Fungi have provided some of the best model systems in genetics, including the first eukaryotic genome to be completely sequenced. Although the capabilities of fungi are well known from laboratory experiments, actual patterns of genetic transmission in natural populations have been less well understood. In this review, we consider recent approaches to two key questions that cannot be answered by direct observation. What are the fundamental units of fungal populations, and how important are asexual and sexual reproduction in determining their genetic structure? As fungal population genetics has been amply reviewed, we focus on recent studies identifying variation in DNA, especially nucleotide sequence data and genealogical analysis.

Fungi do not fit the classical models

Most fungi do not fit the classical models of population genetics. They have novel methods of colonization, dispersal, and their generations overlap. Sexual reproduction can be irregular or absent altogether in some recently derived lineages. Also, most fungi grow indiscretely within opaque substrates making direct measurement of fungal biomass or census of individuals difficult or impossible. Under these conditions, there is no single definition of the fungal individual that serves all purposes. For example, the basidiomycete Armillaria gallica, can legitimately be considered to be one of the smallest organisms. This depends on whether the unit counted is the entire genet or the ramet, which may consist of as little as a single totipotent cell. In fungi, even the fundamental distinction between growth and reproduction is not always clear. For example, several discrete mushrooms can be produced by one continuous mycelium that is hidden from view and is difficult, if not impossible, to trace physically within its substrate. In this case, which is the appropriate unit to count, the mushroom or the mycelium?

Like fungal growth, methods of fungal reproduction are extremely varied. Fungi produce a multitude of different kinds of propagule associated with meiotic and mitotic recombination and the processes that are involved are not always clear. For example, several discrete mushrooms can be produced by one continuous mycelium that is hidden from view and is difficult, if not impossible, to trace physically within its substrate. In this case, which is the appropriate unit to count, the mushroom or the mycelium?

As ubiquitous decomposers, symbionts and parasites, fungi build populations not easily accommodated by population genetic theory. Identifying and delineating individuals and populations is often difficult, and recombination can occur in complex and variable ways. Genotyping and gene genealogies provide the framework for identifying and delineating individuals and for detecting recombination in natural populations. Expanding genomic databases now make fungal ideal subjects for tracking mutation and expression in genes of adaptive importance in experimental populations.

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force in many fungi lacking a sexual cycle, because relatively few recombination events can have a disproportionately large impact on population structure.12.

Genetic clones are units of fungal populations
Given the diverse life histories of fungi, what are the appropriate units to count when characterizing fungal populations? One obvious approach is to identify clones resulting from asexual reproduction. Clones are identified as repeatedly sampled, multilocus genotypes that are unlikely to arise by chance in sexual reproduction.13. Although most clones are of recent origin, some appear to be extremely ancient. For example, certain fungal clones cultivated by highly derived species of leaf-cutting ants could represent exceptional lineages that have managed, as the bdelloid rotifers have, to survive without a sexual cycle for tens of millions of years.15.

The patterns of clonal propagation detected in fungal populations are highly varied. In many fungi, clones are disconnected from their origins by dispersal, which can be local, continental, or even global. In local areas, the spatial distribution of clones can be random or nonrandom and clone frequencies can change with time.11.

In some fungi, however, clones are spatially connected to their origins and are highly territorial.21. In many basidiomycetes, each individual begins with a discrete mating event and then grows vegetatively to occupy a continuous territory. The territoriality of these individuals is evident at strikingly different spatial scales (Fig. 2). Some smaller individuals grow in discrete patches with visible borders that are the result of somatic incompatibility reactions between neighbors, whereas larger individuals grow vegetatively over long periods to occupy many adjacent root systems. In these fungi, somatic incompatibility maintains the integrity of individuals and prevents cytoplasmic continuity among genotypically distinct mycelia. Spatial dispersal by asexual propagules or mycelial fragments in these fungi appears to be rare. Although the dynamics of long-term competition and turnover among adjacent individuals is not known, the best information on the short-term dynamics of colonization and competition among individuals comes from cut spruce stumps and the subsequent colonization of root systems by Heterobasidion annosum.22 Here, many individuals colonize the cut stump surfaces, but usually only one individual predominates in the lower portions of the root system. Whether the outcome of this process is random or determined by fitness differences among genotypes is not known.

