diploid selfing from haploid selfing because of the different effects on genetic structure, in particular the genome-wide distribution of heterozygosity. We therefore argue that, even in ascomycetes, the syngamy between identical haploids should be consistently described by the term ‘haploid selfing’. For the same reason, outcrossing for ascomycetes often refers to mating with a nonidentical haploid (i.e. ‘nonhaploid selfing’), which can actually be either diploid selfing or true outcrossing in the classical sense for plants and animals (i.e. syngamy between products of meiosis of different diploid individuals). The use of nonambiguous terms is important for understanding the underlying concepts and evolutionary consequences, and for rendering the literature on fungi useful, without misunderstandings, for evolutionary biologists less familiar with fungal life cycles.

2. Evolutionary benefits and costs of the different modes of reproduction, breeding systems (homothallism vs. heterothallism) and mating systems (selfing vs. outcrossing)

2.1. Costs and benefits of sex

The costs and benefits of sex have been briefly presented in introduction and have been extensively reviewed elsewhere (e.g. Otto, 2009; Lehtonen et al., 2012). However, some classically assumed costs of sex cannot be applied directly to fungi because many species are isogamous and determine mating compatibility only during the haploid stage through molecular means of nonself-recognition. Furthermore, many sexual fungi undergo multiple rounds of asexual reproduction for each sexual cycle, thus benefitting both from the recombinational advantages of sex and from the demographic advantages of clonality.

2.2. Costs and benefits of homothallism

Although it has been traditionally accepted in mycology that homothallism (i.e. the possibility for an haploid individual to mate with any other haploid individual,
even itself, Fig. 1) evolved to favour haploid selfing, it is important to recognize that the potential for homothallism as determined by haploid selfing in the laboratory does not imply that this is the prominent mating system of the species in nature. The prevalence of haploid selfing in nature is difficult to assess. Indeed, most homothallic species can also reproduce asexually and these two modes of reproduction cannot be distinguished by population genetics analyses. In order to measure the actual frequency of haploid selfing in nature, genetic analyses of progeny in sexual structures collected in nature are required. Moreover, it is not clear why having sex with an identical haploid would be favoured. Two benefits are possible: benefits from segregation and those from recombination. Concerning the segregation advantage, Epinat & Lenormand (2009) showed, in a model with a modifier of haploid selfing partially linked to a locus under diploid selection, that haploid selfing may be favoured when the decrease in the mean fitness of offspring due to inbreeding depression is low relative to the advantages of the transmission of selfing and of the increase in the fitness variance in offspring. Concerning the recombination advantage, there are no models to our knowledge investigating the possible recombination advantage or costs of homothallism but we can expect a priori that haploid selfing should confer no recombinational advantages over asexual reproduction, and it should still incur some of the costs of sex, such as the physiological costs involved in utilizing the meiotic machinery and costly sexual structures. Other costs of sex should, however, be minimal under haploid selfing, in particular avoiding the costs of finding a mate and of risking parasite transmission.

Instead of having evolved to favour haploid selfing, homothallism could alternatively be viewed as a lack of discrimination at syngamy: in homothallic species, each haploid is compatible with all other haploids in the population (including incidentally, genetically identical haploids). In fact, several models suggest that such a universally compatible mutant should easily invade populations when there is a cost for waiting for a compatible mate (Iwasa & Sasaki, 1987). If we consider a fungal species experiencing random encounters of haploids because its gametes widely disperse, homothallism as a mutant form should be selected for because it allows compatibility with all other gametes in the population. An interesting observation is that some species have recently been reported to exhibit polymorphism for homothallism that may reflect the invasion of universally compatible mutants (homothallic mutants): in the pathogens *Candida albicans* and in *Cryptococcus neoformans*, both heterothallism and homothallism via ‘same-sex mating’ are possible (Heitman, 2010 and refs therein; Alby et al., 2009). These species are human pathogens, especially in immunocompromised patients. A possible explanation could be the incipient invasion of universally compatible mutants (homothallic mutants) from an ancestral heterothallic population.

Homothallism, whatever the proximal mechanism, could thus have evolved for universal compatibility of gametes under outcrossing when there is a cost in finding a mating partner and little risk of engaging in haploid selfing, for instance, due to gamete dispersal. In case the gametes remain near their mother for mating, homothallism may be selected against to avoid haploid selfing that brings no recombinational advantage but still incurs some costs of sexual reproduction (section 2.5 below; Giraud et al., 2008). If this is the case, a prediction would then be that homothallic fungi are predominantly outcrossing. This prediction may seem paradoxical at first sight because it is generally believed that homothallism evolved to promote selfing. Studies on the population genetics analyses in homothallic species are rare, but several in fact have found evidence of outcrossing (see section 3.2). In the homothallic *Aspergillus nidulans*, it has been suggested that mating occurs preferentially between genetically different individuals (which has unfortunately and confusingly been called ‘relative heterothallism’, Pontecorvo et al., 1953).

