Chapter 8

Evolution of Human-Pathogenic Fungi: Phylogenies and Species

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Not many of us open medical mycology texts to the chapter on systematics; even I am attracted first to the lurid photos of exotic diseases and the morbid fascination that they provoke. But, sooner or later, we must confront fungal evolution to answer a fundamental question, "Which fungus is distressing my patient?" Simply knowing that a fungus is responsible is not enough, because treatment differs dramatically for different fungi. Neither is knowledge of a few famous pathogenic fungi enough, because today so many fungi can be a threat to the immunodeficient patient. In modern medicine there is no substitute for a broad knowledge of fungi, and the knowledge must go beyond simply knowing the names of fungi. The evolutionary relationships also must be considered because only a few medically important fungi have been thoroughly investigated, and one must often make inferences about the behavior of poorly studied fungi from their better-studied relatives. Obviously, the inference is only as valuable as the evolutionary relationship is accurate.

Our knowledge of the pattern of evolution in fungi has increased dramatically over the past 15 years due to (i) the need to know more about fungal evolution, which was created by the increase in the number of immunodeficient humans owing to the tragedy of AIDS and the promise of organ and bone marrow transplantation, (ii) the spread of cladistic theory (81), and (iii) the technical advances of PCR amplification of DNA (158, 212) and automated DNA sequencing.

The expansion of the population of immunodeficient humans has allowed many fungi to become human pathogens. There are 399 species listed in the *Atlas of Clincal Fungi* (34), and more are reported every month; however, in the interest of brevity, only the 10% that are most commonly encountered as agents of mycoses are considered here (Table 1).

Evolutionary studies of fungi have proceeded at both ends of the phylogenetic tree, i.e., the extremely old speciation events that mark the deep divergences joining the major fungal clades and the recent speciation events that give rise to new species. The deeper divergences are becoming better known as more genes are used (117), but it is proving easier to define major clades than to understand the exact order in which they branch (156). Phylogenetics based on nucleic acid variation is blind to the sexual habits of fungi, and so both meiosporic and mitosporic fungi are treated together and the best-known name, whether it applies to the mitosporic state (anamorph) or the meiosporic state (teleomorph), is used here. Coming forward in time to the most recent divergences at the tips of phylogenetic trees, one finds many new fungal phylogenetic species, whose recognition by concordance of gene genealogies (Fig. 1) was pioneered for fungi with human pathogens (192–194).

A new epidemiological approach, multilocus sequence typing (MLST), has been associated mostly with pathogenic bacteria (120), but there are many fungal examples (191) and none is more fully developed than that for *Penicillium marneffei* (44). MLST has the potential to be the approach that brings together philosophically and geographically disparate research groups working on the same fungus. Some modification of MLST may be needed for fungi, where sequence of housekeeping genes may lack the needed variation; microsatellites, for example, may prove more useful and lead to multilocus microsatellite typing (MLMT) (44, 191).

The studies of species and populations tell us which taxa and genetically differentiated groups are worth identifying, but clinicians still must make the identifications. At the present, it seems as if every research group has devised its own method and only

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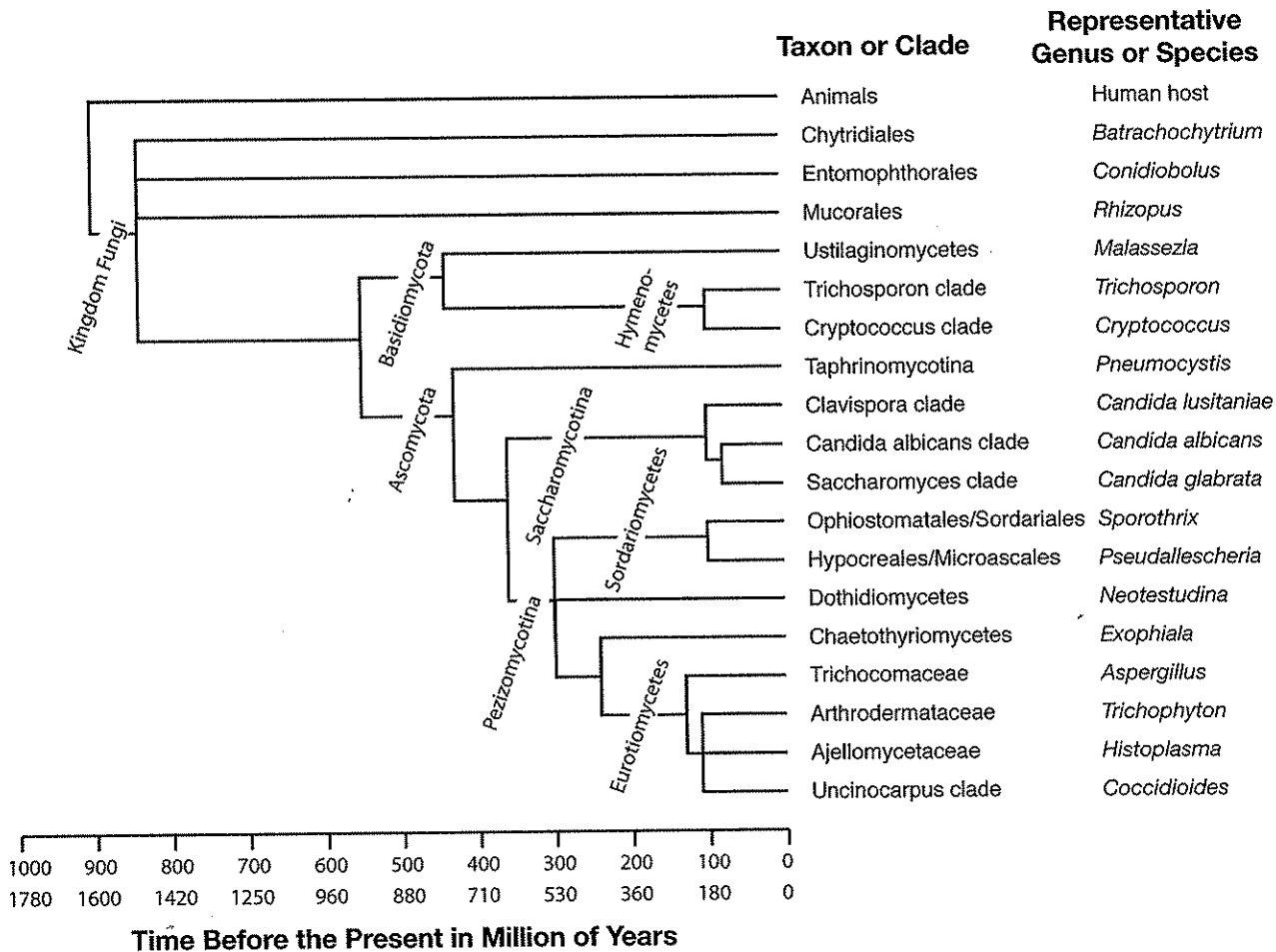
Figure 2. Phylogenetic tree of fungi pathogenic to humans or animals fit to geologic time. A maximum-likelihood phylogeny with an enforced molecular clock based on small-subunit rDNA sequence was used to estimate divergence times for diverse fungi (9), and to this phylogeny were added taxa of commonly encountered pathogenic fungi. Two geologic timescales are used, one reflecting a divergence between animals and fungi at 900 MYA (9) and the other reflecting a divergence at 1,600 MYA (79). Estimates of divergence times are subject to considerable uncertainty, the least of which is due to differences in the estimated time of divergence between animals and fungi (92).

provide a key tool to unraveling the evolution of fungi and their virulence.