Both sexual and asexual reproduction shape population structure
Once genotypes are identified, what are their patterns of descent? Fungal population structures range from almost completely clonal to panmictic.12 The types of population structure described for various fungi could not have been predicted from what was initially known about their life histories. For example, in Sclerotinia sclerotiorum, a pathogen of a wide range of plants, sexual fruiting and meiosis occur every season, but the population structure is highly clonal.15. The reasons for this are prolific asexual reproduction plus sexual reproduction by selfing of haploid mycelia, which results in meioses that are completely homozygous, with no genetic segregation. In Mycosphaerella graminicola, a pathogen of wheat, the sexual stage is not always observed, but the population structure is highly recombed.13. Even for populations in which some individuals are extremely long lived, such as in the hemiomyicyte A. gallica, there is ample evidence of recombination, and no evidence of geographic subdivision, over thousands of kilometers within a continent.14.

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**Fig. 1.** Examples of spore dispersal and vegetative colonization in fungi. (a) Cloud of ascospores released from fruit-bodies of the ascomycete fungus Sclerotinia sclerotiorum. Most fruit-bodies arise from selfing and there is no genetic segregation among ascospores. This mode of reproduction results in a dispersive distribution of clonal genotypes in local areas. Photograph by Jack Kelly Clark, courtesy of the University of California Statewide IPM Project. (b) Rhizomorphs of Armillaria gallica in a 9 cm petri dish with nutrient medium. Vegetative colonization in this fungus results in each clonal genotype occupying a discrete territory. Photograph by J.B.A.
Recombined populations that present no direct evidence of sex

The discovery of recombined population structures in species that present no morphological evidence of mating and meiosis was unexpected. For these fungi, nucleotide sequence data and genealogical analysis allow a test of whether the observed genotypic diversity in a sample of isolates from a population is caused by the accumulation of mutations under a strictly clonal mode of reproduction or by the shuffling of mutations through recombination. The best criterion for detecting whether any recombination has occurred is the same as that used for bacteria: the signature of recombination is incongruency in the genealogies of different genomic regions of DNA (Box 1).

*Coccidioides immitis*, the causal agent of valley fever in humans, is an example of a fungus with no known sexual stage, but with a recombined population genetic structure. A recent study included 30 isolates of *C. immitis* from 25 patients and found 17 polymorphic sites in 14 anonymous regions of nuclear DNA. Twelve of these sites were phylogenetically informative in that each of two alleles was found in two or more isolates in the sample. Two different kinds of analysis of genotypic variation were consistent with genetic exchange and recombination and were irreconcilable with strict clonality. The first test was based on an overall measure of linkage disequilibrium, the index of association ($I_A$) among alleles at different loci. The $I_A$ calculated from the observed genotypic data fell within the range expected under random mating. Although a high mutation rate alone might result in the absence of allelic association, this is difficult to reconcile with the low nucleotide diversity in *C. immitis*.

The more conclusive test of recombination in *C. immitis* is phylogenetic. If evolution is strictly clonal, then it should be possible to use nucleotide positions as characters and the nucleotides as states, and to infer a bifurcating tree of multilocus haplotypes of high internal consistency, but this was not the case. Instead, the shortest trees in *C. immitis* have high levels of homoplasy distributed throughout and are only marginally shorter than the average of those expected under random mating (Fig. 3). The consensus of the best trees from the observed genotypes has essentially no resolution. The homoplasy cannot be attributed to parallel mutation because the proportion of variable sites is low in all DNAs sequenced and only two of the four bases were present at each of these sites.

