doi:10.1111/j.1558-5646.2011.01562.x

WHAT USES ARE MATING TYPES? THE "DEVELOPMENTAL SWITCH" MODEL

Nicolas Perrin[1,2]

[1]*Department of Ecology & Evolution, University of Lausanne, CH 1015 Lausanne, Switzerland*

[2]*E-mail: Nicolas.Perrin@unil.ch*

Received October 27, 2011
Accepted December 6, 2011

Why mating types exist at all is subject to much debate. Among hypotheses, mating types evolved to control organelle transmission during sexual reproduction, or to prevent inbreeding or same-clone mating. Here I review data from a diversity of taxa (including ciliates, algae, slime molds, ascomycetes, and basidiomycetes) to show that the structure and function of mating types run counter the above hypotheses. I argue instead for a key role in triggering developmental switches. Genomes must fulfill a diversity of alternative programs along the sexual cycle. As a haploid gametophyte, an individual may grow vegetatively (through haploid mitoses), or initiate gametogenesis and mating. As a diploid sporophyte, similarly, it may grow vegetatively (through diploid mitoses) or initiate meiosis and sporulation. Only diploid sporophytes (and not haploid gametophytes) should switch on the meiotic program. Similarly, only haploid gametophytes (not sporophytes) should switch on gametogenesis and mating. And they should only do so when other gametophytes are ready to do the same in the neighborhood. As argued here, mating types have evolved primarily to switch on the right program at the right moment.

KEY WORDS: **Diploidy, gametophyte, haploidy, meiosis, sexes, signaling, sporophyte.**

Sexes and Mating Types

Meiotic sex is a complex two-step process, initiated by the fusion of two haploid genomes to form a diploid zygote, and ending with the reduction to haploidy through meiosis, a specialized mechanism that ensures recombination between the two interacting genomes. The haploid genomes resulting from recombination differ from those that entered fusion. Although highly sophisticated, meiotic sex had a very early origin in the history of eukaryotes. Findings of meiosis-related genes in the most basal lineages (e.g., Ramesh et al. 2005; Derelle et al. 2006) show that it dates back to one billion years at least. Sex was a highly successful story right from the beginning, boosting the evolutionary potential of lineages that practiced it. Purely asexual lineages are nowadays extremely rare, and, with very few exceptions, of recent origin (i.e., short lived).

Surprisingly, however, members of sexual lineages are usually not free to recombine with all conspecifics. Depending on lineages, the range of permissible partners is controlled by categories referred to as "mating types" or "sexes" (genders). Both categories may apply at the haploid or diploid level, and certainly present similarities, but should still be kept distinct. "Mating types" refer to incompatibilities between otherwise phenotypically similar partners (isogamy), whereas "sexes" imply some anisogamy, that is, an asymmetry (in terms of size or behavior) between the interacting gametes or partners (reaching an extreme in the oogamy of plants and animals). Anisogamy evolved from isogamous ancestors several times independently. Sexes emerged directly from preexisting mating types in some lineages (e.g., Volvocales; Nozaki et al. 2006; Nozaki 2008), but independently so in others.

Here lies one main reason to keep the distinction: many organisms present both mating types and sexes. The haploid gametophytes of heterothallic filamentous ascomycetes (such as *Podosporina* or *Neurospora*) are hermaphrodite, producing simultaneously both male and female gametes (microconidia and ascogonia, respectively), but microconidia can only fertilize

ascogonia from a different mating type (Coppin et al. 1997). Similarly, the unicellular diploid sporophytes of ciliates (such as *Euplotes*, *Paramecium*, or *Tetrahymena*) are hermaphrodite, producing both a stationary (female) and a migratory (male) micronucleus, but can only exchange male nuclei with a partner from a different mating type (Phadke and Zufall 2009).

Evolution toward anisogamy resulted from disruptive selection (Parker et al. 1972; Bell 1978; Hoekstra 1982; Bulmer and Parker 2002; see other references in Billiard et al. 2011). Given the opposing selective pressures to simultaneously maximize the number of gametes, their encounter rate, and the mass (and ensuing survival) of resulting zygotes, it turns out that the fitness of both partners is maximized when one interacting gamete is small and mobile, while its large and sessile partner provides the resources required for zygote development. Intermediate gametes would do worse than small ones in terms of mobility and numbers, and worse than large ones in terms of provisioning. Furthermore, the developing sporophyte is often directly provisioned by the female gametophyte (such as found in many algae, mosses, or ferns). This obviously requires that the female gamete remains attached to her maternal gametophyte (and consequently that the male gamete actively searches partners). Such developing constraints largely explain why sexes (at the gametic level) are two and only two, and why anisogamy independently evolved in many lineages.

Regarding mating types, however, important questions remain unanswered. Why do isogamous organisms have mating types at all? Why do some lineages that adopted sexes (anisogamy) still maintain mating types? Assuming some costs of finding a mating partner, the best solution would seem to drop entirely mating types, because they drastically reduce the number of compatible partners. An additional (and distinct) question is (Appendix) as follows: If, for some reason, mating types are necessary, why are their numbers often limited to two? Having the largest possible number of mating types would maximize the chance that the first conspecific met is compatible. Two mating types actually seems the worst number, because any individual can only pair with half of its conspecifics (assuming that frequencies are equalized by frequency-dependent selection).

Control over Cytoplasmic Conflicts

Regarding the first question (why having distinct mating types?), one widely shared view posits that mating types are required to control potential conflicts among cytoplasmic organelles (Hurst and Hamilton 1992; Hurst 1995, 1996). Heteroplasmy, which occurs when both gametes contribute mitochondria (or chloroplasts) to the zygote, opens the way to a deadly competition between organelles. These will be selected for better transmission to the next generation, through either active elimination of the competitor or accelerated division (at the cost of efficiency), both resulting in a decrease in host fitness. This opens a wide opportunity for nuclear genes to enforce uniparental inheritance. According to this hypothesis, mating types (and genders) have evolved as a way to prevent costly competition, by fostering uniparental transmission. Thus, whenever mating involves cytoplasmic fusion, mating types or sexes should (1) determine who transmits which organelle and (2) signal who is a potential mating partner or not (to prevent mating between gametes which both suppress or both transmit their organelles).

There is ample support for this prediction across a diversity of phyla. Organelle transmission strongly correlates with sexes in plants, animals, and other anisogamic groups. Organelles are usually transmitted maternally (i.e., through the large gamete), but counterexamples exist (e.g., mitochondria are transmitted paternally in some gymnosperms and chytridiomycetes; Xu 2005). Biparental inheritance occurs in many mussels, but the paternal haplotype is then sequestered in the male germ line, which prevents any conflict (Breton et al. 2007). In isogamous groups also, mating types often correlate with organelle transmission. The locus that determines mating type (MAT) has been found to harbor genes that actively control organelle transmission in several lineages including algae, slime molds, and fungi (see next).