Nevertheless, some homothallic fungi (e.g. homothallic species of *Neurospora*) have been suggested to rely mainly on haploid selfing for reproduction because of the lack of conidia (male gametes) production (Glass & Kuldau, 1992). In some other ascomycetes, such as the sexual *Talaromyces* species (Stolk & Samson, 1972), the male structures surround the female organs, which may in fact promote haploid selfing. In these homothallic species that seem to reproduce sexually only via haploid selfing, it is intriguing why sex is retained instead of asexuality, which would allow bypassing the physiological costs of meiosis and syngamy. If sex via haploid selfing is disadvantageous over the short term compared to asexual reproduction, because it incurs costs without providing recombinational advantages, it should be lost. It has been proposed that haploid selfing could, nevertheless, be maintained if it increased the chance to engage in occasional outcrossing (Lee et al., 2010); this would work only if outcrossing occurs frequently enough to counter the expected rapid invasion by asexuals, in which case we should detect such events of outcrossing in natural populations. Unfortunately, too few studies exist that investigate mating systems in natural populations (see section 3).

Some advantages of sex that are not related to recombination have been proposed to exist in fungi, and they could provide advantages to haploid selfing over asexuality, as explained in the section 2.3 below.

### 2.3. Advantages of haploid selfing vs. asexual reproduction

A possible advantage of haploid selfing over asexual reproduction relies on the advantage of segregation. Indeed, haploid selfing immediately produces diploid
offspring with homozygosity across the whole genome, which can increase the efficacy of selection for deleterious alleles, especially for recessive mutations (e.g. Haag & Roze, 2007). However, this mechanism might be of evolutionary importance only in species with a significant diploid phase under which strong selection can act. This is likely not the case in most ascomycetes, the group of fungi in which homothallism is most frequent: the diploid stage of the life cycle is most often transient in this group, although some exceptions exist, such as in some hemiascomycetous yeasts (Knop, 2006).

Yet, some empirical studies suggest that there indeed might be advantages of haploid selfing vs. asexual reproduction associated with the elimination of newly arisen deleterious mutations. Compared to asexual reproduction, haploid selfing seems to reduce the accumulation of slightly deleterious mutations in the homothallic A. nidulans (Bruggeman et al., 2004). Haploid selfing does not allow the classical purging of deleterious alleles by outcrossing between individuals carrying deleterious mutations at different loci (which is needed to prevent Müller’s ratchet). It has, however, been proposed that haploid selfing would be more efficient at purging newly arisen deleterious mutations from the population than asexual reproduction in A. nidulans. In a mycelium, all the nuclei can form spores because no special germ line exists. Nuclei can be packed to form asexual spores or can fuse to form sexual spores after meiosis. The concept of a selection arena is proposed such that when deleterious mutations appear, they could be eliminated by a selective maternal diversion of resources to the nonmutant fittest progeny, which would develop better while getting rid of lower fitness offspring. This selective resource allocation has been shown to be more stringent in the more costly sexual pathway (Bruggeman et al., 2004). Theoretical work is required to investigate the extent to which deleterious mutations rates are sufficiently high to create an advantage for haploid selfing that balances the cost of sex. The costs of sex are likely much lower in the case of haploid selfing than for outcrossing, being restricted to those related to physiological costs of meiosis and sexual structures. These physiological costs can, however, be significant (Aanen & Hoekstra, 2007).

Sexual reproduction by haploid selfing may also be advantageous compared to asexual reproduction when it allows for the purging of parasites. In some ascomycete species, DNA and RNA viruses are only transmitted via asexual spores. Sexual spores produced by either outcrossing or haploid selfing are free of these parasites (Coenen et al., 1997; van Diepeningen et al., 2008). Similarly, repeat-induced point (RIP mutation) is a mechanism restricting the proliferation of transposable elements, occurs only in association with the dikaryotic stage that follows mating and precedes karyogamy and meiosis (Galagan & Selker, 2004). Also, the sexual cycle is also associated with cell rejuvenation through the resetting epigenetic signalling and the avoidance of senescence. In senescent cultures of Podospora anserina, sexual reproduction restores healthy mitochondria, which is called ‘rejuvenation’ (Silliker et al., 1996). These benefits that are not associated with recombination have likely been linked with sex secondarily, but they can now
2.4. Advantages of sexual reproduction over asexual reproduction, other than those regarding haploid selfing

Most of the advantages of sex other than haploid selfing have been already mentioned above. One of the most important advantages of sexual reproduction between two different genomes is to create new adaptive allelic combinations, often called ‘Fisher–Muller hypothesis’, or to get rid of deleterious alleles, that is, avoiding Muller’s ratchet (Otto, 2009). Most theoretical papers show that this genetic advantage of sex and recombination can explain the evolution and the maintenance of a small rate of sexual reproduction (e.g. Roze, 2009). This is consistent with the observation that many species alternate asexual reproduction and sexual reproduction, especially in fungi. However, it is still not clear why so few species are exclusively asexual given the costs of sex. An explanation may be higher extinction rates in asexual species, which would be less able to adapt to changing environments or are subject to mutational decay of Muller’s ratchet over the long term (Gouyon, 1999). This hypothesis is supported by the observation that exclusively asexual taxa seemed to have appeared relatively recently (Beck et al., 2011).