The failure of parsimony analysis to resolve relationships among haplotypes of *C. immitis* in the conventional phylogenetic sense leaves only one viable explanation: this fungal population is recombining (or at least has recombined in the past) and evolution is reticulate. The different nucleotide sites within an individual do not share one common history of descent, because alleles at different loci are shuffled by genetic exchange and recombination to create new genotypes. Recombination is also the more parsimonious explanation for the observed genotypes. When reticulation is allowed, and haplotypes are not forced to fit the tips of a bifurcating tree, substantially fewer mutational steps are required to explain the observed genotypes (Fig. 3). The recombination could be caused by sexual crossing, which has escaped direct observation, or by parasexual exchange.

Another pathogen of humans, *Candida albicans*, appears to have elements of both clonality and recombination. Because *C. albicans* is diploid, the analyses are more complicated than for haploid fungi such as *C. immitis*. The genetic phases of alleles in *C. albicans* at different loci cannot

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**Fig. 2.** Clonal distribution in basidiomycete fungi at three different spatial scales. (a) Individuals of *Coriolus versicolor* occur within compartments defined by interaction lines within a cut birch stump. (b) Individuals of *Armillaria borealis*. Filled circles represent collection points. Lines encircle identical genotypes. (c) Individuals of *Phellinus weirii*. Filled areas represent infestations of *P. weirii* in a mountain hemlock forest from which cultures were obtained; clear areas represent infestations from which cultures were not obtained. Lines encircle more than one fungal infestation of identical genotype. Ages of the oldest individuals, estimated from observed growth rates, are given in years. Some of the older individuals have become fragmented into two or more infection centers. Reproduced, with permission, from Refs 40–42, respectively.
be determined. Also, gene conversion and mitotic crossing over in *C. albicans* lead to the loss of heterozygosity. The resulting genetic segregation occurs in the absence of any exchange of genes between individuals. We expect that genealogies for multiple nuclear loci will be necessary to resolve completely the question of whether genetic exchange and recombination occurs among individuals of *C. albicans*.

Whether the population structure of pathogenic fungi is recombinating or clonal is important. Identification of fungal genotypes based on neutral markers would only be predictive of their other medically important characteristics, such as pathogenic capabilities or drug resistance, if the population were highly clonal, and this is not always the case. In a recombinating population, any association between neutral markers and other traits would decay with time. Another important implication of recombination is that the fungi are expected to evolve more rapidly with regular genetic exchange and recombination than with strict clonality, because rare mutations to drug resistance or new pathogenic capabilities can be brought together more readily by recombination into new, more threatening genotypes. In a strictly clonal population, the same genotypes could only arise by sequential accumulation of rare mutations within a lineage.

**Mitochondrial genomes also recombine**

Although mitochondrial DNA (mtDNA) evolves clonally in many organisms, in a strictly clonal pattern of evolution, the observed mtDNA genotypes in natural populations of *A. gallica* are best explained by recombination, and not by mutation alone. Recombination in mtDNA is well known from laboratory studies of fungi. How can fungal mtDNAs of different descent come together and recombine? In basidiomycete fungi, there are no morphological sexes, and matings originate with the fusion of vegetatively growing hyphal hyphae. Successful mating produces a dikaryotic or diploid. At the same time, cytoplasmic mixing in the areas of hyphal fusion between paired mates carries the possibility of fusion of mitochondria and mixing of mtDNAs, with subsequent recombination. Because hyphal fusion is common in many fungi as part of the sexual cycle, mtDNA recombination, as described recently in *A. gallica*, could well be common in other fungi. This has two main implications. First, there will be no equivalent of the "mitochondrial Eve" hypothesis for fungi whose mtDNAs recombine, because there is no one genealogical history for the entire molecule. Second, mtDNA
Genealogies reveal barriers to gene flow