However, this model also fails to account for a series of important cases. First are situations where transmission, although uniparental, is random regarding mating types (such as in the slime mold *Didymium iridis*; Silliker et al. 2002; Scheer and Silliker 2006). If uniparental transmission can be controlled by mechanisms other than mating types, these must fulfill a different function. Second, in some organisms (such as in the yeast *Saccharomyces cerevisiae*), both mating types transmit mitochondria. Potential conflicts are solved by mitochondrial fusion and mitochondrial DNA (mtDNA) recombination (Takano et al. 2010). In the brown alga *Ectocarpus siliculosus*, both mating types transmit chloroplasts, which then segregate in different parts of the sporophyte after zygotic division (Peters et al. 2004). Why then maintain mating types? Third, in some lineages (such as the acellular slime molds *Physarum polycephalum*) mitochondrial transmission is controlled by one locus, whereas gametic compatibility and cell fusion are controlled by other independent MAT loci (Moriyama and Kawano 2010). The latter must thus serve a different purpose. Finally, many lineages maintain mating types, despite absence of cytoplasmic fusion. In ciliates and filametous ascomycetes, for instance, organelle transmission is controlled by sexes (inheritance being maternal). The male parent only provides a nucleus, but has to transmit it to a partner with a complementary mating type. That mating types exist independently of sexes show that they must fulfill another (and possibly more fundamental and primary) function. As pointed out in a recent review (Billiard et al. 2011), correlation is not indicative of causes or effects. It might

well be that organelle inheritance was superimposed on preexisting mechanisms (first mating types, then sexes) that evolved for other reasons.

Avoidance of Inbreeding and Same-Clone Mating

In their review, Billiard et al. (2011) evaluate the several hypotheses raised to explain the evolution of mating types and sexes and, as a conclusion, propose these categories to originate from different ultimate causes (respectively, "inbreeding avoidance" and "sex-advantage enhancement"), depending on whether they control mating between diploid or haploid stages. Inbreeding avoidance has often been invoked to account for mating types. Inbreeding depression is largely documented in higher eukaryotes, and is certainly responsible for the incompatibility systems found in angiosperms. It mostly stems from the load of recessive deleterious mutations that accumulate in diploid genomes. However, as noted by Billiard et al. (2011), inbreeding avoidance is unlikely to play a role in predominantly haploid lineages. In many algae, yeasts, or filamentous ascomycetes, the diploid stage is extremely reduced, and its activity largely limited to the initiation of meiosis. Although some inbreeding load might possibly accrue at genes that are only expressed during the short-lived zygotic stage, the main bulk of recessive deleterious mutations is fully exposed to selection in the haploid (hemizygous) state, and should thus be largely purged.

More importantly, haploid mating types cannot prevent inbreeding because any meiosis necessarily produces spores with complementary mating types. Selfing (defined as mating between haploid spores stemming from the same sporophyte) is not only possible in fungi, it is actually frequent (e.g., Giraud et al. 2008). Hence, inbreeding avoidance might have fostered the evolution of mating types (or sexes, or incompatibility systems) in lineages where classes are defined at the diploid stage (such as ciliates, plants, or animals), but not at the haploid one (e.g., fungi or algae). For the latter, Billiard et al. (2011) favor the "sex-advantage enhancer" model, according to which mating types are selected to maximize the recombinatorial advantages of sex (be it DNA repair, breakdown of negative epistasis, increase of genetic variance, or any other). If sex is beneficial for one of these reasons, then syngamy (which is costly) should not occur between genetically identical clones.

However, as I will argue below, some diploid lineages (e.g., ciliates) have evolved a range of sophisticated adaptations (such as epigenetic determination of mating type, or autogamy) precisely aimed at circumventing mating-type constraints and allowing selfing. Inbreeding avoidance therefore cannot be the ultimate cause of mating types in these lineages. Similarly, some lineages where mating types are defined at the haploid level (e.g., yeasts) have evolved diverse and sophisticated adaptations (such as mating-type switch) aimed at circumventing mating-type constraints and allowing mating between genetically identical individuals. Hence, avoidance of "same-clone mating" cannot be the ultimate cause of mating types in these lineages.

Triggers of Developmental Switches

A role for mating types in regulating developmental pathways has been independently proposed in different model organisms. Based on observations of "mixed phases" in red algae (see next), van der Meer and Todd (1977) suggested the sporophytic phase to be triggered by heterozygosity at the mating type, and not by the diploid state per se. A similar suggestion was made for yeasts some 30 years ago, following identification on the MAT locus of transcription factors triggering the differentiation of haploid and diploid cells into mating and meiosis/sporulation stages, respectively (e.g., Herskowitz 1985, 1989, and references therein). More recently, a "ploidy-assessment" hypothesis was proposed for the evolution of mating types in *Chlamydomonas* (Haag 2007). The basic idea is that complementary signals from the two haploid mating types are required to properly inform the zygote of its own ploidy level, and thereby switch on the correct developmental program: "[. . .] the signal for diploidy is often the production of a heterodimeric transcription factor encoded by, or under the direct regulation of, mating type loci. This factor can only be made in zygotes formed from the fusion of individuals of two types, and thus the diploid phase can be inferred and executed properly" (Haag 2007).

Billiard et al. (2011) quickly discarded this hypothesis on the grounds that mating types are not universal. In some lineages, identical genotypes merge to form zygotes, which then develop normally. In the following, I would like to expand on this insightful "trigger" hypothesis, and suggest that the first and main function of mating types is indeed that of controlling key developmental switches along the haploid–diploid life cycle. This includes the ploidy-assessment function (mediated by transcription factors) suggested by the several authors above, as well as additional functions such as mating-partner assessment, mediated by pheromone/receptor systems.

Any given genome encodes a diversity of alternative developmental programs. As a haploid gametophyte, an individual may grow vegetatively (through sequential series of haploid mitoses), produce asexual spores, or initiate the sexual cycle (via gametic differentiation, mating, and zygote formation). Reciprocally, as a diploid sporophyte, it may equally well enter a vegetative program (through diploid mitoses), produce diploid spores, or terminate the sexual program (via meiotic divisions and the formation of haploid spores). A precise coordination is essential, so that developmental switches only occur when the required conditions are

met. In particular, the sexual program leading to gametic differentiation and fusion should only be initiated when a partner is present. Similarly, diploid cells should not directly differentiate into gametes (meiosis is required first) and haploid cells should not enter meiosis (gametic fusion is required first). Here below I will shortly summarize relevant information on the life-cycle and mating-type determinants in a few selected lineages (ciliates, algae, and fungi), showing that most of the genes identified on MAT loci are either transcription factors (which modulate the transcription of genes involved in specific developmental programs), or molecules involved in signaling pathways that activate or inactivate such transcription factors. As further developed below, the main function of such signaling and transcription pathways might be that of coordinating developmental switches along the sexual cycle.

