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Sources of Fungal Genetic Variation and Associating It with Phenotypic Diversity

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ABSTRACT The first eukaryotic genome to be sequenced was fungal, and there continue to be more sequenced genomes in the kingdom Fungi than in any other eukaryotic kingdom. Comparison of these genomes reveals many sources of genetic variation, from single nucleotide polymorphisms to horizontal gene transfer and on to changes in the arrangement and number of chromosomes, not to mention endofungal bacteria and viruses. Population genomics shows that all sources generate variation all the time and implicate natural selection as the force maintaining genome stability. Variation in wild populations is a rich resource for associating genetic variation with phenotypic variation, whether through quantitative trait locus mapping, genome-wide association studies, or reverse ecology. Subjects of studies associating genetic and phenotypic variation include model fungi, e.g., *Saccharomyces* and *Neurospora*, but pioneering studies have also been made with fungi pathogenic to plants, e.g., *Pyricularia* (= *Magnaporthe*), *Zymoseptoria*, and *Fusarium*, and to humans, e.g., *Coccidioides*, *Cryptococcus*, and *Candida*.

SOURCES OF FUNGAL GENETIC VARIATION

Genetic variation is the stuff of evolution: if there is no variation, there can be no evolution. This review of fungal genetic variation begins with a survey of its sources and then discusses means of associating natural genetic variation with phenotypic variation, including phenotypes that are important to fungal adaptation or those that interest cell and developmental biologists engaged in basic or translational research. The tale of the study of fungal genetic variation is also a tale of advances in genomic science, and it is appropriate to note that the first Eukaryote to have its genome sequenced was a fungus, the model yeast *Saccharomyces cerevisiae* (1). Shortly thereafter, yeast was joined by filamentous Ascomycota, e.g., *Neurospora* (2), and Basidiomycota, e.g., *Phanerochaetae* (3). Not long after, three *Aspergillus* species were sequenced—*Aspergillus oryzae* (4), *Aspergillus fumigatus* (5), and *Aspergillus nidulans*—and the comparisons of their genomes (6), along with those of yeasts closely related to *S. cerevisiae* (7), represent landmarks in the field of comparative fungal genomics. A survey

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taken in April 2017 of fungal genomicists associated with the Joint Genome Institute and FungiDB estimated the number of fungal species with sequenced and assembled genomes at 2,000 and estimated that another 1,000 to 1,500 genomes represent multiple individuals from species that are studied by population genomics. No other group of eukaryotes enjoys as deep a genomic database as is seen for the fungi.

Gene Gain and Loss

The sources of genetic variation covered in this review are listed in Table 1. One of the first types of genetic variation discoverable by comparing genomes was gene gain and loss by duplication or excision. Again, the model fungus *S. cerevisiae* was involved because it showed abundant gene duplication. The debate that ensued over the source of gene duplication, whether by whole-genome duplication (8) or by the accrual of smaller duplications (9), was settled in favor of whole-genome duplication when genomes of outgroup yeasts were analyzed (10, 11). This episode made two seminal contributions that will be revisited in this article: that interspecific hybridization is an important source of genetic variation in the Fungi (12, 13) and that duplications as large as chromosomes are routinely gained and lost throughout the Fungi (14, 15).

Most duplications are rapidly lost, and where duplicated regions are retained, natural selection is likely responsible, as will be seen below. As more representatives of Ascomycota were sequenced, broader comparisons became possible (16). These comparisons revealed abundant examples of gene gain and loss and made two additional discoveries. First, duplications are more common in proteins that respond to stress than in those involved in core metabolism and, second, where duplications are retained, they result in the evolution of differential expression of the paralogs more often than the evolution of new functions. Where the duplicated protein formerly participated in more than one pathway, duplication allowed the copies to evolve to focus on just one pathway or the other (16). This disentangling of pathways may explain why more duplicated genes are retained, and in a recognizable form, than would be predicted by evolutionary theory that assumed retention would require neofunctionalization (17). Although gene gain clearly gets more attention than gene loss, when it comes to plant-pathogenic fungi, gene loss can be an important contributor to infection of novel hosts through the loss of genes whose products engage host defenses (18, 19).

TABLE 1 Genetic variation and its use in associating genotype and phenotype

Sources of genetic variation in fungi
Single nucleotide mutations and recombination (76–82, 98)[a]
Gene gain and loss (7–17)
Gene family expansion and contraction (20–26)
Horizontal gene transfer (33–38, 45–53)
Selfish DNA (39–43)
Aneuploidy (54–60, 148)
Conditionally dispensable chromosomes (59)
Loss of heterozygosity (61–64)
Genome rearrangements (18, 19, 65–75)
Epigenetic modifications (83, 84)
Endofungal bacteria (85–90)
Fungal viruses (91–94)
Mitochondria (95–97)
Hybridization and introgression (111–141)
Using natural variation to associate genotype and phenotype
QTL, GWAS, and reverse ecology (98–110)
Hypothesis testing in wild fungi (142–147)

[a]References germane to topic.