Speaking of virulence, what better place to begin than with the Onygenales, the fungal group that has most of the bad actors, those fungi capable of causing systemic mycoses in otherwise healthy individuals. From there, we will work outward at increasing phylogenetic distances, moving through other groups of Ascomycota, then to Basidiomycota, and ending with the earliest diverging fungal groups, Zygomycota and Chytridiomycota (Fig. 2).

PHYLUM ASCOMYCOTA

Two of every three described fungi are members of the Ascomycota, which is divided into three principal groups, all of which harbor pathogens: Taphrinomycotina, Saccharomycotina, and Pezizomycotina.

In Pezizomycotina, pathogens are found in Eurotiomycetes, Sordariomycetes, Chaetothyriomycetes, and Dothidiomycetes. In Eurotiomycetes, pathogens are found in Eurotiales and Onygenales (181). The Onygenales contains the Trichocomaceae, Arthrodermataceae, and our starting place, the Ajellomycetaceae and the Uncinocarpus clade.

Subphylum Pezizomycotina
Class Eurotiomycetes
ORDER ONYGENALES

The fungi that cause systemic disease in otherwise healthy humans are found in the Onygenales and are classified in the genera *Coccidioides*, *Histoplasma*, *Blastomyces*, *Paracoccidioides*, and *Lacazia* (11, 15, 83, 145, 146). The Ajellomycetaceae (65, 69, 203) comprises the last four genera, along with *Emmonsia* species, one of which has been reported to cause disease in immunodeficient

Table 1. Fungi and other agents of the most common human mycoses

Fungi
Ascomycota
  Pezizomycotina
    Eurotiomycetes
      Onygenales
        Uncinocarpus clade
          Coccidioides immitis
          Coccidioides posadasii
        Ajellomycetaceae
          Histoplasma capsulatum
          Blastomyces dermatitidis
          Paracoccidioides brasiliensis
          Lacazia loboi
        Arthromdermataceae
          Epidermophyton floccosum
          Trichophyton rubrum
          Trichophyton interdigitale
          Trichophyton schoenleinii
          Trichophyton tonsurans
          Trichophyton violaceum
          Microsporum audouinii
          Microsporum canis
    Chaetothyriomycetes
      Herpotrichiellaceae
        Exophiala jeanselmei
        Exophiala dermatitidis
        Cladophialophora bantiana
        Cladophialophora carrionii
        Phialophora verrucosa
        Phialophora americana
        Fonsecaea pedrosii
    Dothidiomycetes
      Dothidiales
        Hortea werneckii
        Neotestudina rosatii
      Pleosporales
        Madurella grisea
    Sordariomycetes
      Sordariales
        Madurella mycetomatis
      Ophiostomatales
        Sporothrix schenckii
      Microascales
        Pseudallescheria boydii
        Scedosporium prolificans
      Hypocreales
        Fusarium solani
        Fusarium oxysporum
  Saccharomycotina
    Candida albicans clade
      Candida albicans
      Candida dubliniensis
    Saccharomyces clade
      Candada glabrata
      Candida krusei
      Candida tropicalis
    Clavispora-Metchnikowia clade
      Clavispora lusitaniae
  Taphrinomycotina
    Pneumocystis jiroveci

Continues

Table 1. *Continued*

Basidiomycota
  Hymenomycetes
      Cryptococcus neoformans
      Trichosporon asahii
      Trichosporon inkin
      Trichosporon mucoides
      Trichosporon cutaneum
      Trichosporon ovoide
  Ustilaginomycetes
      Malassezia furfur
Zygomycota
  Mucorales
      Rhizopus oryzae
  Entomophthorales
      Conidiobolus coronatus
Chytridiomycota
  Chytridiales
      Batrachochytrium dendrobatidis

Stramenopila
Oomycota
      Pythium insidiosum

Mezomycetozoea
Dermocystidia
      Rhinosporidium seeberi

its members can implement it (mea culpa). A global solution to the problem of clinical identification, at least using DNA variation, may be at hand thanks to microarray technology. Researchers working with bacteria have used ribosomal DNA (rDNA) to identify species collected in air samples (214), and a system for fungi involving a sufficiently variable region that is easily PCR amplified, perhaps the internal transcribed spacer (ITS) of the rDNA, could provide rapid identification using one or a few sets of PCR primers. Once the fungus was identified, and cultures were available, then MLST or MLMT could follow.

As noted at the outset, the field of fungal evolution has made tremendous strides in the past 15 years, but bigger challenges and opportunities await mycologists in the form of genomics, where the comparative approach so familiar to evolutionary biologists will
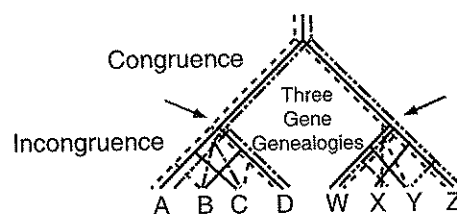

Figure 1. Phylogenetic species recognition by congruence of gene genealogies. Within recombining species, different genes have conflicting genealogies. Between genetically isolated species, drift and selection lead to fixation of ancestral polymorphism and congruence of genealogies. The point where congruence gives way to conflict is the point where species are recognized (arrows). Reprinted from reference 194 with permission.

humans (39). Nearby is the Uncinocarpus clade, home to *Coccidioides* species (15, 145, 168).

The most infamous member of the group is *Coccidioides immitis*, which was the only human pathogenic fungus to be considered a "select agent of terrorism" by an act of the United States Congress. The first MLST study of fungi showed that this fungus harbored two phylogenetic species, *C. immitis* in California and northern Mexico and *C. posadasii* in the rest of the range of this New World endemic fungus (48, 99). Easily recognized phenotypic differences between the two species were not initially apparent; however, subsequent research has discovered that only *C. immitis* can make hyphae at 37°C (M. Orbach, personal communication). The *Coccidioides* species also were the first fungi subjected to MLMT (Color Plate 5), which was used to determine that there were at least two genetically differentiated populations in *C. immitis* and at least three in *C. posadasii* (46, 47). Although coccidioidomycosis is acquired only from the environment, the distribution of microsatellite genotypes in South America, Mexico, and Texas suggests that the South American populations represent a relatively recent introduction (8,000 to 130,000 years ago), perhaps aided by human migration (47). The database of nine microsatellites typed for 167 individuals permits the assignment of newly collected isolates (whether obtained from clinics or the environment [62]) to species and to population (50). Given that the two species diverged as many as 10 million years ago (MYA), many loci can be used for species identification (12). Although *Coccidioides* species are mitosporic, population genetic tests of association of alleles among loci in both species have supported a significant role for recombination (18, 49).