CILIATES

Although unicellular, ciliates represent a highly evolved lineage of eukaryotes. Mating types are defined at the diploid level. At conjugation, diploid individuals of complementary mating types meet, enter meiosis, and exchange haploid micronuclei. After nuclear fusion, the two exconjugants divide mitotically, producing a total of four genetically identical cells (caryonids). In *Euplotes*, all four caryonids display the same mating type, determined by codominant alleles at a multiallelic MAT locus ("synclonal" determination; Phadke and Zufall 2009). Each mating type produces specific pheromones and receptors that determine complementarity. Interestingly, both the pheromone and the receptor are produced by the alternative splicing of a single MAT gene (Vallesi et al. 2005). One transcript of the gene translates into a soluble pheromone that is released in the environment. The alternative transcript translates into a transmembrane receptor, with an extracellular C-terminal sequence corresponding to the pheromone, and an additional intracellular N-terminal sequence. This receptor acts both as an autocrine growth factor and as a paracrine mating signal. When binding their own pheromones (constitutively secreted into the extracellular environment), cells grow vegetatively and divide mitotically. When binding a nonself pheromone (produced by cells from a different mating type), they arrest growth and develop competence for mating (Vallesi et al. 2005).

Hence, a crucial function of *Euplotes* mating type appears to be that of distinguishing self from nonself. Individuals must be informed that a conspecific is present in their immediate neighborhood, so that meiosis and conjugation can safely proceed. If all individuals were displaying the same mating type, they would all express the same pheromone and receptor, and would thus be unable to detect the presence of conspecifics in their environment. In other words, an individual would be triggered by its own pheromone to enter the meiosis/conjugating program.

Mating types in ciliates are clearly not involved in controlling mtDNA transmission. Only micronuclei are exchanged during conjugation, so that mitochondria are inherited maternally (i.e., according to sex, not to mating type). Similarly, mating types play no role in inbreeding avoidance. In fact, many ciliates have evolved epigenetic mating-type determination (e.g., cytoplasmic or caryonidal in *Paramecium* and *Tetrahymena*; Preer 2000) that precisely allow mating compatibility between genetically identical cells resulting from the same conjugation event. Even with synclonal determination, individuals from a given clone may still switch mating types as a result of maturation (random segregation of alleles during successive macronucleus fissions) or autogamy (within-individual fusion of two genetically identical meiotic products from the same nucleus), with the main consequence of allowing selfing (i.e., subsequent mating between individuals stemming from the same caryonid; Phadke and Zufall 2009).

ALGAE

Contrasting with ciliates, mating types in algae are defined at the haploid level. Here I focus on one of the best-studied species, the chlorophyte *Chlamydomonas reinhardtii*, arguably similar to the ancient common ancestor of plants, animals, and fungi (Goodenough et al. 2007). The haploid stage is unicellular, and reproduces vegetatively as long as conditions are favorable. In response to stressful conditions (e.g., nitrogen depletion) haploid cells differentiate into phenotypically similar gametes of complementary mating types (either MAT+ or MAT−). After fusion, the diploid zygote differentiates into a resistant cell, with a zygote-specific cell wall, which allows survival through the stressful period. Once conditions improve, the zygote enters meiosis, producing two MAT+ and two MAT− haploid spores.

Mating type is determined by a biallelic MAT locus, comprising a relatively large non-recombining region (~1 Mb). Genes at the MAT locus are involved in three main functions (Goodenough et al. 2007), respectively, (1) gametogenesis and fertilization competences, (2) zygote differentiation, and (3) control of organelle inheritance (zygotic mtDNA is inherited from the MAT− parent, and chloroplast from the MAT+). Gametogenesis and mating-type signaling is controlled by the activation of a gene (*MID*, for *minus* dominance) on the MAT− allele (Ferris and Goodenough 1997). The MID protein (a bZIP transcription factor) is upregulated within 30 min of nitrogen depletion, and initiates a cascade of events by activating *minus* genes and repressing *plus* genes.

In particular, MID represses the autosomal gene encoding the *plus* agglutinin glycoprotein (*SAG1*), and activates the *minus* agglutinin gene (*SAD1*), localized on the same MAT− locus. As a consequence, the MAT+ and MAT− cells express (when needed) the complementary proteins required for the recognizing and fusing of individuals of opposite mating types (Goodenough et al. 1978). In parallel, the *plus* gametes upregulate a gene (*FUS1*)

on the MAT+ locus, required for cell fusion (Ferris et al. 1996). Upon fusion, adhesion of MAT+ and MAT− agglutinins induces a cascade of signal transduction events that prepare for zygote formation. As in *Euplotes*, this complementarity ensures that the mating/fusion program is not self-induced.

A second crucial role of MID is that of regulating additional autosomal genes that encode mating-type specific homeodomain transcription factors (Kurvari et al. 1998). Specifically, it activates Gsm1 (expressed by the *minus* gamete) and represses Gsp1 (expressed by the *plus* gamete). Upon cell fusion, these transcription factors form heterodimers that enter the diploid nucleus and initiate zygote differentiation. The Gsm1/Gsp1 heterodimer is both necessary and sufficient to repress the genes involved in mating behavior, and to switch on the zygotic program (in particular the genes involved into the building of cell wall and stress resistance), and later drive the meiotic/sporulation phase (Zhao et al. 2001). If Gsm1/Gsp1 heterodimerization fails, the zygote will initiate a diploid vegetative growth once nitrogen levels are restored (instead of entering meiosis). If starved again, this zygote will differentiate as a *minus* gamete (MAT− being dominant over MAT+) and subsequently mate with a haploid *plus* gamete, a deadly mistake given that triploid cells cannot complete meiosis (Ebersold 1967; Galloway and Goodenough 1985). Reciprocally, transformed cells that constitutively express Gsm1 and Gsp1 do not differentiate into gametes, but directly enter the zygotic program and form a cell wall (Goodenough et al. 2007).