2.5. Advantages of heterothallism over homothallism

An interesting question is why heterothallism is sometimes favoured over homothallism, whereas heterothallism restricts the number of potentially compatible partners for any gamete. Some authors proposed that gamete classes such as mating types evolved as a means to avoid cytoplasmic conflicts between organelles by enforcing their uniparental inheritance (Hurst & Hamilton, 1992). The idea behind this hypothesis is that uniparental inheritance should discourage the evolution of selfish cytoplasmic elements, which would replicate faster but would be less efficient for providing energy to the organism. Indeed, it seems highly advantageous for nuclear genes to limit the opportunity to be associated with selfish organelles. Uniparental inheritance of organelles is efficient for this and in fact most often seems controlled by a mechanism associated with gamete classes, such as the size of the gametes or mating types (Hurst & Hamilton, 1992). However, the association between uniparental inheritance of organelles and gamete classes is not necessarily evidence for gamete classes having evolved for controlling uniparental inheritance. The evolution of uniparental inheritance should be easier if it can use a pre-existing system of gamete classes that may have evolved for other reasons. In fact, we showed in a previous review (Billiard et al., 2011) that evidence exists in fungi suggesting that gamete classes may have evolved independently of the system regulating uniparental inheritance of organelles. Some species do exhibit mating types, but nevertheless show biparental inheritance of organelles (e.g. Coprinus cinereus, May & Taylor, 1988; Neurospora crassa, Yang & Griffiths, 1993), or show uniparental inheritance but with a system independent of mating types (e.g. A. bisporus, Jin & Horgan, 1994). On the other hand, in some other fungal species without mating types, organelles are inherited uniparentally, which means that mechanisms other than mating types exist that ensure uniparental inheritance (e.g. in yeasts with mating type switching mitochondria actively segregate during the first few rounds of cell division; Berger & Yaffe, 2000). Also in many ciliates, mating types exist...
Despite the lack of any cytoplasmic exchange during syngamy, and thus the impossibility for the evolution of selfish organelles (Phadke & Zufall, 2009). Therefore, the absence of strict association between organelle uniparental transmission and gamete classes as would have been predicted by the hypothesis of Hurst & Hamilton (1992) suggests that other evolutionary pressures are involved in the appearance and maintenance of gamete classes, such as mating types. The most likely evolutionary sequence thus seems that gamete classes evolved first and that they allowed the evolution towards uniparental transmission of the organelles (Maynard Smith & Szathmáry, 1995).

A number of other hypotheses have been proposed to explain the evolution of mating types, defined here as a molecular mechanism of the gametes allowing discrimination for syngamy, independent of size dimorphism. We summarize below only the main hypotheses and their limits (see Billiard et al., 2011, for more details).

The advantages put forward to explain the evolution of mating types depend on the specificities of mating types in different organisms. Mating types indeed do not restrict the possibility of syngamy in similar ways in all organisms. In plants, for instance, mating types are called self-incompatibility systems and they prevent diploid selfing (i.e. syngamy between gametes produced by the same diploid individual; Figs 1 and 2). In such cases where mating types prevent diploid selfing, it is generally assumed that they evolved precisely to avoid diploid selfing, and thus promote outcrossing to limit inbreeding depression. This probably also applies to oomyces.

In fungi however, mating types are effective at the haploid stage and therefore do not prevent diploid selfing: any diploid stage of a heterothallic species is heterozygous for the mating type and can therefore produce haploids that can undergo diploid selfing (Fig. 2). Mating types in fungi will only prevent haploid selfing. Besides, inbreeding depression is not expected to act in predominantly haploid organisms, such as ascomycetes, because deleterious mutations are not sheltered and can be purged from the population as they appear. Inbreeding depression therefore has likely played much less of a role in the evolution of fungal mating types as it does in plants. A possible explanation for the evolution of mating types in fungi would therefore be to prevent haploid selfing, thus affording the benefits of sex associated with recombination (Czaran & Hoekstra, 2004). Indeed, if recombination has an advantage, whatever it is, individuals gain this advantage only when the haploid genomes that are recombining are not strictly identical. For example, the prevention of Müller’s ratchet (i.e. the restoration of haplotypes free of deleterious mutations in a population with no individual carrying zero mutations) requires mating between nonidentical haploids. This hypothesis remains poorly investigated, but if valid, then the fitness of progeny produced by haploid selfing should be lower than the fitness of individuals produced by diploid selfing (see section 2.6 below). We see here that the distinction between diploid selfing and haploid selfing contributes to understand why mating types exist in fungi.

2.6. Advantages of diploid selfing over haploid selfing

As we have seen in the previous sections, we expect little or no advantage of sex when it occurs as haploid selfing, especially regarding those advantages of sex that rely on recombination. In the case of diploid selfing, genetic differences exist between the two haploid genomes undergoing syngamy and recombination, allowing some of the recombinational advantages of sex to be realized. In this case, the diploid or haploid progeny produced by diploid selfing should have a higher fitness than that produced by haploid selfing.