Family Ajellomycetaceae. Nearly as infamous as *Coccidioides* is the genus *Histoplasma*. MLST analysis of 130 individuals from the three traditional varieties, *H. capsulatum* var. *capsulatum*, *H. capsulatum* var. *duboisii*, and *H. capsulatum* var. *farciminosum*, showed that there are at least eight species in the genus and that the present concept of varieties is insupportable (90, 91). For example, the African clade of *Histoplasma* contains fungi that both do and do not show the disease phenotype of *H. capsulatum* var. *duboisii*, and in *H. capsulatum* var. *farciminosum* the isolates collected from horses and related mammals have arisen from three other genetically isolated clades (91). Most of the isolates received as *H. capsulatum* var. *farciminosum* were European or Asian and had identical genotypes, a finding that is consistent with their production of skin lesions and associated transmission from animal to animal. In fact, the entire Eurasian clade emerges from within the large, tropical South American clade and therefore has originated more recently than the older radiation of the other species, some 3 to 13 MYA (91). As with *C. posadasii*, perhaps humans were involved in the transport of *Histoplasma*, but in this case it was a migration out of Latin America to Europe. Humans are not the only agents of long-distance dispersal in *Histoplasma*; bats certainly are involved and may have helped spread members of the Latin American clades to Mexico and vice versa (196, 197). Microsatellites have been discovered for *Histoplasma* and used to show genetic differentiation within one of two North American clades as well as between North and South American clades (21). As would be expected for a fungus that makes both mitospores and meiospores (*Ajellomyces capsulatus* is the meiosporic state) (102, 103), population genetic analysis of MLST data has supported significant recombination (90).

The third North American member of this group is *Blastomyces dermatitidis*, the mitosporic state of the meiosporic fungus *Ajellomyces dermatitidis*. A population genetic study of 59 isolates from North America, India, and Africa, using restriction fragment length polymorphisms (RFLPs) of the rDNA region and PCR fingerprinting, found that there were genetically isolated groups within the species and that genetic differentiation could be associated with geographic distances as short as that between the northern and southern United States of America (125). Although the fungus is capable of sexual reproduction (126), it also was found that the same genotype could predominate at one geographic locale, presumably through clonal spread (125).

Although *Coccidioides* and *Histoplasma* are found in Latin America, the most important mycotic species in this continent is *Paracoccidioides brasiliensis* (160). Calcagno et al. (19) used randomly amplified polymorphic DNAs (RAPDs) to show that there was genetic variation in this species and that the variation seemed to correlate with the geographic origin of the isolates. One interesting environmental reservoir for the species is the armadillo, and molecular data have been used to confirm the identity of isolates from these animals (7). A very recent MLST study has found what appear to be three phylogenetic species in *Paracoccidioides*, and two of them are sympatric (123a). Additionally, microsatellite markers have been described and used to ask if clinical isolates are genetically distinct from environmental isolates; as it turned out, they are not (137). The microsatellite markers certainly will be useful in future population genetic studies of this fungus from every corner of South America.

Rounding out the group is *Lacazia loboi*, an enigmatic pathogen if ever there was one (189). It causes infections of humans and freshwater dolphins (78) and cannot be cultivated. DNA of *L. loboi* is unstable in clinical samples, and only rapid freezing in liquid nitrogen permitted the recovery of sufficient DNA for phylogenetic studies (83). The first case of mycosis caused by *L. loboi* in the United States has been reported, but it almost certainly was acquired during travel in Venezuela (17). The difficulty in obtaining good-quality DNA samples has hampered population studies of this fungus, but an intrepid medical mycologist tackling this fungus might be rewarded by an interesting evolutionary history.

From the Ajellomycetaceae and Uncinocarpus clade, we now move back to the node that joins them with the Arthrodermataceae, some 120 MYA, and from that node forward in time to the home of the ascomycete dermatophytic fungi.

Family Arthrodermataceae. Among the many species in the family Arthrodermataceae, eight are considered important associates of humans (34, 93): two species of *Microsporum*, *M. audouinii* and *M. ferrugineum*, and six species of *Trichophyton*, *T. tonsurans*, *T. interdigitale*, *T. rubrum*, *T. violaceum*, *T. schoenleinii*, and *T. concentricum*. In addition, it is impossible to treat this group without considering the zoophilic species, *M. canis*. All of these names apply to mitosporic states, and where there is a meiosporic state it is in the genus *Arthroderma*. Close relationships among mitosporic and mitosporic species are seen for *A. simii* with *T. schoenleinii* and for *A. vanbreuseghemii* with *T. tonsurans* and *T. interdigitale* (93). *A. benhamiae* has a close relationship with *T. concentricum*, and *A. otae* is a close relative of *M. audouinii*, *M. canis*, and *M. ferrugineum*. Mitosporic dermatophytes without close meiosporic relatives include *T. rubrum* and *T. violaceum*.

Summerbell has hypothesized that the mitosporic, human dermatophytes have evolved from meiosporic species associated with animals that live in the soil and that the human dermatophytes now are spread exclusively by mitospores (184). In framing his hypothesis, Summerbell knew that human dermatophyte individuals typically are exclusively, or nearly exclusively, of one mating type (MAT) and that there is very little variation among individuals (as judged by ITS sequence, fingerprints of repeated DNA, or amplification fragment length polymorphism [AFLP] analysis) (84). Subsequently, a very thorough study from Y. Gräser's laboratory of over 200 individuals of the *A. otae* complex (*M. canis, M. ferrugineum,* and *M. audouinii*), using 10 polymorphic microsatellites and 3 sequenced coding regions, provided even more

compelling evidence for the relationship of clonal lineages to sexual species (93). *A. otae* individuals are heavily biased to *MAT* (−), with only a few *MAT* (+) individuals known from Japan, and the genetic distance between the two mating types is considerable. *M. canis* individuals are genetically nearly indistinguishable from *A. otae MAT* (−), and both *M. canis* and *A. otae MAT* (−) genotypes are quite distinct from that of *A. otae MAT* (+). It seems likely that *M. canis* is a single clonal lineage derived from one *A. otae MAT* (−) individual. Classification of clonal lineages that lack nearly identical sexual relatives is not straightforward, because each tiny branch could merit its own name (139). If the mitosporic lineage has a close, but genotypically distinct, sexual relative, it is possible to define a mitosporic species in relation to the meiosporic relative (194) (Fig. 3). However, if the clonal lineage emerges from a sexual species, some might argue that the clonal lineage is one, long-lived individual (see the discussion of *H. capsulatum* var. *farciminosum* above). To complicate matters further, neither *M. ferrugineum* nor *M. audouinii* has as close a sexual relative as does *M. canis*, and their closest meiosporic relative is *A. otae* mating type (+). Gräser and colleagues favor the interpretation that meiosporic *A. otae* has spawned three clonal species, *M. canis, M. audouinii,* and *M. ferrugineum,* implying that *A. otae* harbors considerable variation (93). It is possible, however, that *A. otae* contains more than one species, a hypothesis that would become testable only if populations of *A. otae* are found with more balanced distributions of mating-type alleles.