Similar patterns are found in red algae (Rhodophycaea), where Florideophycean evolved multicellularity and anisogamy. Sex in *Gracilaria* is determined by two alleles at the mating type locus (mt^m and mt^f; van der Meer and Todd 1977). The carpogonia of female gametophytes (mt^f) are fertilized by spermatia produced by male gametophytes (mt^m). The resulting diploid carposporophytes (mt^m/mt^f) release diploid spores that develop into tetrasporophytes (mt^m/mt^f). Meiosis then produces haploid tetraspores (two mt^m and two mt^f per meiosis, developing into male and female gametophytes, respectively). Interestingly, red algae occasionally present "mixed phases," with sexual organs (spermatia and carpogonia) developing directly from diploid tetrasporophytic tissues, together with tetrasporangia. As shown in *Gracilaria*, this results from asymmetric allelic segregation during the differentiation of reproductive structures, stemming from mitotic recombination (van der Meer and Todd 1977) or random segregation of chromosomes during depolyploidization (Goff and Coleman 1986; Haig 1993). Tetrasporophytic tissues that are recombinant for mating type (and thus homozygous mt^m/mt^m or mt^f/mt^f) enter the male or female gametophytic program, respectively. The resulting diploid spermatia (mt^m/mt^m) can fertilize haploid carpogonia (mt^f), but the resulting triploid tetrasporophytes are not fertile, due to the problems inherent to triploid meiosis. As put by van der Meer and Todd (1977), this clearly shows that "heterozygosity for mating type, rather than the diploid state, triggers development of the tetrasporophytic phase." This second function of mating types corresponds to the ploidy-assessment hypothesis independently proposed by Herskowitz (1985) or Haag (2007), according to which complementary transcription factors formed by the two mating types allow informing the zygote of its diploid state, so that it can correctly enter the zygotic program.

ASCOMYCETES

As in algae, mating types in fungi are determined at the haploid (gametophytic) stage. Heterothallic ascomycetes are bipolar (i.e., two complementary mating types are found among the spores resulting from one meiotic event; Lee et al. 2010). In the yeast *S. cerevisiae*, the two mating types **a** and α are determined by a biallelic MAT locus. This locus encodes only transcription factors, two on the MATα allele (one from the homeodomain HD1 class, the other from the high-mobility group (HMG)/α-box class) and one on the MAT**a** allele (homeodomain HD2 class). Autosomal genes encode mating-type specific pheromones and cell-surface receptors (Lee et al. 2010), the **a**-pheromone and α-receptor being expressed constitutively. The mating program is initiated by up-regulation of the MATα locus. The α2 (HD1) transcription factor represses the **a**-specific genes (in particular *STE2*, which encodes the α-receptor), whereas the α1 factor (HMG/α-box) activates the α-specific genes (in particular *STE3*, which encodes the **a**-receptor). Here again, specific pheromones and receptors ensure that fusion only occurs between cells of opposite mating types (i.e., that gametogenesis is not self-induced).

Upon formation of the α/**a** zygote, the transcription factors **a**1 (HD2 class, provided by the **a** gamete) and α2 (HD1 class, provided by the α gamete) form heterodimers, which bind to specific DNA sites of the diploid nucleus, repressing the genes involved in the haploid cycle, and ensuring that only diploid cell functions are expressed after mating (Galitski et al. 1999; Galgoczy et al. 2004; Lee et al. 2010). In *Candida albicans*, **a**1/α2 heterodimers are also required to repress mating competence: experimentally produced **a/a** or α/α diploids enter a mating program, just as haploids (Miller and Johnson 2002). This function is fundamentally similar to that of the Gsm1/Gsp1 heterodimer formed in the *Chlamydomonas* zygote, ensuring that diploid cells correctly enter the zygotic program.

Interestingly, *S. cerevisiae* also evolved the ability to switch mating types and undergo a self-fertile sexual cycle (homothallism; Haber 2003). The current mating type of a gametophyte is actually controlled by the allele (MAT**a** or MATα) present at the MAT locus. However, all cells also possess (on the same chromosome) a silent copy of MATα on the left (called HML, for homology to MAT left) and a silenced copy of MAT**a** on the right (called HMR, for homology to MAT right). Mating-type switches are initiated by the gene *HO* (only activated in the haploid phase,

being repressed by the **a**1/α2 heterodimer; Ezov et al. 2010). *HO* encodes a highly specific endonuclease, which cleaves DNA at the MAT locus. Once cut, the DNA is degraded by exonucleases, and the resulting gap is filled by a copy of either HML or HMR. This gene conversion is strongly biased toward the alternative mating type (i.e., previously α tends to become **a**, and vice versa; Wu and Haber 1996). Hence, a colony founded by a single haploid spore will soon restore both α- and **a**-mating types (many laboratory strains, which have lost the *HO* gene, remain indefinitely either α or **a**). Similar mechanisms were derived independently in other yeasts such as *Schizosaccharomyces pombe* (Holmes et al. 2005). In *Kluyveromyces lactis* (which lost the *HO* gene) mating-type switches are mediated by a transposable element (Barsoum et al. 2010).

Such exquisite adaptations make clear that mating types have not evolved in yeasts to prevent same-clone mating. Quite to the contrary, the highly specialized mechanisms of mating-type switching precisely allow same-clone mating, despite the necessity of maintaining complementary mating types for proper zygotic development. As argued in the present article, mating types are required to ensure that individual cells switch on the right program at the right moment. In particular, to enable haploid cells to correctly identify nonself haploid cells in the neighborhood before entering gametic differentiation, and zygotes to correctly identify their own diploid state before entering sporophytic differentiation.

In filamentous ascomycetes (e.g., *Podospora anserina* and related species), the MAT locus also encodes transcription factors only, all from the HMG family (FPR1 on the MAT+ allele; FMR1, SMR1, and SMR2 on the MAT− allele). These transcription factors also induce mating-type specific expression of autosomal pheromones and receptors (Coppin et al. 1997). However, nuclear fusion (karyogamy) is not immediate after cell fusion. The resulting sporophyte instead develops first as a multinuclear mycelium, which then differentiates into series of binucleate ascogonial cells. Nuclei from the two haploid gametophytes perfectly segregate in these dikaryons (i.e., each cell has one nucleus of each mating type), which obviously requires nuclear signaling and recognition. The MAT+ gene *FPR1* governs the expression of MAT+ nucleus-specific proteins, whereas the MAT− genes *FMR1* and *SMR2* form heterodimers that control the expression of proteins specific to MAT− nuclei. Karyogamy only occurs in ascus mother cells and is immediately followed by meiosis.

Interestingly, meiosis in *P. anserina* and other filamentous ascomycetes is followed by a postmeiotic mitosis, hence producing four pairs of identical haploid nuclei. Each of the four ascospores resulting from a single meiosis then receives two nuclei, one of each mating type (which also requires nuclear signaling/recognition). As a consequence, cultures initiated by a single dispersing ascospore are self-fertile heterokaryons ("pseudohomothallic;" Coppin et al. 1997). This pattern again clearly shows that the function of mating types is not that of preventing inbreeding. Pseudohomothallism in filametous ascomycetes has evolved just to allow selfing (in that case, mating between two gametophytes stemming from the same spore), despite the need to mate with a partner of a different mating type.