2.7. Advantages and drawbacks of diploid selfing over outcrossing

A long-standing question is why so many species undergo outcrossing instead of diploid selfing. Indeed, there is an automatic advantage of diploid selfing relative to outcrossing: an individual undergoing diploid selfing transmits two copies of its haploid genome in selfed progeny and in many species they can in addition sire offspring by fertilizing outcrossers, whereas these outcrossers cannot fertilize selfers (Fisher, 1941). Furthermore, in species with a predominant diploid phase, the ability to undergo diploid selfing avoids mating partner limitation, that is, selfing provides reproductive assurance. Diploid selfing may, however, be disadvantageous relative to outcrossing because of inbreeding depression, that is, when recessive deleterious mutations are present in a population and are exposed to selection in the homozygous condition due to mating with close relatives. However, in fungi with a predominantly haploid life cycle, such as most ascomycetes, the importance of inbreeding depression should be restricted to loci with expression limited to the dikaryotic or diploid stages. This has been observed, for example, in N. crassa, the only ascomycete species where inbreeding depression has been investigated: crosses between highly related individuals exhibited reduced fertility; inbred lines produced deficient perithecia with no or few viable ascospores or presented ascospore maturation defects (Leslie & Raju, 1985). In basidiomycetes in contrast, the dikaryotic stage is predominant in the life cycle; inbreeding depression may therefore have important consequences and be a significant evolutionary force. Inbreeding depression has in fact been found in the basidiomycetes A. bisporus where outcrossed populations showed higher fitness than inbred ones in several fitness components (Xu, 1995). Some mechanisms in fungi may have in fact evolved to control the mating system by limiting diploid selfing or promoting outcrossing (see section 3 below).
2.8. Conclusions and prospects

We argue that answering the questions raised above about the evolution of asexual vs. sexual reproduction, homothallism vs. heterothallism, haploid selfing vs. diploid selfing and diploid selfing vs. outcrossing requires (i) measuring what types of syngamy occur in nature (see section 3 below), (ii) retracing their evolution using phylogenies and identifying associated life-history traits (see section 4) and (iii) experimentally assessing the benefits and costs of the different possible modes of reproduction, asexual or sexual, and the mating systems of haploid selfing, diploid selfing and outcrossing (see section 5).

3. Modes of reproduction and mating systems in nature

In order to understand why particular breeding systems (homothallism vs. heterothallism), mating systems and modes of reproduction have evolved, we need to assess what types of reproduction and syngamy are actually performed in natura. Indeed, in vitro observations do not allow for strong inferences of the reproductive mode in natural conditions. For instance, homothallism have been suggested to evolve either for allowing haploid selfing or, on the contrary, for allowing higher mate availability under outcrossing (Billiard et al., 2011). Disentangling between these hypotheses requires assessing how often in nature homothallic fungi undergo haploid selfing, diploid selfing or outcrossing. Similarly, the frequency of sexual reproduction in nature cannot be inferred from in vitro observations. Below, we briefly review the modes of reproduction and mating systems of fungi (sensu lato, i.e. including oomycetes, and the two main groups of true fungi, ascomycetes and basidiomycetes), as revealed in natura using population genetics approaches, although such studies remain scarce.

3.1. Modes of reproduction: sexual vs. asexual reproduction

As a measure of relative time or growth, most ascomycetes are haploid during almost their entire life cycle, as mycelium or yeast, and often they are capable of both asexual and sexual reproduction. Ascomycetes include yeasts, filamentous species and many species responsible for crop or tree diseases. Basidiomycetes include mushrooms, smuts, yeasts and rusts, the latter being also responsible for plant diseases and producing large numbers of asexual spores, as do ascomycetes. Linkage disequilibrium among genetic markers and the number of repeated haplotypes isolated from natural populations have been used to infer the occurrence and degree of clonality, although this latter phenomenon is not distinguishable from haploid selfing by these methods (Giraud et al., 2008; Gladieux et al., 2010). A great diversity of reproductive systems has been revealed (Giraud et al., 2008; Taylor et al., 1999), from exclusively clonal propagation, at least in some introduced ranges, to a high prevalence of sex, with many surprising results compared to what was expected from in vitro observations. In the ascomycete Botrytis cinerea, responsible for grey mould, the human pathogen Coccidioides immitis or the Macrotermes natalensis fungus grown by termites, sexual structures have never been observed and these species were long thought to be strictly clonal. However, molecular markers revealed pervasive recombination (Burt et al., 1996; Fournier & Giraud, 2008; Giraud et al., 1997; De Fine Licht et al., 2006). In contrast, sexual forms have been observed in the wheat leaf rust Puccinia triticina (Wahl et al., 1984), yet genetic analyses of French populations revealed a completely clonal structure, with no or very little evidence of genetic recombination (Goyeau et al., 2007). In the fungus responsible for the yellow rust of wheat, Puccinia striiformis f.sp. tritici, studies measuring linkage disequilibrium in European populations similarly indicated prominent clonality (Hovmöller et al., 2002). Moreover, the high degrees of heterozygosity observed at microsatellite markers (Enjalbert et al., 2002) and in sequences associated with ribosomal RNA genes (Roose-Amsaleg et al., 2002) provided evidence for a Meselson Effect, where ancient asexual lineages exhibit high divergence between their homologous chromosomes due to the accumulation of independent mutations on different alleles (Halkett et al., 2005). A Meselson effect has also been detected in Glomales (Kuhn et al., 2001; Schurko et al., 2009), similarly indicating very ancient clonality, but recent data showing recombination events and that the meiotic machinery is conserved might suggest that these fungi undergo cryptic sex (Halary et al., 2011). Sex seems to have been lost several times independently in certain groups, such as in the Penicillium genus (Lobuglio et al., 1993; López-Villavicencio et al., 2010).