A benefit of the research described above is that classification of *Microsporum* species has been simplified by synonymizing taxa that are phylogenetically close or indistinguishable so that fewer taxa remain; e.g., *M. canis* (60), *T. rubrum* and *T. violaceum* (59),
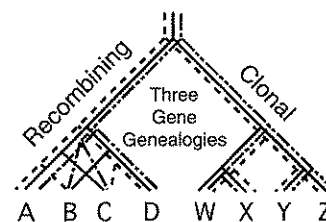

Figure 3. Phylogenetic species recognition applied to species that are exclusively clonal. Exclusively clonal species would not exhibit the change from congruent to conflicting gene genealogies seen in recombining species, which would make species recognition arbitrary. However, if clonal species are uncommon compared to recombining species, then clonal species can be recognized in comparison to their nearest recombining relatives, in this case a sister species. Reprinted from reference 193 with permission.

T. schoenleinii (149), and other species (121). The data used for classification also provide aids to the identification of dermatophytes, in particular: regions of the rDNA, e.g., ITS (121) or intergenic spacers (76), or of protein-coding genes, e.g., those encoding topoisomerase (88) or chitin sythase (89), or fingerprints of repetitive DNA (84). In search of ever more polymorphic markers, microsatellites now are being developed for *T. rubrum* and *T. violaceum* (144), in addition to those used for the *Microsporum* research discussed above.

We now travel back to 130 MYA to the node joining Onygenales to Eurotiales and from there forward on the branch leading to the Trichocomaceae, home to the genera *Aspergillus* and *Penicillium*.

ORDER EUROTIALES

Family Trichocomaceae. *Aspergillus* species form a monophyletic clade in or near the *Eupenicillium* group of penicillia, and *Aspergillus* species have become important opportunistic pathogens as the number of immunodeficient hosts has increased. There is a real and as yet unmet need for a broad, multigene phylogeny of *Aspergillus* species (208, 211). *A. fumigatus* is by far the most important human pathogen, followed by *A. flavus* and *A. terreus* (34). Protein-coding gene phylogenetics of the *A. fumigatus* group have shown that this mitosporic fungus has a close meiosporic relative, *Neosartorya fischerii* (56), and similar studies have shown that *A. flavus* has a close meiosporic relative, *Petromyces alliaceus* (207). Phylogenetic species recognition of *A. flavus* found four cryptic species (57), and later analysis of reproductive structures and aflatoxin production showed that these four phylogenetic species could be recognized by a combination of toxin production and sclerotium size (55). One of the cryptic species produces both aflatoxins B and G, and it appears to be more common in the Southern Hemisphere (147, 200).

Fingerprinting studies using repeated DNA failed to find any cryptic species in *A. fumigatus* or any correlation between fungal genotype and either pathogenicity or geographic origin (27). Researchers performing a subsequent study of Canadian isolates by using microsatellite markers came to the same conclusion (157), as did the workers performing a European study involving presumed single-nucleotide polymorphisms scored by PCR amplification (188). However, a study of *A. fumigatus* from French hospitals, using microsatellites as well as RAPDs and single-nucleotide polymorphisms, concluded that isolates did differ by geographic origin (10). A recent study using the DNA sequence flanking four microsatellites and a global sample of *A. fumigatus*

found one new cryptic species (it and *A. fumigatus* are sympatric) and no population structure in *A. fumigatus*. As a result, identical genotypes are found on different continents and there is no evidence that certain genotypes are associated with pathogenicity (148a). *A. fumigatus* appears to be a globally distributed fungus with no geographic population structure. Workers performing a similar and contemporary study using a different collection of global isolates and a different set of nucleic markers have arrived at the same conclusion (145a). It seems as if *A. fumigatus* is unlike any other fungal species that has been examined by a thorough MLST approach, although it is impossible to rule out the existence of a structured population somewhere on Earth from which this global expansion has emanated. Even if the latter scenario should prove to be true, the size of the clonal expansion would be unprecedented. The third *Aspergillus* species, *A. terreus*, has not been the subject of a thorough phylogenetic or population genetic study, but studies using an RAPD approach have begun and show considerable variation in the genotypes of isolates collected from different cystic fibrosis patients (24), but similar genotypes in serial isolates from the same patient (187).

Although both *A. fumigatus* and *A. flavus* are mitosporic, analyses of the sequenced genes used to search for phylogenetic species in both taxa have found evidence for recombination, in addition to evidence for clonality (57, 148a). In the case of *A. fumigatus*, one mating-type gene was found from a search of the sequenced genome (148) and the other was found in a second individual (40), indicating that the fungus may achieve recombination by sexual reproduction through outbreeding (heterothallism). The aforementioned phylogenetic study of *A. fumigatus* and its relatives (56) indicated that heterothallic *Aspergillus* species in the *A. fumigatus* group evolved from homothallic relatives. This result is surprising because heterothallism is ancestral in Ascomycota as a whole, but in this group of *Aspergillus* species, homothallism appears to be ancestral. The recent completion of genome sequences for *A. fumigatus* (138a), *A. oryzae* (117a), and *A. nidulans* (54a) should provide the information to address questions about the evolution of reproductive modes (5, 41) and about pathogenesis. Here, the ability of *Aspergillus* species to make secondary compounds that are toxic to humans (e.g., aflatoxin) (14, 20, 206) not only makes these fungi different from yeasts or Basidiomycota, which, for example, seem to lack genes for polyketide synthases (100), but also provides them with the potential for a very different mode of virulence.

From *Aspergillus*, we move to the closely related genus *Penicillium*. Several *Penicillium* species are

capable of causing disease in immunodeficient humans, but only *P. marneffei* presents a significant danger to public health (34). This fungus is not a member of the clade of well-known *Penicillium* species, i.e., those that make penicillin and that may have meiosporic states in the genus *Eupenicillium*, but, rather, is in the clade with meiosporic states in the genus *Talaromyces* (114). No member of the Eupenicillium clade is known to grow as single cells, but *P. marneffei* apparently can convert to a single-celled form. Able to produce single cells at 37°C, *P. marneffei* has become the third most important opportunistic pathogen in Thailand, behind only tuberculosis and cryptococcosis (185). The fungus was described from a captive bamboo rat, and a recent study of *P. marneffei* infection in India showed that nearly 10% of some rat species are infected with the fungus (72). *P. marneffei* had been studied by a variety of molecular approaches (RFLP, M-13 fingerprinting, electrophoretic karyotyping, and ITS-PCR [72]), but the recent discovery and application of microsatellite markers shows the potential of these highly polymorphic markers to reveal the biogeography of a fungus (44, 45, 108).