BASIDIOMYCETES

After nuclear exchange between two complementary gametophytes, the sporophyte of basidiomycetes develops into a filamentous dikaryon (each cell harboring one nucleus of each partner). Karyogamy and meiosis only occur in the final fruiting body (the carpophore of mushrooms). Mating-type determination is tetrapolar (i.e., four mating types may occur among the spores resulting from a single meiosis), and is encoded by two independent loci. One locus determines a pheromone/receptor system, whereas the other encodes transcription factors, which again act as heterodimers to regulate proper development of the dikaryon. The two loci interact to control mtDNA inheritance. In a few lineages, these two loci have fused into a single diallelic locus to form a bipolar system similar to the one found in ascomycetes (e.g., Bakkeren and Kronstad 1994; Lengeler et al. 2002).

In the corn smut *Ustilago maydis* (hemibasidiomycetes), mating specificity is ensured by the biallelic locus *a*, comprising two tightly linked genes that encode a mating-type specific pheromone and the corresponding receptor. The *a*1 allele includes *mfa1* (for mating factor *a*1) and *pra1* (for pheromone receptor *a*1), while the *a*2 allele encodes the corresponding genes *mfa2* and *pra2* (Banuett 1995). The *a*2 locus furthermore harbors two more genes (*lga2* and *rga2*) controlling gametic differentiation, and activated in response to pheromone stimulation. In addition, *lga2* is strongly upregulated after cell fusion (being a target of the bE/bW heterodimer complex, see next), where it mediates the loss of the *a1*-mtDNA during diploid (pathogenic) development (Fedler et al. 2009).

Mating-type specific transcription factors are encoded by the multiallelic locus *b* (with > 30 alleles known; Puhalla 1970). Each allele encodes two homeodomain transcription factors (bW and bE, belonging to the different functional classes HD1 and HD2, respectively). After fusion of two compatible cells (decided by alleles at the *a* locus), the bW allele from one mating type binds to the compatible bE allele from its partner to form two active heterodimers (e.g., bE1/bW2 and bE2/bW1; Gillissen et al. 1992; Kämper et al. 1995). These heterodimers then enter the diploid nucleus to activate the zinc finger transcription factor Rbf1 (Heimel et al. 2010), which in turn controls the switch from yeast-like to filamentous growth, the maintenance of the dikaryotic state, and the subsequent pathogenic and sexual (meiotic) development (Heimel et al. 2010; Wahl et al. 2010).

Importantly, heterodimers formed by HD1/HD2 transcription factors from the same MAT allele are not active in zygotic

differentiation. In addition, the bW and bE genes encoding these factors are tightly linked (~260 bp of each other) and thus inherited together. This avoids generating haploid progeny that would produce active bW1/bE2 heterodimers, which would switch on the diploid developmental program in haploid cells.

Mating types in smuts did not evolve to prevent selfing. Parasitic sporophytes produce diploid dispersing teliospores (e.g., the bipolar *Microbotryum violaceum*, cause of anther smut disease; Giraud et al. 2008). Meiosis occurs only after the spore has infected a new host, making sure that complementary mating types are found among the haploid yeasts resulting from a single dispersing spore. This mechanism evolved to promote selfing (which is actually the dominant type of mating in *M. violaceum*; Giraud et al. 2008), despite the need to display complementary mating types.

The homobasidiomycetes (exemplified, e.g., by *Coprinus cinereus*) are also mostly tetrapolar, with two independent MAT loci, one coding for the pheromone/receptor pathway (B), the other (A) for transcription factors. Interestingly, haploid mycelia may fuse and exchange nuclei independent of mating types. Pheromone stimulation (encoded by locus B) rather plays a role in organizing the growth of the dikaryon (nucleus signaling), ensuring that two nuclei of complementary mating types are present within each cell. This system organizes in particular clamp cell fusion, a process bearing similarity with the mating of two haploid gametes in *U. maydis* (Casselton and Olesnicky 1998).

The HD1 and HD2 transcription factors (encoded by locus A) play a role both as homodimers in the gametophyte (where they express mating-type specific genes and repress asexual sporulation; Tymon et al. 1992) and as heterodimers in the sporophyte, where they specifically repress the haploid program and initiate the diploid transcription program (development of the dikaryonic mycelium; Banham et al. 1995; Casselton and Olesnicky 1998; Spit et al. 1998).

In *Cryptococcus neoformans* (where a bipolar system emerged secondarily by linkage of the A and B MAT loci), mating occurs between mating types **a** and α. Experimentally produced α/α zygotes cannot proceed further to complete the dikaryon stage. This requires the heterodimeric complex formed by the complementary transcription factors Sxi1α (homeodomain HD1) and Sxi2a (homeodomain HD2). This transcription complex specifies the dikaryotic state by upregulating genes required for the development of dikaryotic filaments, basidia, and spores, and repressing mating-specific genes (in particular the pheromones MFa and MFα) to prevent additional fusion of dikaryons with haploids (Stanton et al. 2010).

Interestingly, this heterodimeric complex also controls mtDNA inheritance (Yan et al. 2007). Although both types initially transmit their mitochondria, the MAT**a** mtDNA is quickly eliminated a few hours after cell fusion. Disruption of Sxi2**a** results in biparental mtDNA inheritance. Similarly, progeny from artificially induced same-sex mating show biparental inheritance.

What Uses Are Mating Types?

As illustrated through several of the examples above, mating types did not evolve to control organelle inheritance. Such types also exist in organisms that do not practice cytoplasmic fusion (e.g., ciliates or filamentous ascomycetes, which control organelle transmission via sexes). Empirical correlations more likely arise because mating types (and sexes) were often co-opted secondarily to control organelle inheritance. Similarly, mating types did not evolve to avoid inbreeding or same-clone mating. Many lineages (including ciliates, yeasts, filamentous ascomycetes, or bipolar basidiomycetes) have evolved highly complex adaptations precisely to allow inbreeding or same-clone mating, despite the need to have mating types.

Expanding a seminal idea independently proposed by several authors, I suggest instead that mating types evolved primarily to trigger major developmental switches along the sexual cycle. This includes a ploidy-assessment function, mediated by complementary heterodimers of transcription factors, which allows sporophytic development and meiosis to be triggered in diploid cells only, not in haploids (and reciprocally, gametophytic development, gametogenesis, and mating to be triggered in haploid cells only, not in diploids). This also includes a mate-assessment function, mediated by complementary pheromones and receptors, which allows the mating program to be switched on when conspecific haploid cells are ready to do the same in the immediate environment (rather than being self-triggered). These successive switches may be controlled by a single MAT locus (like in many ciliates, algae, and ascomycetes), or by several independent loci, such as in some basidiomycetes (where the locus B controls proper development of the dikaryon and locus A subsequent karyogamy and meiosis) or acellular slime molds (where the locus matB controls cell fusion, and locus matA nuclear fusion and mitochondrial inheritance; Moriyama and Kawano 2010).