However, exclusively clonal fungi are scarce, molecular markers having most often revealed the occurrence of at least some degree of recombination, even in species where no sexual stages are known (Burt et al., 1996; Lee et al., 2010). Besides footprints of recombination based on population genetics data, another type of evidence indicating that sex occurs regularly in most fungi comes from the apparent functionality of the mating types genes, even in species without known sexual structures. Virtually, all the mating type genes analysed so far indeed present apparent functional sequences (Debuchy et al., 2010). Most ascomycetes actually exhibit mixed reproductive systems, with indications of both sexual and asexual reproduction (e.g. Dutech et al., 2008; Kiss et al., 2011). However, as mentioned above, a problem is that haploid selfing cannot be distinguished from clonality in homothallic fungi using most common approaches of population genetics.
In contrast with ascomycetes, mushrooms (homobasidiomycetes) do not produce asexual spores, so that clonal propagation is restricted to dispersal via mycelia. Clonality appears therefore of minor importance in most mushrooms at the population level (Prospero et al., 2008), although clone growth can occur over quite large distances (Anderson & Kohn, 1995; Smith et al., 1992).

3.2. Mating systems in fungi: Haploid selfing vs. diploid selfing vs. outcrossing

In the following sections, we review briefly the evidence for the occurrence of the different types of syngamy in natural populations.

Diploid selfing vs. outcrossing

The relative importance of diploid selfing vs. outcrossing is most easily examined in basidiomycetes and oomycetes because, with their main state being respectively dikaryotic and diploid, the \( F_{IS} \) index estimated using codominant genetic markers can give direct indications about the degree of diploid selfing. Surprisingly however, such studies are still quite rare compared to those in plants or animals.

Most basidiomycetes disperse primarily as haploid basidiospores just before mating, which greatly favours outcrossing. In fact, heterozygote deficiencies are quite rare in the few studies having measured \( F_{IS} \) in nature in sexual basidiomycetes (Barrés et al., 2008; Bergemann & Miller, 2002; Engh et al., 2010; Franzen et al., 2007; Kauzerud & Schumacher, 2002; Kretzer et al., 2004; Rosewich et al., 1999; Roy et al., 2008). Some sexual basidiomycetes showed heterozygote excess (Amend et al., 2010; Engh et al., 2010; Rosewich et al., 1999), which has been interpreted as due to disassortative mating (preferential mating with individuals genetically more different than the average of the population) or linkage of markers with the \( MAT \) or \( vic \) (vegetative incompatibility) loci (which maintains high heterozygosity as fusion can only occur between cells carrying different alleles at these loci, Hood & Antonovics, 2000).

In contrast to most basidiomycetes, the heterothallic Microbotryum violaceum, population genetics studies have shown strong heterozygote deficiency (Delmotte et al., 1999; Giraud, 2004), indicating high rates of diploid selfing. Diploid selfing may be common in natural populations because of a lack of available mating partners or because of mechanisms favouring diploid selfing compared with outcrossing. In \( M. \) violaceum, diploid spores are dispersed and only one may arrive at a time on a new resource for growth (i.e. a host plant) (Fig. 3), and because further dispersal of the haploid products of spore germination and meiosis is limited, diploid selfing may often be the primary option available. Some data, however, also indicate that a preference for diploid selfing vs. outcrossing exists (Giraud et al., 2005; Hood & Antonovics, 2000) and that this is mediated through developmental patterns of the promycelium where meiosis takes place (Hood & Antonovics, 2000).

In oomycetes, an example of contrasted mating systems in two closely related species is given by \( Plasmopara viticola \) and \( Plasmopara halstedii \), respectively, responsible for the downy mildews of grapevine and sunflower. Molecular markers showed that the two species displayed similar levels of genetic diversity (Chen et al., 2007; Delmotte et al., 2006). The homothallic (\( sensu \) oomycetes) species \( P. \) halstedii showed considerable heterozygote deficiency (\( F_{IS} \approx 0.95 \)), probably due to the limited dispersal ability before mating and thus lack of available sexual partners in the field (Giresse et al., 2007), whereas the populations of the heterothallic (\( sensu \) oomycetes) species \( P. \) viticola only slightly deviated from Hardy–Weinberg proportions (Chen et al., 2007; Delmotte et al., 2006).

Haploid selfing vs. clonality vs. outcrossing

Haploid selfing is difficult to distinguish from clonality using molecular markers as both yield progeny not segregating for any marker and yield linkage disequilibrium over the long term at the population level. Sampling and genotyping the progeny within sexual structures in natural populations should allow determining whether haploid selfing actually occurs in nature.