Fisher et al. have found 20 useful microsatellite markers in *P. marneffei* and used them with an MLMT approach to study 24 isolates from Asia, including individuals from China, Vietnam, Indonesia, and India (44). They found two well-separated clades, one from China plus India and the other from Vietnam plus Indonesia. There is no evidence for recombination, which could be due to its absence or to fine-scale population structure. Arguing in favor of clonality are the lack of observed sexual reproduction in *P. marneffei* and the fact that identical MLMT genotypes have been found in rats caught at the same locations and in humans and rats living in the same area (72). Of course, it also is possible that *P. marneffei* is clonal in part of its geographic range and sexual in another, as has been seen for a number of plant-pathogenic fungi (193), as well as for *Cryptococcus neoformans* var. *grubii*, as described below (113). To address the question of reproductive mode, we need more isolates from throughout the area of endemic infection. There is every prospect that more isolates will be found and typed and that analyses of the growing data set will answer questions about *P. marneffei* reproduction, speciation, and population structure. The MLMT approach is ideal for this research because different research groups can pool their microsatellite data using the *P. marneffei* MLMT website (44). Not incidentally, the MLMT data are ideal for typing new isolates; the markers are so variable that finding two isolates with identical

genotypes makes it almost certain that they are from the same individual (45).

From the Trichocomaceae, we move back through the Eurotiales and Eurotiomycetes to the node that joins them all to the Chaethothyriomycetes, some 250 MYA, and then forward to the modern members of this group of darkly pigmented fungi that cause diseases known as phaeohyphomycoses and chromoblastomycoses.

Class Chaetothyriomycetes

The fungi that cause chromoblastomycosis or phaeohyphomycosis are found in the family Herpotrichiellaceae of the Chaetothyriomycetes and have melanized cell walls. They can cause disease in otherwise healthy humans, but in order to do so they require a wound to bypass the defense offered by skin (16, 22). They are becoming more common, and more dangerous in terms of dissemination, due to the rise in the number of immunocompromised hosts. Although these fungi are far more common in the tropics, they are not unknown in the Northern Hemisphere (16).

Chaetothyriomycetes is one of two great groups of fissitunicate ascomycetes that make flask-shaped ascoma, the other being Dothidiomycetes, home to many plant-pathogenic fungi. de Hoog et al. (34) consider 10 species of Chaetothyriomycetes to be important human pathogens in that they are common or particularly severe. They are found in five genera: *Exophiala* with *E. oligosperma* (the description of which reduced the medical importance of *E. jeanselmei* sensu stricto [33]), *E. dermatitidis*, and *E. spinifera*; *Phialophora* with *P. verrucosa*, *P. americana*, and *P. europaea*; *Cladophialophora* with *C. carrionii* and *C. bantiana*; *Ramichloridium* with *R. mackenziei*; and *Fonsecaea* with *F. pedrosoi*. Another fungus growing in skin and making darkly pigmented hyphae is *Hortaea werneckii*, the agent of tinea nigra; this fungus, however, is a member of the Dothidiomycetes (along with a *Madurella* species to be mentioned below).

Phylogenetic studies of Chaetothyriomycetes, performed using regions of the rDNA repeat, have shown that the features of conidiation used to define the different genera in this class are not reliable indicators of phylogenetic relationships (1, 202). In some cases, one individual fungus can make both annelloconidia (associated with *Exophiala*) and sympoduloconidia (associated with *Rhinocladiella*) (30, 33). Therefore, the genera *Phialophora*, *Exophiala*, *Chadophialophora*, and *Rhinocladiella* need revision, and all of these mitosporic taxa have close relatives or meiosporic states among meiosporic Chaetothyriomycetes in the genus *Capronia*.

Comparisons of rapidly evolving sequences of large collections of isolates of these fungi are now appearing and have resulted in (i) a better understanding of described *Exophiala* species (122), (ii) the discovery of cryptic *Exophiala* species (210), and (iii) the discovery of a cryptic *Fonsecaea* species (6, 32). Given that considerable variation is seen within these taxa, it seems likely that phylogenetic species recognition studies involving many individuals and several protein-coding genes would discover even more species.

From the Chaetothyriomycetes, we move back in time, past even their divergence from the Eurotiomycetes, to a point between 270 and 330 MYA when these two classes diverged from Dothidiomycetes, Pezizomycetes, Lecanoromycetes, and our next destination, the Sordariomycetes.

Class Sordariomycetes

In the class Sordariomycetes are the fungi responsible for mycetomas and sporotrichosis. These filamentous ascomycetes can infect otherwise healthy humans, but only if they are implanted past the skin barrier by trauma. Among the many fungi capable of causing this type of disease, five species are considered important (34). Four are Sordariomycetes, *Sporothrix schenckii, Pseudallescheria boydii, Scedosporium prolificans,* and *Madurella mycetomatis,* but one is a fissitunicate ascomycete, *Madurella grisea* (Pleosporales). Of course, these fungi also cause disease in immunocompromised hosts, where dissemination of the fungus from the initial wound is the greater threat.

There are two main clades of Sordariomycetes, one with Ophistomatales and Sordariales and the other with Hypocreales and Microascales. In the Ophiostomatales is *S. schenckii,* which causes sporotrichosis, an infection of lymph nodes or skin. This mitosporic fungus is the sister species to the meiosporic fungus *Ophiostoma stenoceras* plus species in the *O. nigrocarpum* complex (28). In the Sordariales is found *M. mycetomatis,* the principal agent of black-grained mycetoma, which is most common in Africa (31). In the Microascales is found *P. boydii* (whose anamorphic state is *Scedosporium apiospermum*), the principal agent of white-grained mycetoma, which is common in North America (86). Also in this order is *S. prolificans,* which affects the skin and joints (66). *S. prolificans* is a close relative of *P. boydii* and a closer relative of meiosporic *Petriella* species (86). Molecular identification schemes have been proposed for *M. mycetomatis* (116), *S. prolificans* (170), and *P. boydii* (153), and all show genetic variation among individuals of each species. In the case of *P. boydii,* studies of cystic fibrosis patients have shown that although this fungus is difficult to isolate from air, a single patient can host multiple isolates (23, 29, 221). Here, again, are fungi that would be candidates for phylogenetic species recognition. Another agent of black-grained mycetoma is *M. grisea,* which, as mentioned above, is a dothidiomycete along with the genus *Hortaea* (31).

The last order of Sordariomycetes hosting pathogens is the Hypocreales, home to the genus *Fusarium.* Species of *Fusarium* can cause infection in immunodeficient hosts, and the outcome is grave. The most commonly reported species is *F. solani,* which embraces at least 25 species (140), but there are also many cases involving the *F. oxysporum* complex (also very speciose) and *F. verticilliodes* (likewise very speciose) (37). In addition, fungi classified in other mitosporic genera, i.e., *Cylindrocarpon lichenicola* and *Acremonium falciforme,* have proved to be members of the *F. solani* clade (3). *Fusarium solani* has been recovered from the water supplies of hospitals where *Fusarium* infections have been reported (3), as have isolates of *F. oxysporum* (143) and *F. verticilloides* (63).