In fungi with genetic exchange and recombination, genealogical analysis can identify genetic barriers between groups of individuals22. In C. immitis, a second, sample of isolates showed the existence of two groups that are found in different geographical regions but are morphologically indistinguishable23. When nucleotide variation in five different regions of DNA is considered jointly, the genealogy of 17 isolates has only a single internal branch, which separates these two groups (Fig. 3). Within each group, genealogies are highly incongruent, indicating the prevalence of genetic exchange and recombination. Between groups, gene flow is absent. Consequently, these cryptic species within C. immitis are highly differentiated with many fixed differences and no shared polymorphisms. A similar situation occurs in Aspergillus fumigatus, except that one of the groups is clonal and the other recombining24. Complete genetic isolation carries the epidemiological implication that the fixed genetic differences might predict differences in pathogenicity or drug resistance.

Fungi as ideal subjects for experimental population genetics

Most previous studies of fungal population genetics have focused on selectively neutral variation in DNA (Ref. 35). The emergence of the field of genomics facilitates a significant new avenue of research in fungal population genetics. Genes of adaptive importance can now be more easily focused on selectively neutral variation in DNA (Ref. 35). Gene genealogies reveal barriers to gene flow and the definition of biological species,25 and the definition of the species Ainsworth and Bisby’s Dictionary of the Fungi, 4th ed. (Michod, R.E. and Levin, B.R., eds), pp. 106–125, Sinauer

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Recent advances in South American mammal paleontology

John J. Flynn and André R. Wyss

Review

South America was an island continent for most of the past 80 million years, from its separation from most other Pangaeum landmasses in the Late Cretaceous 65–80 million years ago (MYA) until the beginning of its full reconnection with North America via the Isthmus of Panama about 3.5 MYA. This long-term isolation produced a highly peculiar terrestrial biota, of which the paleontologically best known component is a diverse array of endemic mammals (marsupials, edentates, primates, rodents, and numerous ‘ungulate’ groups). Much has changed since Patterson and Pascual’s landmark paper in 1968 summarizing knowledge of the South American mammalian fossil record. At that time, there existed only one Mesozoic mammal specimen, a single age-calibrating radiometric date and scant information from outside the high temperate latitudes (Argentina, Patagonia). Recent efforts have resulted in vast improvements, including better age control, enhanced geographical, temporal, paleoecological and paleoenvironmental sampling and a greater understanding of the phylogenetic diversity of South American mammals.

Showing remarkably few signs of its age, Simpson’s ‘three-phase’ or ‘3-stratum’ concept remains a serviceable summary of South American mammal evolution (Fig. 1). Simpson’s Stratum 1 corresponds to the continent’s early phase of isolation, and the establishment of basal clades within many of its indigenous, ‘archaic’ lineages (e.g. edentates, notoungulates and xerotherm ungulates, and various marsupials). This Late Cretaceous to Early Cenozoic (~60–80 MYA) interval was dominated by warm and humid, tropical–temperate forest environments throughout South America1. The arrival of immigrant taxa (rodents and primates, most likely from Africa), and modernization of other faunal aspects during the mid-Cenozoic (reflecting adaptation to major environmental changes, including increased aridity and cooling) marks the base of Stratum 2. Stratum 3 corresponds to development of the Recent (Holocene) fauna, beginning with a few North American immigrants (‘waid dispersers’ or ‘heralds’ appearing sporadically, well before the main influx of ‘legions’ of diverse other taxa) around 10 MYA, culminating in the Great American Biotic Interchange (~3.5 MYA to the Present)16, Isotopic data from paleosols16 and mammalian dental enamel17 support the idea that climate and habitat change (including major changes in plant communities (from C3 to C4 dominated) beginning ~4 MYA) continued during Stratum 3. Recent work somewhat complicates this simple picture, providing important new fossil material (Fig. 2) and welcome details about the timing, patterns and processes explanations for evolutionary changes embodying the 3-stratum phases.