The literature on mating systems in fungi is, however, unfortunately blurred by the pervasive confusion between haploid selfing and diploid selfing. For instance, a study on the ascomycete pathogen \( Cryphonectria parasitica \) responsible for the chestnut blight, claims to have shown that this ascomycete has a predominantly outcrossing mating system (Marra et al., 2004). In fact, the authors looked at whether the progeny within perithecia segregated for some highly polymorphic markers and could thus only infer the occurrence of haploid selfing vs. either diploid selfing or outcrossing. Their results interestingly showed that sampling progeny in natural populations can reveal whether haploid selfing occurs, and it was in fact rare in this species (although not completely absent, which is surprising for a supposedly heterothallic species). Such studies estimating the rate of haploid selfing are, however, still too rare, and the questions are confused by ambiguous terms like ‘selfing’ and ‘outcrossing’. Another example is the study by Pérez et al. (2010) which also called ‘selfing’ the phenomenon of haploid selfing, and estimated that ‘outcrossing’ (i.e. in fact either diploid selfing or outcrossing) was frequent in the homothallic plant pathogen \( Mycosphaerella nubilosa \).

Recent population genetics studies in yeasts have managed to estimate the rates of haploid selfing, diploid selfing and outcrossing at the same time by using linkage disequilibrium with the mating type locus in these species capable of mating type switching. Tsai et al. (2008) estimated that a sexual cycle occurred every 1000 asexual cycles in \( Saccharomyces paradoxus \). The sex events were estimated to be 94% from within the same tetrad (i.e. the automixis for diploid selfing), 5% with a
clonemate after switching the mating type (i.e. haploid selfing) and 1% outcrossed. This was consistent with another study of natural populations estimating outcrossing at 1.1% in this species (Johnson et al., 2004).

Murphy & Zeyl (2010) have shown, using an experimental approach, that *S. cerevisiae* and *S. paradoxus* undergo even higher rates of outcrossing (ca. 10–25%) when mates are available, much higher than what was long expected based on the existence of a capsule-protecting whole ascus. Earlier experimental studies had also estimated intratetrad mating rates at about 75–80% *in vitro* (Knop, 2006).

Several other studies have showed high rates of outcrossing in homothalic or pseudo-homothalic species, in particular in *Cryptococcus* and *Agaricus* (Bui et al., 2008; Callac et al., 2006; Heitman, 2010; Hiremath et al., 2008; Saul et al., 2008), supporting the idea that homothallism may have evolved for higher mate availability, instead of and/or in addition to promoting haploid selfing.

### 4. Utility of phylogenies in the study of sex, breeding systems and mating systems

Phylogenetic studies can enlighten the evolutionary history of mating systems and modes of reproduction in fungi. Some studies have shown, for example, that sex has been independently lost several times in fungal groups such as *Penicillium* (Lobuglio et al., 1993; López-Villavicencio et al., 2010). Phylogenetic analysis is also a powerful tool to study the relative ages of clades depending on the type of reproduction and thereby to assess whether different reproductive systems are advantaged over the long term. For example, the advantage of sex and outcrossing would be supported if asexual clades and selfing clades appeared younger than sexual outcrossing clades.

Phylogenies also allow estimating the ancestral and derive modes of reproduction in given clades. Phylogenies have shown that homothallism has probably appeared from a heterothallic ancestor in some groups of fungi (*Pusarium*, O’Donnell et al., 2004; Neurospora, Nygren et al., 2011; *Penicillium*, López-Villavicencio et al., 2010). In other specific groups such as *Aspergillus*, sequencing and analyses of mating type genes suggest that heterothallism may have reappeared from homothallism via gene loss from a homothallic ancestor (Galagan et al., 2005; Geiser et al., 1998; Ramirez-Prado et al., 2008). Phylogenetic studies also revealed that homothallism has evolved in some cases by lateral transfer of mating type genes: both mating type idiomorphs were acquired by horizontal gene transfer in *Stemphylium* (Inderbitzin et al., 2005), and clonal populations of the Dutch elm disease agent *Ophiostoma novo-ulmi*, presenting a single mating type for many years, recovered a sexual cycle after acquiring the MAT1-2 idiomorph from the closely related species *O. ulmi* (Paolletti et al., 2006). Phylogenies have also revealed the history of the acquisition of mating type switching in yeast (Butler et al., 2004).

Another interesting possibility offered by phylogenies would be to assess whether associations exist between the evolution of certain mating or breeding systems and other traits. For instance, if homothallism evolves for universal compatibility at syngamy, whereas heterothallism evolves for preventing haploid selfing, we may expect that homothallism evolved in outcrossing species in which other mechanisms prevent haploid selfing, such as rapid gamete dispersal after meiosis or lack of clonal multiplication of gametes. If selfing ensures reproductive success, we expect that it evolves in species where finding a mate is challenging. These approaches can be applied with great power to diverse organisms (e.g. in corals Kerr et al., 2011) but we first need to acquire data on fungal mating systems in nature (haploid selfing vs. diploid selfing vs. outcrossing) and on multiple life-history traits, in order to analyse them on phylogenies together with the mode of reproduction and breeding system (heterothallism vs. homothallism).

### 5. Experimental designs to distinguish between hypotheses

Most of the models proposed to explain the origin and maintenance of sexual reproduction and mating types assume fitness differences between the progeny depending on the mode of reproduction and the mating system (see section 2). Experimental approaches may therefore allow testing for the existence and the amount of these differences using fungi.