Fusarium species are far better known as plant pathogens, and a very thorough study of *F. oxysporum* complex isolates from humans, set in the context of plant and other environmental isolates, has demonstrated the value of a broad sampling of individuals combined with multiple, sequenced loci (143). The authors studied 88 patient isolates and 18 environmental isolates from four continents, using DNA sequences from protein-coding genes, nuclear rDNA, and mitochondrial rDNA, as well as 173 biallelic AFLP loci. Each of the four previously known clades of *F. oxysporum* (141) proved to harbor human-pathogenic isolates, although the vast majority came from just one of the clades. Among these individuals was a large clone, as judged by its DNA sequence, which proved to embrace seven smaller clones when examined with the more polymorphic AFLP loci. The largest of the AFLP clones are found both in North America and Europe, and all but one of the AFLP clones contained both human and environmental isolates. The low variation among individuals is consistent with the clonal spread of these fungi; alas, such low variation makes it difficult to determine the source of infection when environmental isolates from within and without hospitals have the same genotype as patient isolates. Arguments have been made for waterborne infection (3), airborne infection, or carriage of *F. solani* prior to elective immunosuppression (152); all seem possible.

Now, we will proceed toward the divergence of Pezizomycotina and Saccharomycotina, some 370 MYA, and then move forward in time to the Saccharomycotina, home to the most famous fungus, *Saccharomyces cerevisiae,* and the most important opportunistic pathogenic fungus, *Candida albicans.*

Subphylum Saccharomycotina

The Saccharomycotina contains a group of yeasts that, in what is now a recurrent theme, have become increasingly important human pathogens as the population of immunodeficient hosts has grown. Among the many yeasts that have been found in humans, six are considered to be most important (34): *Candida albicans, C. dubliniensis, C. glabrata, C. krusei, C. tropicalis,* and *C. lusitaniae* (= *Clavispora lusitaniae*). These yeasts belong to three clades, the *Saccharomyces cerevisiae* clade (*C. glabrata* and *C. krusei*), the *Candida albicans* clade (*C. albicans, C. dublinensis,* and *C. tropicalis*), and the *Clavispora-Metschnikowia* clade (*C. lusitaniae*) (101). These yeasts are part of the normal commensal mycota of humans, and, recently, PCR fingerprinting has been used to show that different body regions have different complements of species (the mouth has the lowest diversity), that the community composition of *Candida* species changes over time on a single host individual, and that isolates may be transmitted among family members (87).

C. albicans is the most important of the yeasts because it is the most common opportunistic pathogen of immunodeficient hosts. It was in *C. albicans* that the first phylogenetically recognized, cryptic fungal species was found, *C. dubliniensis* (183). Studies of nucleic acid variation in *C. albicans* have uncovered genetically isolated populations in Africa (51) that may prove to be another cryptic species, *C. africana* (199). Other studies have found variation that correlates with geography; for example, an RFLP study showed five clades (124), a DNA fingerprinting study found five groups (172), and a comparison of microsatellite and single nucleotide polymorphisms found a recently evolved North American clade. As Soll and Pujol (172) wrote, "these findings lay to rest the idea that one strain represents all strains of *C. albicans*, support the need for a worldwide analysis of populations structure and clade-specific phenotypic characteristics, and demonstrate that in the future, pathogenic characteristics must be analyzed in representatives from all five clades." Clearly, *C. albicans* would be an excellent candidate for a global MLST (190) or MLMT effort.

The possibility of sexual reproduction in *C. albicans* was raised as a result of multilocus electrophoresis studies (151) and supported by studies of nucleic acid variation (61). Studies of mitochondrial DNA variation supported a hypothesis of ancient recombination followed by exclusively clonal reproduction in present-day populations (4). Developmental biologists have shown that *C. albicans* can be induced to mate in mice or in vitro, but meiosis has not been detected (85, 118). Two recent reviews tell the story very well (86a, 119). *C. albicans* individuals are normally diploid, and after mating they become tetraploid, only to then lose chromosomes to restore diploidy. The ability to mate is correlated with a switch in colony morphology from white to opaque. Natural diploid individuals that have both mating-type alleles produce a heterodimer of the mating-type-like locus (*MTL*) a1 and MTLα gene products, which prevents the switch from white to opaque colony morphology. Preventing the transition from white to opaque severely inhibits mating because white cells mate at a frequency three orders of magnitude lower than that of opaque cells. When individuals lose *MTLa* or *MTLα* and become either hemi- or homozygous for one *MTL*, they switch to the opaque form and mate; it has just been shown that plasmogamy is followed by karyogamy (8a). The loss of alleles seems to be caused by mitotic recombination or gene conversion, which makes it more difficult to interpret population studies aimed at detecting recombination (190). Interestingly, for a human commensal, growth at 37°C promotes the switch from opaque to white and the inhibition of mating. Even more interestingly (and somewhat contradictory), mating proceeds better on the skin of mammals at 32°C than it does in the laboratory at 37°C (107). The relationship of mating competence, mating itself, and pathogenicity is complex and surely will be the focus of many future studies (171). As a taste of what is to come, a recent comparison of these processes in *C. albicans* and *S. cerevisiae* shows that developmental paths and circuits have evolved to employ different components to achieve similar developmental outcomes in these two yeasts (201).

C. dubliniensis, the aforementioned sister species of *C. albicans*, also has been found to mate and even can mate with *C. albicans*; in fact, interspecific mating can be more efficient than intraspecific mating (150). *C. albicans* is a more virulent pathogen than *C. dubliniensis* (182), and a recent comparative study of the genomes of these two species by competitive DNA hybridizaton to a *C. albicans* microarray identified suites of genes missing from *C. dubliniensis*; these missing genes might help explain the superior virulence of *C. albicans* (135).

C. glabrata, the closest pathogenic relative of *S. cerevisiae*, also has been the subject of population genetic studies. A study of French isolates by using multilocus enzyme electrophoresis and RAPD markers found some population structure in the form of reduced gene flow between isolates collected in Paris and those collected in Montpellier, as well as an association among markers consistent with clonal reproduction in this haploid yeast (36). However, analysis of the

genome of *C. glabrata* has discovered representatives of most of the genes known to be involved in *S. cerevisiae* sexual reproduction, indicating that the last word on reproduction in *C. glabrata* has yet to be written (215). DNA sequence variation also showed population structure in *C. glabrata* between North and South America (161). An MLST scheme has been developed for *C. glabrata*, using six sequenced loci, and has found six clades that correlate with the geographic origin of the isolates; e.g., clade I is most common in Europe, clade IV is most common in Japan, and clade III is most common in North America (38).

C. *krusei* and *C. lusitaniae* have not received the attention paid to their more clinically important relatives, but population genetic studies have begun that use both sequenced markers (106) and microsatellites (167).

