Pondering Mating: *Pneumocystis jirovecii*, the Human Lung Pathogen, Selfs without Mating Type Switching, in Contrast to Its Close Relative *Schizosaccharomyces pombe*

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Almeida et al. (1) have interrogated the genomes of two *Pneumocystis* species, *Pneumocystis jirovecii* and its sister *Pneumocystis carinii*, for genes known to be involved in sexual reproduction in the widely studied fission yeast *Schizosaccharomyces pombe*, with the hope that defining genetic pathways governing sexual reproduction in *Pneumocystis* will inform disease prevention strategies. *Pneumocystis* spp. cause host-specific lung infections in mammals, and sexual reproductive propagules appear to be the infectious stage of the life cycle (2). *P. jirovecii*, a genetically intractable obligate human pathogen, causes pneumonia in immunosuppressed individuals, with an estimated 400,000 life-threatening infections reported annually worldwide and a mortality rate of up to 80% (3). *P. carinii* inhabits the lungs of rats (4).

P. carinii and relatives were long thought to be protozoan parasites until molecular phylogenetic analysis (1988) clearly placed them within the ascomycetes (5, 6), together with baker's yeast (*Saccharomyces cerevisiae*), the human pathogen *Candida albicans* (in the subphylum *Saccharomycotina*), the human pathogen *Coccidioides immitis*, pricey European truffles (*Tuber* spp.) and morels (*Morchella* spp.), and familiar contemporary genetic models, such as the saprobes *Neurospora* spp. and the destructive cereal pathogens *Cochliobolus heterostrophus* and *Fusarium graminearum* (all *Pezizomycotina*). Although in the same phylum, *Pneumocystis* is only distantly related to these other fungi. In fact, it is associated with a diverse group of ancient lineages at the base of the ascomycete phylogenetic tree collectively known as the *Taphrinomycotina* (7, 8). The *Taphrinomycotina* include, in addition to the *Pneumocystis* mammalian pathogens, *Taphrina deformans*, a dimorphic plant pathogen that causes leaf curl disease of peach, and *S. pombe*, used in the fermentation of millet beer and a genetic model second only to *S. cerevisiae* (9, 10). Molecular requirements for *S. pombe* sexual reproduction were elucidated more than 25 years ago (11).

Unlike *S. pombe* and *T. deformans*, *Pneumocystis* species are obligate pathogens and thus cannot be cultured. This element complicates the study of *Pneumocystis* biology, including its possible sexual cycle, and is challenging from a clinical perspective, because sex is thought to play a crucial role in the survival of *Pneumocystis*. Only the cysts, which are considered to be asci containing the sexual spores, are infectious and able to spread to new hosts (2). Despite the crucial potential importance of sex to the epidemiology of *Pneumocystis* pneumonia, little is known about molecular mechanisms associated with this developmental pathway in *Pneumocystis*. Earlier studies hinting at a sexual lifestyle include a report on the possible observation of synaptonemal complexes (12), a report identifying conserved mating and meiotic genes that are functional when heterologously expressed in *S. pombe* mutants (13), and evidence that the meiotic recombinase Dmc1 is expressed in cysts (14). The study by Almeida et al. (1) offers significant insight into the mechanism by which sexual reproduction might occur in *Pneumocystis*.

Almeida et al. (1) queried genome sequences of *P. jirovecii*, *P. carinii*, and their relative *T. deformans* with genes known to be involved in sexual reproduction in *S. pombe* and identified candidate homologs. Mating in *S. pombe* is controlled by the single mating type locus *mat1* and is successful when strains of opposite mating type, designated P and M, pair. P and M cells differ in gene content at *mat1* (15, 16). Furthermore, as with the budding yeast, *S. cerevisiae* (17, 18), *S. pombe* has, in addition to the active *mat1* mating type locus, two linked but silent mating type loci, one containing the P and the other the M gene content. By programmed interconversion, one of the silent copies can change places with the active copy at the *mat1* locus, leading to "switching" of cell type. Thus, homothallism in both yeasts refers to a change in mating type in some of the cells within a culture of a formerly uniform mating type, followed by mating of "switched" cells with "unswitched" cells within the culture, culminating in the production of sexual spores. This type of homothallism with mating type switching has not been described in *Pezizomycotina* to date.