Gene Family Expansion and Contraction

As regions of the fungal tree of life became more densely populated with whole genomes, comparative phylogenomics uncovered gene family expansions and contractions that could be correlated with adaptation (Fig. 1). Using an approach developed for all eukaryotes (20), plant pathologists compared the rice blast fungus, *Magnaporthe*, with *Neurospora* and *Aspergillus* and found expansion in nearly 10 gene families, including those associated with pathogenesis, e.g., cytochrome p450 genes and G-protein-coupled receptors (21). Subsequent addition of genomes expanded the search to more pathogens and the discovery of more expanded gene families including those whose products populate the secretome (22). The approach was refined (23) and expanded to animal-pathogenic fungi, where the shift from eating plant cell walls to eating animal protein was documented by expansions in genes coding for proteinases and contractions in those coding for cellulases and other enzymes that deconstruct plant cell walls (Fig. 1) (24). Gene family expansion and contraction have even been applied to major shifts in the geologic record, such as the end of the coal age (25). Here, while studying genes coding for enzymes that saprotrophic fungi used to decay lignin, mycologists found an expansion that they could correlate with the end of the Carboniferous age, thereby supporting speculation that these fungi helped spell the end of widespread coal formation. Of course, expansions and contractions are relative events, and

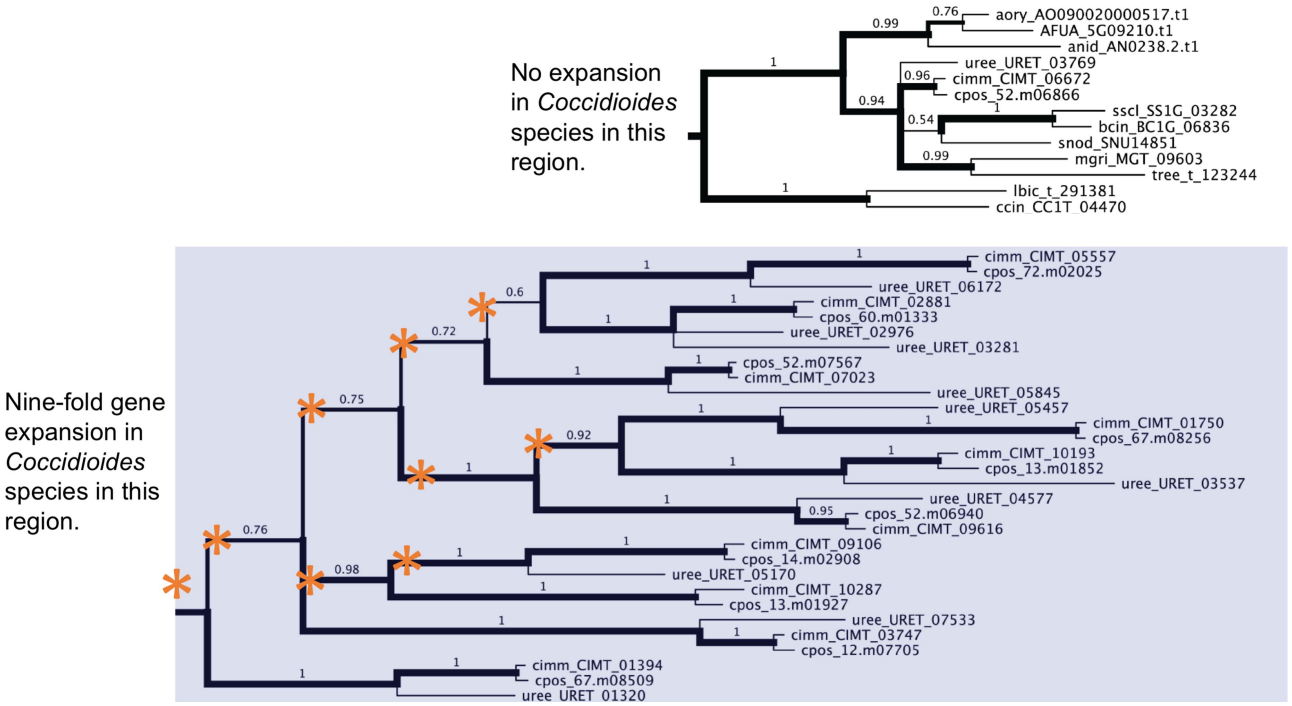


FIGURE 1 Gene family Bayesian phylogeny for proteinase genes with S8 domains showing (top) some phylogenetic lineages with no expansion and (below) others with a large expansion due to nine gene duplications (asterisks). Key to taxon abbreviations preceding gene identifiers: *Aspergillus oryzae* (aory), *Aspergillus fumigatus* (afum), *Aspergillus nidulans* (anid), *Uncinocarpus reesii* (uree), *Coccidioides immitis* (cimm), *C. posadasii* (cpos), *Sclerotinia sclerotiorum* (sscl), *Botrytis cinerea* (bcin), *Stagonospora nodorom* (snod), *Magnaporthe grisea* (mgri), *Trichoderma reesii* (tree), *Laccaria bicolor* (lbic), and *Coprinopsis cinerea* (ccin). Adapted from reference 24.

a caveat in the search for gene family expansion and contraction was recently sounded, where what was thought to be an expansion of genes coding for proteins with LysM domains in a group of dermatophytic fungi proved rather to be a contraction in a related fungal clade that harbors the systemic pathogens *Coccidioides immitis* and *Coccidioides posadasii* and their nonpathogenic relatives (26).