We now leave the Saccharomycotina to move back to the earliest divergence in the Ascomycota at 430 MYA, the one that leads to the Taphrinomycotina, an assemblage of species that have very long branches and whose monophyletic nature is not as well supported as that of the other two subphyla, Saccharomycotina and Pezizomycotina.

Subphylum Taphrinomycotina

In the Taphrinomycotina is the model fungus, *Schizosaccharomyces pombe*, and the species of *Pneumocystis*, which are found in the lungs of mammals and one of which causes human disease if the host becomes immunodeficient (169). Until the late 1980s, these fungi were considered to be protists, but comparison of rDNA sequences showed them to be fungi (42, 175); to be fair, earlier work by electron microscopists already had found the truth (209). *Pneumocystis* species recognition and phylogenetic relationships are based on nucleic acid variation, undoubtedly because none of the species can be cultivated axenically. There is a close correlation between species of *Pneumocystis* and their mammalian host; the most famous species, *P. carinii*, is found in rats, as is a more recently described species, *P. wakefieldii* (26). Mice have a species, *P. murina* (95), and there are many species associated with primates (35), including the human-associated species, *P. jiroveci*. Phylogenetic species recognition using a combination of rDNA regions and protein-coding genes is now the norm for this genus, and studies of multiple isolates from primates (macaques) have shown that two phylogenetic species of *Pneumocystis* can be associated with a single macaque species and vice versa (73). *Pneumocystis* species and their mammalian hosts are a study in coevolution, and dates for divergences among the fungal species correlate with those proposed for the mammalian hosts (96). MLST

would be very difficult with *Pneumocystis* species because individuals cannot be cultivated. Instead, typing has focused on the rDNA ITS sequence. A recent study of South African *P. jiroveci* ITS sequences (155) showed how useful even one sequenced allele can be: individual hosts can be infected by up to six *P. jiroveci* strains, the same *P. jiroveci* individual can infect several host individuals, and analysis of the ITS sequences shows that recombination is a likely event in its evolution, consistent with earlier electron microscopic reports of meiosis in *Pneumocystis* (123).

We now move back along the branch that unites all Ascomycota to its divergence from the Basidiomycota, about 550 MYA, and then come forward in time to the Basidiomycota.

PHYLUM BASIDIOMYCOTA

In the phylum Basidiomycota is found about one-third of the described fungi, but only a few basidiomycete species cause disease in otherwise healthy humans. Basidiomycota comprise three major lineages, one with the rusts and other simple-septate basidiomycetes (Urediniomycetes), a second with the monocot smuts (Ustilaginomycetes), and a third (Hymenomycetes) with the hymenium-forming basidiomycetes, including the "jelly" fungi, bracket fungi, and mushrooms (117, 186).

Class Hymenomycetes

The best-known basidiomycete human pathogens are found in the Hymenomycetes. Within this class there are two lineages, the subclass Hymenomycetidae, with the orders Dacrymycetales, Auriculariales, Aphyllophorales, and Agaricales, and the subclass Tremellomycetidae, with the order Tremellales. In the Tremellomycetidae are found two genera of pathogenic basidiomycetes, *Filobasidiella* and *Trichosporon*. *Filobasidiella neoformans*, better known by the name for its mitosporic state, *Cryptococcus neoformans*, is the agent of cryptococcosis and is the best-known basidiomycete human pathogen. There are five serotypes of *C. neoformans*, A, B, C, D, and AD, which are partitioned among three genetically isolated groups, *C. neoformans* var. *neoformans* (serotype D), *C. neoformans* var. *grubii* (serotoype A) (52), and *C. neoformans* var. *gattii* (serotypes B and C), and individuals that are hybrids between *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* (serotype AD) (13, 110, 217). The divergence times between the varieties are estimated to be in the tens of millions of years, long enough for them to be considered phylogenetic species (219). In fact, *C. neoformans* var. *gattii*, long ago, was described as a species, *C. bacillisporus* (104), and the proposal to conserve *C. gattii* as the name for this taxon emphasizes its true rank (105).

C. neoformans var. *neoformans* is known to mate in cultivation, and individuals of mating types **a** and α are found in nature, although mating type α (*MAT*α) is far more commonly encountered (*MAT*α: *MAT*a, 49:1). In *C. neoformans* var. *grubii* the distribution of *MAT* is even more skewed; in fact, this species was known only from *MAT*α individuals until recently. However, the ability of *C. neoformans* var. *grubii MAT*α individuals to hybridize with *C. neoformans* var. *neoformans MAT*a individuals indicated that *C. neoformans* var. *grubii* maintained the capacity for sexual reproduction (80). The discovery of *C. neoformans* var. *grubii MAT*a individuals (112) led to laboratory demonstrations of mating in this variety (138), and population genetic analysis of multiple loci in serotype AD hybrids supported a history of recombination in nature for both parent varieties (218). The recent discovery of a *C. neoformans* var. *grubii* population in Botswana in which *MAT*a individuals account for ca. 10% of the population and for which population genetic tests are consistent with recombination (113) indicates that this fungus is a sexual basidiomycete. The discovery of a natural, sexual *Cryptococcus* population was a first for the genus. A previous study of Australian *C. gattii* had discovered a population with a balance of both mating types, but population genetic analysis detected only clonal reproduction (77). When *C. neoformans* var. *grubii* was known only from *MAT*α individuals, it was hypothesized that only fungi of that mating type were virulent. However, when a *MAT*a individual was discovered, experimental infections of mice showed that the *MAT*a individual also was virulent (97, 138). Adherents of phylogenetic species recognition would consider each variety to be a species, whereas those who favor biological species recognition might retain varieties *grubii* and *neoformans*, because they can mate (80). However, it seems likely that these two lineages were kept apart by geographic isolation and that when their allopatric distribution was changed to one of sympatry (at least for *MAT*α individuals of *C. neoformans* var. *grubii*), hybrid progeny followed. As more populations are discovered, the taxonomic situation is certain to become more complex; e.g., the aforementioned discovery of recombination in Botswanan isolates also uncovered several genetically isolated populations (113). The failure of some *C. neoformans* var. *grubii* pairs to mate is consistent with cryptic species (chapter 2, this volume), and recent investigations of *C. gattii* suggest that it, too, embraces several genetically isolated lineages (94, 98, 176).

The global effort made to genotype *Cryptococcus* individuals by using repetitive DNA sequences is unprecedented in pathogenic fungi, and phylogenetic analysis of the genotypes shows good agreement with the varieties (109, 131). Given the global distribution of this fungus (130) and its sexual nature, *Cryptococcus* would be another ideal candidate for a large, multinational, fungal MLST approach (176). *C. neoformans* also is becoming a model for basidiomycete evolution, judging from recent studies of mutation, mating loci (53, 111), mitochondrial inheritance (220), and genotype by environment studies (216). *Cryptococcus* also was instrumental in the development of a very interesting hypothesis about the origins of pathogenicity resting in interactions between pathogenic yeasts, such as *Cryptococcus*, and simple eukaryote hosts, such as soil amoebae (173, 174).