As correctly noted by Billiard et al. (2011), the main challenge to the present hypothesis is posed by homothallism (same-clone mating). If complementary molecules are required to trigger developmental switches, how could the fusion of genetically identical gametes produce a fully viable zygote? A first point to note is that seemingly homothallic systems sometimes turn out to be pseudohomothallic, as exemplified by filamentous ascomycetes, which manage to pack two nuclei of complementary mating types within the same spore.

Second, genetically identical gametes produced by the same clone might still express different mating types. This may arise from mating-type switches such as documented in yeasts, where

gene conversion at the mating-type locus organizes same-clone mating. Similarly, mating types in ciliates are determined by the macronucleus, which goes through series of epigenetic differentiation and maturation events allowing individuals from a same clone (i.e., with genetically identical micronuclei) to display complementary mating types. In most cases of homothallism, haploid cells have been shown to possess more than one mating types in their genome (Lin and Heitman 2007; Ramirez-Prado et al. 2008). Differential gene silencing in otherwise genetically identical gametes might thus allow expressing a variety of complementary mating types.

This might seem a plausible scenario in anisogamic species, where genetically identical cells undergo highly differentiated gametogeneses to develop into either male of female gametes. Many filamentous ascomycetes are homothallic (with ascogonia fertilized by microconidia from the same gametophyte). It might be more than a coincidence that ascomycetes also have sexes. The highly differentiated gametogenesis of microconidia and ascogonia should facilitate sex-specific imprinting of transcription factors. Differential silencing might suffice to generate the required complementarity (i.e., prevent the unwanted development of unfertilized ascogonia). A crucial test of the present model will come from investigations on the way developmental switches are controlled in homothallic lineages.

ACKNOWLEDGMENTS

I am grateful to Leo Beukeboom, Susana Coelho, Tatiana Giraud, David Haig, Laurent Keller, John Pannell, Claus Wedekind, and two anonymous referees for comments on a first version of this paper, as well as to the Swiss National Science Foundation for funding (grant 31003A- 129894).

LITERATURE CITED

Akada, R. 1985. Mating types and mating-inducing factors (gamones) in the ciliate *Euplotes patella* syngen 2. Genet. Res. 46:125–132.

Bakkeren, G., and J. W. Kronstad. 1994. Linkage of mating-type loci distinguishes bipolar from tetrapolar mating in basidiomycetous smut fungi. Proc. Natl. Acad. Sci. U. S. A. 91:7085–7089.

Banham A. H., R. N. Asante-Owusu, B. Göttgens, S. A. J. Thompson, C. S. Kingsnorth, E. J. C. Mellor, and L. A. Casselton. 1995. An N-terminal dimerization domain permits homeodomain proteins to choose compatible partner and initiate sexual development in the mushroom *Coprinus cinereus*. Plant Cell 7:773–783.

Banuett, F. 1995. Genetics of *Ustilago maydis*, a fungal pathogen that induces tumors in maize. Annu. Rev. Genet. 29:179–208.

Barsoum, E., P. Martinez, and S. U. Aström. 2010. α3, a transposable element that promotes host sexual reproduction. Genes Dev. 24:33–44.

Bell, G. 1978. The evolution of anisogamy. J. Theoret. Biol. 73:247–270.

Billiard, S., M. Lopez-Villaviciencio, B. Devier,M. E. Hood ,C. Fairhead , and T. Giraud. 2011. Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. Biol. Rev. 86: 421–442.

Bloomfield, G., J. Skelton, A. Ivens, Y. Tanaka, and R. R. Kay. 2010. Sex determination in the social amoeba *Dictyostelium discoideum*. Science 330:1533–1536.

Breton, S., H. Doucet Beaupré, D. T. Stewart, W. R. Hoeh, and P. U. Blier. 2007. The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? Trends Genet. 23:465–474.

Bulmer, M. G., and G. A. Parker. 2002. The evolution of anisogamy: a game-theoretic approach. Proc. R. Soc. B 269:2381–2388.

Casselton, L. A., and N. S. Olesnicky. 1998. Molecular genetics of mating recognition in basidiomycete fungi. Microbiol. Mol. Biol. Rev. 62:55–70.

Coppin, E., R. Debuchy, S. Arnaise, and M. Picard. 1997. Mating types and sexual development in filamentous ascomycetes. Microbiol. Mol. Biol. Rev. 61:411–428.

Derelle E., C. Ferraz, S. Rombauts, P. Rouze, A. Z. Worden, S. Robbens, F. Partensky, S. Degroeve, S. Echeynie, R. Cooke, *et al*. 2006. Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. Proc. Natl. Acad. Sci. U. S. A. 103:11647–11652.

Ebersold, W. T. 1967. *Chlamydomonas reinhardtii*: heterozygous diploid strains. Science 157:447–449.

Ezov, K. T., S. L. Chang, Z. Frenkel, A. V. Segre, M. Bahalul, A. W. Murray, J. Y. Leu, A. Korol, and Y. Kashi. 2010. Heterothallism in *Saccharomyces cerevisiae* isolates from mature: effect of HO locus on the mode of reproduction. Mol. Ecol. 19:121–131.

Fedler, M., K. S. Luh, K. Stelter, F. Nieto-Jacobo, and C. W. Basse. 2009. The a2 mating-type locus genes lga2 and rga2 direct uniparental mitochondrial DNA (mtDNA) inheritance and constrain mtDNA recombination during sexual development of *Ustilago maydis*. Genetics 181:847–860.

Ferris, P. J., and U. W. Goodenough. 1997. Mating type in *Chlamydomonas* is specified by *mid*, the minus-dominance gene. Genetics 146:859–869.

Ferris, P. J., J. P. Woessner, and U. W. Goodenough. 1996. A sex recognition glycoprotein is encoded by the plus mating type gen *fus1* of *Chlamydomonas reinhardtii*. Mol. Biol. Cell. 7:1235–1248.

Galgoczy, D. J., A. Cassidy-Stone, M. Llinas, S. M. O'Rourke, I. Herskowitz, J. L. DeRisi, and A. D. Johnson. 2004. Genomic dissection of the cell-type-specification circuit in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U. S. A. 101:18069–18074.

Galitski, T., A. J. Saldanha, C. A. Styles, E. S. Lander, and G. R. Fink. 1999. Ploidy regulation of gene expression. Science 285:251–254.

Galloway, R. E., and U. W. Goodenough. 1985. Genetic analysis of mating-locus linked mutations in *Chlamydomonas reinhardtii*. Genetics 111:447–461.

Gillissen, B., J. Bergemann, C. Sandmann, B. Schroeer, M. Bölker, and R. Kahmann. 1992. A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell 68:647–657.