The experimental designs we propose are based on the comparison of the fitness of the diploid or haploid individuals produced by different modes of reproduction and by different types of syngamy. We note $W_{AR}$, $W_{HS}$, $W_{DS}$ and $W_{OC}$ the fitnesses of progeny produced by asexual reproduction, haploid selfing, diploid selfing and outcrossing, respectively. Different fungal models may be used to test these different hypotheses. It should be noted that these fitnesses can be measured either in haploid or in diploid individuals, depending on the biology of the species under investigation.

Does haploid selfing afford the advantages of sex not related to recombination? $W_{AR} < W_{HS}$ would then be expected

Testing the existence of fitness differences between progeny produced by haploid selfing vs. asexual reproduction can provide evidence of advantages of sex that are unrelated with recombination. By definition, haploid selfing is possible in homothalic ascomyces and it is not clear why some species would only undergo haploid selfing, because it has no recombinatorial advantages over asexual reproduction and is at the same time more costly. However, nonrecombinatorial advantages may also exist (section 2). This question can be investigated using a homothalic species able to reproduce both sexually and
aseline. Some experimental work has already been carried out on these aspects in *A. nidulans* (Bruggeman et al., 2004): several lineages were started, some transferred between plates exclusively by sexual spores resulting from haploid selfing, and others transferred between plates exclusively by asexual spores. After a certain number of replating events (i.e. several generations), fitness comparisons were made based on sizes of the colonies. Results indicated that haploid selfing slowed down the accumulation of deleterious mutations. Similar experiments are needed on additional species, and other traits can be used to estimate the fitness depending on the species, such as asexual spore production, sexual reproduction, viability of the progeny or competitive ability. Other features unrelated to the recombinational advantages of sex could also be compared in the progeny resulting from asexual reproduction vs. haploid selfing, such as the protection from the proliferation of parasites or transposable elements in sexual and asexual structures, or senescence.

**Is haploid selfing only an artefactual by-product of the evolution of homothallism for higher mate availability?** \( W_{\text{AR}} \geq W_{\text{HS}} \) would then be expected

Testing the existence of fitness differences between progeny produced by haploid selfing vs. asexual reproduction as above can also allow testing the hypothesis that homothallism evolved not to allow haploid selfing but to allow universal mating compatibility in outcrossing species. In this case, one does not expect any advantage of haploid selfing over asexual reproduction, and even some costs of meiosis under haploid selfing as compared to asexual reproduction. We would also expect that homothallic species outcross in nature (see section 3).

**Does diploid selfing provide the recombinatorial advantage of sex?** \( W_{\text{HS}} < W_{\text{DS}} \) would then be expected

We hypothesize, with others (Czaran & Hoekstra, 2004), that the origin and maintenance of mating types might be explained because they allow benefiting from the recombinatorial advantages of mating between genetically different gametes. This can be tested in haploid organisms using homothallic species, by testing fitness differences between lines from sexual reproduction by haploid selfing vs. diploid selfing. These experiments can be performed in a fixed or variable controlled environment, in order to distinguish whether recombination benefits rely on the decrease in mutation accumulation or on the increase in adaptation speed. A fitness comparison between offspring produced by haploid vs. diploid selfing is possible only if the parents show a reasonable level of heterozygosity. Hence, in both types of experiments, a first generation of outcrossing, preferentially between individuals from different populations, should be performed.

**Deleterious mutation accumulation experiments**

Recombination is considered to be fundamental to prevent the accumulation of deleterious mutations in sexual populations. Haploid selfing does not allow recombinination between different genetic haploid genomes; it therefore does not allow the elimination of deleterious mutations, except in the meiosis where they appear (Bruggeman et al., 2004). Experimental protocols using fungi can allow testing the importance of recombination for the fitness of the progeny after several generations of mutation accumulation. Different lineages can be created with individuals grown in a controlled environment for a given number of generations. A generation begins with a single individual produced by haploid or diploid selfing. Lineages can be maintained for enough generations to permit mutations to appear or mutational load could be artificially increased. To detect advantages of recombination, the fitness of the progeny formed by haploid vs. diploid selfing could be compared. If differences exist (\( W_{\text{HS}} < W_{\text{DS}} \)), this could support the idea that sex is advantageous only when recombination exists.

**Adaptation speed experiments**

Sexual recombination between genetically different genomes is also considered to increase the rate of adaptation in populations. In yeast, sex has been shown to increase adaptation compared to asexual reproduction in nonfavourable environments (Goddard et al., 2005). This advantage is expected only under diploid selfing, whereas no differences should be expected between asexual reproduction and haploid selfing under which no effective recombination occurs. Different lineages formed via either haploid or diploid selfing can be created. These different lineages can be submitted to different environmental conditions, in harsh or mild environments (by modifying nutrients, temperature or by adding antifungal compounds that could make environmental conditions more stringent and require adaptations). The fitness of these two lineages can be measured at some time points comparatively in the different environments.