Given that *Pneumocystis* is related to *S. pombe*, one might expect these fungi to have similar mating systems, but this is not what Almeida et al. (1) found. Instead, they detected a single mating type locus in the two *Pneumocystis* species, one or two loci in *T. deformans* (short contig sequences make linkage uncertain), and no silent loci (Fig. 1). This configuration indicates that these fungi are unable to switch mating type using an *S. pombe*-type mechanism. Also, the *Pneumocystis* and *Taphrina* mating type loci contain both P and M mating type genes, an arrangement, denoted as primary homothallism, known to enable selfing in *Pezizomycotina*. Where it has been examined carefully in *Pezizomycotina*, all instances of primary homothallism arose from a genetic recombination event (and loss in some cases) between heterothallic relatives.

Evidence for primary homothallism is new to the *Taphrinomycotina*, but homothallism was inferred previously in population genetics studies which demonstrated widespread clonality in *P. jirovecii* (19, 20). As noted above, primary homothallism has been

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observed in the largest group of ascomycetes, the *Pezizomycotina*. Examples include the mostly saprobic, but sometimes opportunistic, human pathogens *Aspergillus nidulans* (21, 22) and *Neosartorya fischeri* (23, 24) and various plant pathogens and saprobes. One of the best-studied examples is *Cochliobolus* spp. All heterothallic *Cochliobolus* species have a single mating type locus (*MAT1*), with a single gene, either *MAT1-1* or *MAT1-2*, and only isolates that differ at *MAT1* are able to mate (Fig. 1). Like *Pneumocystis*, the primary homothallic *Cochliobolus* species have both mating type genes in their genomes, generally arranged side by side at a single locus (25). Functional analyses involving swapping of heterothallic for homothallic *MAT* genes and of homothallic for heterothallic *MAT* genes demonstrate that mating lifestyle can be altered by an exchange of *MAT* genes. Heterothallic *C. heterostrophus* can be rendered homothallic by introduction of the homothallic *Cochliobolus luttrellii MAT* genes, and homothallic *C. luttrellii* can be rendered heterothallic by introduction of the heterothallic *C. heterostrophus MAT* genes (25, 26). We note that homothallism without switching using silent mating type cassettes has also been described in the *Saccharomycotina*. Examples include strains of predominantly heterothallic *C. albicans* that become capable of self-mating through alterations in pheromone signaling (27) and the recently described novel switching mechanism in *Hansenula polymorpha*, in which only one of two linked *MAT1* and *MAT2* genes is expressed in a single nucleus (28, 29). Homothallism in *H. polymorpha* is achieved by a chromosomal inversion of the *MAT* region.

How do primary homothallic fungi evolve? The origin of the mating type gene arrangement in *Pneumocystis* and *Taphrina* is unknown, because these are the first and only *MAT* configurations described, but there is evidence in the *Pezizomycotina* that primary homothallic species originated from heterothallic ancestors by means of recombination between DNA motifs shared between opposite mating type alleles (25, 30–33). Opposite mating type alleles differ in DNA sequence, but when, for example, both *MAT1-1* and *MAT1-2* of heterothallic *C. heterostrophus* are aligned with the fused *MAT1-1*/*MAT1-2* sequence of primary homothallic *C. luttrellii*, all sequences are identical across an 8-nucleotide stretch that, in *C. luttrellii*, is located at the fusion junctions between opposite mating type alleles. This suggests that recombination between *MAT* genes of a *Cochliobolus* heterothallic ancestor resulted in the fused mating type arrangement found in *C. luttrellii* today. For some primary homothallic representatives, no recombination sites have been identified, and in some, only one *MAT* gene is present (e.g., *Neurospora africana* has only *matA* [*MAT1*], while *Huntiella moniliformis* has only *MAT2*) (30, 34–41). In the genomes of other primary homothallic ascomycetes and possibly in *T. deformans*, the opposite mating type alleles are unlinked. This configuration can be explained by hypothesizing that heterothallic *MAT* genes are first linked by recombination and then rendered unlinked via a double-strand break between the linked *MAT* genes and a chromosomal translocation event. Examples include *A. nidulans* (22), *N. fischeri* (24), and possibly one species of *Cochliobolus* (25).