Giraud, T., R. Yockteng, M. Lopez-Villavicencio, R. Refrégier, and M. E. Hood. 2008. Mating system of the anther smut fungus *Microbotryum violaceum*: Selfing under heterothallism. Eukaryot. Cell 7:765–775.

Goff, L. J., and A. W. Coleman. 1986. A novel pattern of apical cell polyploidy, sequential polyploidy reduction and intracellular nuclear transfer in the red alga *Polysiphonia*. Am. J. Bot. 73:1109–1130.

Goodenough, U. W., C. Hwang, and A. J. Warren. 1978. Sex-limited expression of gene loci controlling flagellar membrane agglutination in the *Chlamydomonas* mating reaction. Genetics 89:235–243.

Goodenough, U. W., H. Lin, and J.-H. Lee. 2007. Sex determination in *Chlamydomonas*. Semin. Cell Dev. Biol. 18:350–361.

Haag, E. S. 2007. Why two sexes? Sex determination in multicellular organisms and protistan mating types. Semin. Cell Dev. Biol. 18:348–349.

Haber, J. E. 2003. Mating-type gene switching in *Saccharomyces cerevisiae*. Annu. Rev. Genet. 32:561–599.

Haig, D. 1993. Alternatives to meiosis: the unusual genetics of red algae, microsporidia, and others. J. Theor. Biol. 163:15–31.

Heimel, K., M. Scherer, M. Vranes, R. Wahl, C. Pothiratana, D. Schuler, V. Vincon, F. Finkernagel, I. Flor-Parra, and J. Kämper. 2010. The

transcription factor Rbf1 is the master regulator for *b*-mating type controlled pathogenic development in *Ustilago maydis*. PLoS Pathog 6:e1001035.

Herskowitz, I. 1985. Master regulatory loci in yeast and lambda. Cold Spring Harb. Symp. Quant. Biol. 50:565–574.

Herskowitz, I. 1989. A regulatory hierarchy for cell specialization in yeast. Nature 342:749–757.

Hoekstra, R. F. 1982. On the asymmetry of sex: evolution of mating types in isogamous populations. J. Theor. Biol. 98:427–451.

Holmes, A. M., A. Kaykov, B. Arcangioli. 2005. Molecular and cellular dissection of mating-type switching in *Schizosaccharomyces pombe*. Mol. Cell. Biol. 25:303–311.

Hurst, L. D. 1995. Selfish genetic elements and their role in evolution: the evolution of sex and some of what that entails. Phil. Trans. R. Soc. Lond. B 349:321–332.

Hurst, L. D. 1996. Why are there only two sexes? Proc. R. Soc. Lond. B 263:415–422.

Hurst, L. D., and W. D. Hamilton. 1992. Cytoplasmic fusion and the nature of the sexes. Proc. R. Soc. B 247: 189–194.

Kämper, J., M. Reichmann, T. Romeis, M. Bölker, and R. Kahmann. 1995. Multiallelic recognition: nonself-dependent dimerization of the bE and bW homeodomain proteins in *Ustilago maydis*. Cell 81:73–83.

Kües, U., and L. A. Casselton. 1993. The origin of multiple mating types in mushrooms. J. Cell. Sci. 104:227–230.

Kurvari, V., N. V. Grishin, and W. J. Snell. 1998. A gamete-specific, sex-limited homeodomain protein in *Chlamydomonas*. J. Cell Biol. 143:1971–1980.

Lee, S. C., M. Ni, C. Shertz, and J. Heitman. 2010. The evolution of sex: a perspective from the fungal kingdom. Microbiol. Mol. Biol. Rev. 74:298–340.

Lengeler, K. B., D. S. Fox, J. A. Fraser, A. Allen, K. Forrester, F. S. Dietrich, J. Heitman. 2002. Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. Eukaryot. Cell 1:704–718.

Lin, X., and J. Heitman. 2007. Mechanisms of homothallism in fungi and transitions between heterothallism and homothallism. Pp. 35–57 *in* J. Heitman, J. W. Kronstad, J. W. Taylor, and L. A. Casselton, eds. Sex in fungi: molecular determination and evolutionary implications. ASM Press, Washington, DC.

Miller, M. G., and A. D. Johnson. 2002. White-opaque switching in *Candida albicans* is controlled by mating-type homeodomain proteins and allows efficient mating. Cell 110:293–302.

Moriyama, Y., and S. Kawano. 2010. Maternal inheritance of mitochondria: multipolarity, multiallelism and hierarchical transmission of mitochondrial DNA in the true slime mold *Physarum polycephalum*. J. Plant Res. 123:139–148.

Nozaki A. 2008. A new male-specific gene " *OTOKOGI* " in *Pleodorina starrii* (Volvocaceae, Chlorophyta) unveils the origin of male and female. Biologia 63:772–777.

Nozaki, H., T. Mori, O. Misumi, S. Matsunaga, and T. Kuroiwa. 2006. Males evolved from the dominant isogametic mating type. Curr. Biol. 16:R1018–R1020.

Parker, G. A., R. R. Baker, V. G. F. Smith. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. J. Theor. Biol. 36:181–198.

Peters, A. F., D. Scornet, D. G. Mueller, B. Kloareg, and J. M. Cock. 2004. Inheritance of organelles in artificial hybrids of the isogamous multicellular chromist alga *Ectocarpus siliculosus* (Phaeophycaea). Eur. J. Phycol. 39:235–242.

Phadke, S. S., and R. A. Zufall. 2009. Rapid diversification of mating systems in ciliates. Biol. J. Linn Soc. 98:187–197.

Preer, J. R. 2000. Epigenetic mechanisms affecting macronuclear development in *Paramecium* and *Tetrahymena*. J. Eukaryot. Microbiol. 47: 515–524.

Puhalla, J. E. 1970. Genetic studies of the b incompatibility locus of *Ustilago maydis*. Genet. Res. 16:229–232.

Ramesh, M. A., S. B. Malik, and J. M. Logsdon. 2005. A phylogenetic inventory of meotic genes; evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. Curr. Biol. 15:185–191.

Ramirez-Prado, J. H., G. G. Moore, B. W. Horn, and I. Carbone. 2008. Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet. Biol. 45:1292–1299.

Raper, J. R., G. S. Kronbelg, and M. G. Baxter. 1958. The number and distribution of incompatibility factors in *Schizophyllum commune*. Am. Nat. 92:221–232.

Scheer, M. A., and M. E. Siliker. 2006. Mitochondrial inheritance patterns in *Didymium iridis* are not influenced by stage of mating competency. Mycologia 98:51–56

Silliker, M. A., J. L. Liles, and J. A. Monroe. 2002. Patterns of mitochondrial inheritance in the myxogastrid, *Didymium iridis*. Mycologia 94:939–946.