**Do progeny produced by diploid selfing suffer from inbreeding depression?** \( W_{\text{DS}} < W_{\text{OC}} \) would then be expected in diploid progeny

Inbreeding depression is a frequent phenomenon in plants and animals (Charlesworth & Willis, 2009). Inbreeding depression is easily measured in controlled experiments, by comparing the fitness of outcrossed vs. selfed offspring, and can be estimated in natural populations using genetic markers, by estimating the relatedness between individuals and the fitness of their offspring. Surprisingly, although numerous measures of inbreeding depression have been performed for decades in plants and animals, it has rarely been measured
in fungi. Yet, estimating inbreeding depression in basidiomycetes and oomycetes would be straightforward, as they have a life cycle with a predominant dikaryotic and diploid stage, respectively. Inbreeding depression indeed results from the homozygosity of deleterious rare mutations in inbred offspring, and can be estimated by comparing the fitness between outcrossed dikaryotic or diploid progeny and progeny produced by diploid selfing. Traits that can be measured to estimate fitness in basidiomycetes and oomycetes include heterokaryon growth rate, primordium formation, number of fertile fruiting bodies and average weight per fruiting body.

**Conclusion**

This study had two main goals: first, clarifying the concepts and terminology used to describe sexual reproduction in fungi by placing them in an evolutionary perspective, and explaining the theoretical costs and benefits of each mode of reproduction and mating system, and second, proposing some general experimental considerations and population genetics analyses in fungi to help disentangle hypotheses about the evolution of the mode of reproduction and mating systems. In the evolutionary biology literature, many models and experiments have focused on the question of why many species undergo outcrossing instead of diploid selfing. So far however, very few studies have used fungi as models, and even worse, the mating systems and modes of reproduction of fungi in nature have been poorly investigated. Yet, fungi have particularities that can give valuable insights into long-standing questions in evolutionary biology, especially the ability of most fungi to undergo both asexual and sexual reproduction, and to undergo sexual reproduction via three mating systems: haploid selfing, diploid selfing and outcrossing. Original questions can even be addressed in fungi: do many species undergo sexual reproduction through haploid selfing? And in this case, why is it so, while they are able of performing asexual reproduction? Are benefits of recombination sufficient to explain the evolution of heterothallism, with the evolution of complex molecular mechanisms restricting syngamy between identical haploids? We argue that these questions can be reasonably addressed using population genetics, phylogenetics and experimental approaches and would give invaluable insights into the evolution of sex and mating systems.

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exchange in sexual, alpha mating type populations. 


**Glossary**

**Anisogamy:** a feature of species where the two fusing cells at syngamy are of different sizes or morphologies and often do not provide the same amount of resources to the zygote (e.g. large and small gametes, or as in some fungi where syngamy involves a spore fusing with a hypha or mycelium).

**Automixis:** syngamy between haploid cells or haploid nuclei derived from the same meiosis, also referred to as intratetrad mating when involving syngamy. Breeding system: the cellular or developmental mechanisms that determine how syngamy can be achieved for an organism (e.g. homothallism vs. heterothallism in fungi, hermaphroditism vs. dioecy in plants).

**Diploid selfing:** syngamy between haploid cells derived from meioses of a single diploid individual.

**Haploid selfing (or same-clone mating, intragametophytic mating, gametophytic selfing):** syngamy between haploid cells that are mitotic descendants of a common haploid progenitor, often the cells are genetically identical except for mutation and at the mating type locus in the case of species with mating type switching.

**Homothallism:** for fungi, the potential to undergo haploid selfing; for oomycetes, the potential to undergo diploid selfing without the presence of another individual with another mating type.

**Heterothallism:** for fungi, the requirement for genetic differences between haploid cells to undergo syngamy; for oomycetes, the requirement of hormonal signals from a diploid individual carrying a different mating type to produce gamete and undergo either diploid selfing or outcrossing.

**Idiomorph:** sequences present at the same locus but that are not derived from a common ancestral sequence (i.e. in contrast to alleles; idiomorphs at the mating type locus in ascomycete fungi determine the compatibility for syngamy.

**Isogamy:** a feature of species where the two fusing cells at syngamy are of the same size and morphology, often yielding the same amount of resources to the zygote (e.g. in basidiomycete fungi, syngamy can be between two mycelia).

**Mating system:** the realized type of syngamy in nature in terms of the relatedness of the cells involved (e.g. inbreeding or outcrossing).

**Mating type:** molecular recognition systems determining the compatibility between cells for syngamy where haploid cells expressing identical mating types at syngamy cannot fuse.

**Mating type switching:** a system found in some ascomycete yeasts where the determinants of alternate mating types are present but the determination of which mating type is expressed can change by localized genetic rearrangements.

**Modes of reproduction:** a basic distinction between asexual and sexual reproduction.

**Outcrossing:** syngamy between haploid cells derived from meioses that occurred in different diploid individuals.

**Pseudo-homothallism:** ability of some fungi to complete the sexual cycle without the apparent need for a syngamy, which is achieved by the presence of two haploid nuclei of opposite mating types and from a single meiosis in the dispersed spore.

**Syngamy:** fusion of haploid cells (often gametes, but sometimes mitotically replicating haploid cell or hyphae, as in homobasidiomycetes) for fertilization, which leads to zygote formation after karyogamy; karyogamy can occur long after syngamy in fungi, for example, in basidiomycetes.

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