How the primary homothallic mating type arrangement in *Pneumocystis* evolved is unknown. The P and M *mat1* genes are present on the same *Pneumocystis* chromosome; thus, recombination between the *mat* genes in an as-yet-undiscovered heterothallic ancestor is the most likely mechanistic scenario. It is curious, however, that the *Pneumocystis* mating type genes are more closely

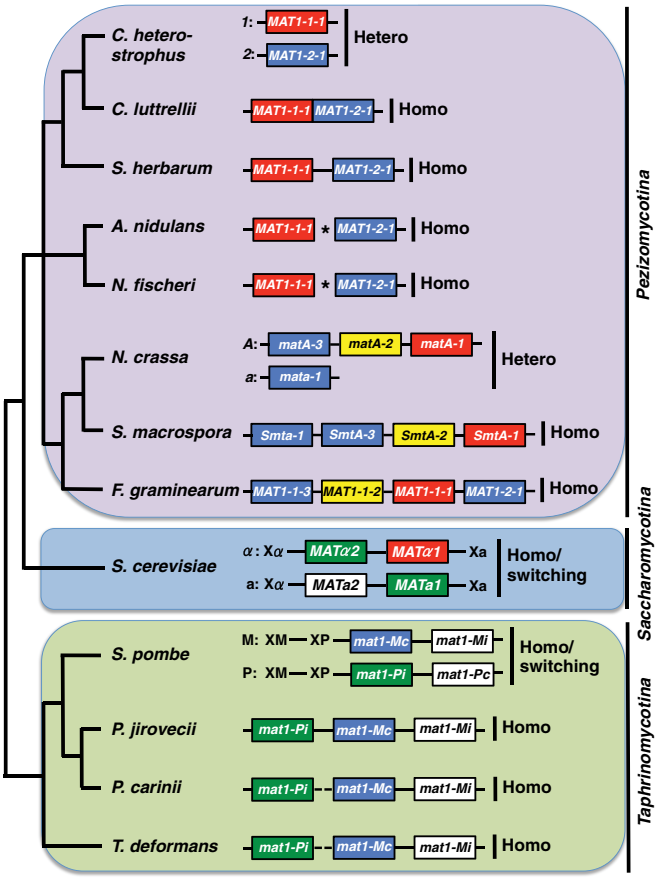

FIG 1 Mating type locus organization in select species mentioned in the text. The phylogenetic relationship of the species is given on the left, and mating type arrangements are on the right. Boxes correspond to mating type genes, and gene names are within the boxes. Red boxes encode alpha1 (α1) domain proteins, blue boxes high-mobility-group (HMG) box proteins, yellow boxes amphipathic alpha-helix proteins, green boxes homeobox proteins, and white boxes proteins with other or unknown domains. An "X" signifies a silent mating type locus. Horizontal lines between the boxes correspond to noncoding regions or non-mating type genes. Dashed lines indicate unknown DNA sequence, and an asterisk between boxes means that mating type genes are unlinked. For species with more than one mating type allele, both alleles, including allele designations, are provided. Gene diagrams are not to scale. "Hetero" stands for heterothallic, "Homo" for primary homothallic, and "Homo/switching" for homothallic by switching. Ascomycete subphyla are indicated by vertical lines on the right. For references, see the text. The phylogenetic topology is based on the work of Schoch et al. (42). *S. herbarum*, *Stemphylium herbarum*; *S. macrospora*, *Sordaria macrospora*; *N. crassa*, *Neurospora crassa*.

related to their homologs in *T. deformans* than to homologs in *S. pombe* (1) (Fig. 1), because *Pneumocystis* is more closely related to *S. pombe* than to *T. deformans* (7, 10). This suggests that the mating type arrangement of *Pneumocystis* and *T. deformans* may have evolved following the separation of these two lineages and then was transferred horizontally from one lineage to the other, as demonstrated for *Stemphylium MAT* genes (30). Alternatively, the mating type arrangement of *Pneumocystis* may have evolved before the separation of the *Taphrinomycotina* lineages. The *S. pombe* silent mating type cassettes used to effect switching and homothallism may have been acquired later (Fig. 1).

In conclusion, the evidence generated by Almeida et al. (1) and

the body of genetic and phylogenetic evidence from the study of other ascomycete species, strongly suggest that the examined *Pneumocystis* species are primary homothallic species. Whether this is the case for all *Pneumocystis* species and how primary homothallism evolved in *Pneumocystis* and the *Taphrinomycotina*, in general, require additional studies. Given the clinical importance of *Pneumocystis* and the plant-pathogenic nature of *Taphrina*, molecular understanding of their reproductive strategies and evolutionary trajectory may have substantial practical implications.

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