Spit, A., R. Hyland, E. J. C. Mellor, and L. A. Casselton. 1998. A role for heterodimerization in nuclear localization of a homeodomain protein. PNAS 95:6228–6233.

Stanton, B. C., S. S. Giles, M. W. Staudt, E. K. Kruzel, and C. M. Hull. 2010. Allelic exchange of pheromones and their receptors reprograms sexual identity in *Cryptococcus neoformans*. PLoS Genet. 6:e1000860.

Takano, H., K. Onoue, and S. Kawano. 2010. Mitochondrial fusion and inheritance of the mitochondrial genome. J. Plant Res. 123:131–138.

Tymon, A. M., U. Kues, W. V. J. Richardson, and L. A. Casselton. 1992. A fungal mating type protein that regulates sexual and asexual development contains a POU—related domain. EMBO J. 11:1805–1813.

Vallesi, A., P. Ballarini, B. di Pretoro, C. Alimenti, C. Miceli, and P. Luporini. 2005. Autocrine, mitogenic pheromone receptor loop of the ciliate *Euplotes raikovi*: pheromone-induced receptor internalization. Eukaryot. Cell 4:1221–1227.

van der Meer, J. P., and E. R. Todd, E. R. 1977. Genetics of *Gracilaria* sp. (Rhodophyceae, Gigartinales) IV. Mitotic recombination and its relationship to mixed phases in the life history. Can. J. Bot. 55:2810–2817.

Wahl, R., A. Zahiri, and J. Kämper. 2010. The *Ustilago maydis* b mating type locus controls hyphal proliferation and expression of secreted virulence factors in planta. Mol. Microbiol. 75:208–220.

Wu, X., and J. E. Haber. 1996. A 700bp *cis*-acting region controls mating-type dependent recombination along the entire left arm of yeast chromosome III. Cell 87:277–285.

Xu, J. 2005. The inheritance of organelle genes and genomes: patterns and mechanisms. Genome 48:951–958.

Yan, Z., C. M. Hull, S. Sun, J. Heitman, and J. Xu. 2007. The mating type-specific homeodomain genes Sxi1α and Sxi2a coordinately control uniparental mitochondrial inheritance in *Cryptococcus neoformans*. Curr. Genet. 51:187–195.

Zhao, H., M. Lu, R. Singh, and W. J. Snell. 2001. Ectopic expression of a *Chlamydomonas* mt^+-specific homeodomain protein in mt^- gametes initiates zygote development without gamete fusion. Genes Dev. 15:2767–2777.

Associate Editor: S. West

Appendix: How Many Mating Types?

If mating types cannot be avoided, then their numbers should be increased whenever mating partners are limiting. Selection should

be strongest if mobility is restricted by low dispersal ability or high habitat structure. However, adding a third mating type to a dual system might not be an easy task. The new mating type has to display hormones that activate all preexisting receptors but not self, and receptors that react to all preexisting pheromones but not self. Similarly, the new transcription factors should function as active heterodimers with all preexisting ones, but not self. As put by Haag (2007), the biochemical problem of making specific, functional heterodimers becomes increasingly difficult with increasing numbers of mating types.

This problem can be exemplified by the cellular slime mold *Dictyostelium discoideum*, which displays three mating types. The MAT locus of mating types I and III (thought to be ancestral) harbor a single gene each (respectively, *matA* and *matS*), encoding small hydrophilic proteins. The matA and matS proteins are nonhomolog (idiomorphs), and must interact to allow mating and subsequent formation of a diploid resistant zygote (macrocyst). The new mating type II apparently emerged by capturing a copy of both *matA* (from type I) and *matS* (from type III). Its matB protein (homolog of matA) interacts with matS when mating with type III, and its matC protein (homolog of matS) interacts with matA when mating with type I. Importantly, matB and matC are sufficiently diverged from their original copies (matA and matS) that they cannot interact to foster zygotic development and macrocyst development in haploid type II cells (Bloomfield et al. 2010). Such evolutionary constraints (need to distinguish self from nonself) might however be alleviated if the two interacting molecules (pheromone and receptor) are produced by the alternative splicing of a single gene, as occurs in *Euplotes* (Vallesi et al. 2005).

An additional difficulty is that of controlling organelle transmission whenever mating types were co-opted for this function. A classical example is provided by the true (acellular) slime mold *Physarum polycephalum*. Cell fusion is decided by the MatB locus, with 15 alleles known (the two haploid cells must differ at this locus to mate). After cell fusion, nuclear fusion only occurs if the two nuclei also differ at their matA allele (16 alleles known); otherwise, cells separate again and go their own way. If (and only if) nuclear fusion occurs, all mitochondria from one parent will be selectively digested. Which parent transmits its mitochondria to the plasmodium is also decided by alleles at the matA locus, which establish a linear hierarchy ("pecking order") among haplotypes. The exact mechanisms, however, are unknown, and apparently not perfect, resulting in paternal leakage or biparental transmission in some crosses (Moriyama and Kawano 2010).

Increasing the numbers of mating types was achieved independently in many groups, through a diversity of mechanisms. This may be simply achieved by increasing the number of alleles at one locus, as exemplified in cellular and acellular slime molds mentioned here and above. This strategy is also practiced by ciliates with synclonal mating type determination. The 10 mating types of *Euplotes patella* are determined by four codominant alleles at the MAT locus (i.e., four mating types are homozygous at this locus and six are heterozygous; Akada 1985). An alternative strategy was adopted by ciliates with cytoplasmic or caryonidal mating type determination (such as *Tetrahymena*), which capitalize on differential epigenetics of the macronucleus to generate different mating types in genetically identical clones (Preer 2000).

Basidiomycetes achieved similar results by relying on functional redundancy (Casselton and Olesnicky 1998). Karyogamy and meiosis in this group is controlled at locus A (active heterodimers must be formed by combining an HD1 transcription factor from one partner with an HD2 factor from the other). The potential for compatibility was multiplied by several gene duplications, each gene copy encoding one HD1 and one HD2 factor. It is enough for the interacting gametophytes to be compatible at one copy only to produce active HD1/HD2 heterodimers. This allows *Coprinus cinereus*, for instance, to display as much as 160 mating types at it's A locus (Kües and Casselton 1993), and up to 288 in *Schizophyllum commune* (Raper et al. 1958).

This is in sharp contrast with yeasts, filamentous ascomycetes, or bipolar basidiomycetes, which mostly present two mating types only (as do some Ciliates). However, multiplying the numbers of mating types might be less (or even not) required in lineages that evolved competences for same-clone mating (as allowed by mating-type switches in yeasts), same-spore mating (as in pseudohomothallic filamentous ascomycetes or bipolar hemibasidiomycetes), or autogamy (which evolved in Ciliates as an alternative to mating-type multiplication; Phadke and Zufall 2009).