Leaving the genus *Cryptococcus*, we move back to its divergence from the genus *Trichosporon* and then move forward in time to the several species of this genus of keratinophilic fungi known for their ability to grow on hair or skin. *Trichosporon* species are known to make only mitospores, and so assignment of *Trichosporon* species to the Basidiomycotina could not rely on sexual characteristics. The chemical composition and laminated form of the cell wall suggested that this fungus was a basidiomycete, and this hypothesis was confirmed by comparison of partial 28S rRNA gene sequences (68). From phenotype, site of infection, DNA-DNA hybridization, and 28S rRNA gene sequence, at least five *Trichosporon* species capable of infecting humans have been proposed (71). Two cause white piedra, one on pubic hair, *T. inkin*, and the other on capital hair, *T. ovoides*; three cause systemic disease, *T. mucoides, T. asahii*, and *T. cutaneum*; a sixth was isolated from skin, *T. asteroides*. The *Atlas of Clinical Fungi* considers *T. inkin, T. asahii*, and *T. mucoides* to be the most commonly encountered human pathogenic species in the genus (34). The familiar *T. beigelli* is now considered a doubtful name because it was improperly described, and isolates formerly classified as *T. beigelli* should be referred to one of the other species. *Trichosporon* phylogeny (43, 133) and taxonomy (71) are well studied, with new species being added with regularity. Methods have been designed for the identification of medically important *Trichosporon* species by substrate utilization (132) or by sequencing of rDNA ITS (177). However, all species recognition in *Trichosporon* is by phenotype, and no studies have been conducted to examine genetic variation within species or to recognize species by phylogenetics.

Moving back in time along the branch leading to the divergence between Hymenomycetes and Ustilaginomycetes at 440 MYA, we then join the Ustilaginomycetes and move forward in time to the genus *Malassezia*.

Class Ustilaginomycetes

In the Ustilaginomycetes (8) is found another genus of animal pathogenic yeasts, *Malassezia*. Species of *Malassezia* can cause disease of the stratum corneum of the body and head, mostly in the tropics (25, 67, 74). Many isolates are lipid dependent and can also cause deeper mycoses in temperate climates if the host is taking lipid intravenously. Traditionally, isolates were classified into two species, *M. furfur* and *M. pachydermatis*, which were thought to be restricted to humans and animals, respectively. However, studies of nuclear large-subunit rRNA gene sequences, morphology, growth substrate, and catalase production have shown that there were at least seven species, all of which could be found in human clinical samples (although *M. furfur* was by far the most common) and several of which also were associated with animals (70). The seven species recognized by Guého and colleagues are *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae*. New species continue to be described, e.g., *M. dermatis*, *M. yanatoensis*, and *M. japonica* (178–180), and both phenotype and molecular methods of identification have been proposed (58, 75, 198). Species recognition is by phenotype and by phylogenetic relationships using variable regions of the rDNA repeat. Intraspecific variation of protein electrophoretic mobility (134) and of chitin synthase gene sequence in *M. pachydermatis* (2) suggest that species recognition by concordance of gene genealogies might discover more genetically isolated groups.

Other basidiomycetes that occasionally have been reported to cause disease include *Rhodotorula rubra*, a member of the Urediniomycetes clade, *Ustilago* spp., which are found on the Ustilaginomycetes clade, and *Coprinus cinereus* and *Schizophyllum commune*, hymenomycetes in the Agaricales and Aphyllophorales, respectively. That mushrooms such as *Coprinus* or wood decay fungi such as *Schizophyllum* can cause human disease shows that almost any fungus is a potential danger to a host whose immune system is sufficiently degraded.

Finished with the Basidiomycota, we move back past its divergence with the Ascomycota at 550 MYA to divergences involving clades of morphologically simpler Zygomycota and Chytridiomycota between 660 and 830 MYA.

PHYLUM ZYGOMYCOTA

Molecular phylogenetic studies have shown that neither Zygomycota nor Chytridiomycota is likely to be monophyletic. It is prudent, therefore, to consider theses fungi to be members of well-supported, order-level clades (165). In Zygomycota, there are two prominent orders, Mucorales and Entomophthorales, that harbor human pathogens. Many members of the Mucorales may be responsible for mycoses, but *Rhizopus oryzae* most commonly is the cause. The phylogenetic relationships of *Rhizopus* species to other Mucorales have been analyzed (142), and genetic variation within *Rhizopus* species has been detected (159, 204). The other order harboring pathogens, Entomophthorales, is well known for its pathogens of insects, and one species, *Conidiobolus coronatus*, is known to cause a subcutaneous infection in humans and animals (64, 154). Species of *Conidiobolus* have not been recognized phylogenetically, and there are suggestions that the species contain considerable variation (54, 166, 213). Another genus capable of causing human disease, *Basidiobolus*, is classified in the Entomophthorales, but phylogenetic studies of 18S rDNA have shown that it does not belong there and suggest that, instead, it may belong in the Chytridiales (136). However, more recent research with both 18S and 28S rDNAs and a number of protein coding genes indicates that *Basidiobolus* is a member of the Entomophthorales (T. Y. James, R. J. Vilgalys, J. E. Longcore, S. E. Mozley-Standridge, and the Assembling the Fungal Tree of Life Working Group, abstract, *Inoculum* 56:28, 2005). Population genetic and phylogenetic studies of *Basidiobolus* species indicate that human pathogenic isolates are distinct from those that are saprobic (138).

PHYLUM CHYTRIDIOMYCOTA

Going back along the entomophthoralean branch, we come to the polytomy of zygo- and chytridiomycete orders at about 830 MYA. Picking the lineage leading to the Chytridiales, we head toward the most important animal pathogenic chytrid, *Batrachochytrium dendrobatidis*, which appears to be responsible for the worldwide amphibian decline (115). With the return of *Basidiobolus* species to the Entomophthorales, no chytrid is known to cause human disease. It had been suggested that *Rhinosporidium seeberi*, the mycotic agent of rhinosporidiosis, was a chytridiomycete; however, it is not a fungus but a mesomycetozoean (82, 129). With the Chytridiales, we have completed our phylogenetic tour of fungal pathogens of humans. The only pathogen studied by mycologists that we have missed is *Pythium insidiosum*, a member of the Oomycota, a phylum now recognized to belong to the Stramenopila, home also to brown algae and diatoms. As with the fungi, studies of *P. insidiosum* have focused on phylogenetics (164), population structure (162), and molecular means of identification (163, 205). Conversely, there is a group of fungal or near-fungal pathogens that have not been studied

by mycologists: Microsporidia (94a, 198a). These obligate parasites of many animal groups are capable of causing human disease (51a, 94b), and methods of their identification using nucleic acid variation are being developed (139a).

CONCLUSION

If this short treatment of the evolution of fungi pathogenic on humans has whetted your appetite for more information, you should examine recent works on the place of fungi in *Assembling the Tree of Life* (195), the *Mycota* volumes on fungal systematics (127, 128), and, of course, the *Atlas of Clinical Fungi* (34).

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