Interkingdom Horizontal Gene Transfer

The same genomes that permitted the discovery of gene duplication within a genome also permitted the discovery of genes gained from outside the species by the process of horizontal gene transfer (HGT). An early and prescient review discussed the possibilities in fungi (27) at a time when HGT was being studied in whole genomes of prokaryotes (28), and there have been a number of subsequent reviews (e.g., 29). To demonstrate HGT, it is necessary to show that the phylogeny of the transferred genes is truly in conflict with the organismal phylogeny as inferred from other genes. Before genomes, it was not easy to demonstrate HGT, but whole genomes provide more than enough data to achieve a statistically significant result. Plant-fungal HGT has been shown to work in both directions and, although infrequent and apparently ancient, exchanges of transport proteins and siderophores are thought to have aided the invasion of the terrestrial environment by both fungi and plants (30). Far more common are HGT events between the kingdom Fungi and Oomycota; here it can be fairly said that HGT from Fungi, mostly Ascomycota, enabled Oomycota to become plant parasites (31). Among the genes transferred from Ascomycota to Oomycota are those that code for proteins responsible for deconstruction of plant cell walls, uptake of nutrients, resistance to plant defenses, and the aggressiveness of the parasitism. Traffic from Oomycota to Fungi is far less frequent, with the best example being a monooxygenase-like protein involved in antibiotic synthesis (31).

Fungi living in the guts of animals also experience HGT, and one of the most remarkable examples of interdomain HGT concerns the Chytridiomycota that live in the stomachs of ruminants. These fungal symbionts clearly received glycosyl hydrolase genes from

bacteria, a transfer that helped them adapt to feeding on plant cell walls in the stomachs of ruminants (28). Gut fungi in the Zoopagomycota also have experienced HGT in the form of a ubiquitin gene received from their mosquito hosts (32). Could these genes, known to mark proteins for degradation or to alter their function or location, be involved in manipulating the host? As related above, interkingdom and interdomain HGT is well documented, but the mechanisms of gene transfer among distantly related organisms are not understood.

Transposable Elements

Not all HGT involves genes that confer an advantage to the recipient, at least initially. Some involves selfish nucleic acids, and the most prevalent types are transposons and retrotransposons, which are capable of moving and copying themselves in one genome or among genomes (33–35). Transposable elements (TEs) provide genetic variation by disruption of existing genes and gene order, by stimulating rearrangements and duplications, as well as by simply adding genetic material that can then evolve to new functions. Many fungi have an active defense against TEs, e.g., repeat induced point mutations (RIP) (36). RIP targets regions of repeated DNA sequence and in both copies mutates C-G pairs to A-T, sometimes spilling over into an adjacent, unduplicated sequence. Thus, it can reduce genetic variation by restricting gene duplication and also enhance it by generating mutations (37). However, the strength of RIP varies widely among fungi, as does the presence of TEs, and many symbionts, whether mutualists, i.e., ectomycorrhizal fungi, or pathogens, have large genomes due to an abundance of these mobile elements (38). A recent study of a Basidiomycota plant parasite, *Microbotryum*, showed that, despite RIP, TEs show bursts of activity that periodically increase the size of fungal genomes (39). Another recent study, focused on the Ascomycota plant pathogen *Fusarium*, investigated the role of TEs in a type of aneuploidy that will be discussed below (40). A different type of TE is a homing endonuclease, which enters recipient genomes at extremely conserved sites, e.g., ribosomal RNA genes, and which spreads faster than Mendel imagined by being transmitted to all progeny. Homing endonucleases have a "life cycle" of transmission to a new species followed by the spread among the individuals of the new species until all available invasion sites are filled. At that point, there can be no additional transmission and no selection for active endonuclease, so the elements degenerate and disappear from the species, making it ripe for new introduction (41, 42). Work on these elements in yeast led to a proposal that homing endonucleases could be used to extirpate insect vectors of disease, such as the mosquitoes that vector malaria (43), an idea that has gained popularity with the application of the CRISPR-associated protein-9 nuclease system to genetic manipulation (44).

HGT Among Fungi

Fungus-to-fungus HGT of coding genes is also a source of genetic variability involved in adaptation, and none is more spectacular than the transfer among Ascomycota of a toxin gene, *ToxA*, that instantly confers on the recipient the ability to attack wheat cultivars that possess the toxin-sensitive allele of the plant gene *Tsn1* (45). In this case, the direction of HGT could be determined by assessing, in the two fungi, the amount of genetic variation in the transferred toxin gene, *ToxA*. Using the logic that the more variable species was the donor and the species with little to no variation was the recipient, the flow could be shown to be from *Parastagonospora nodorum* to *Pyrenophora tritici-repentis* (45). Remarkably, the plant gene targeted by the toxin is a member of a wall-associated class of kinase receptors that help plants resist rusts and biotrophic fungal pathogens by killing affected cells and starving the fungus. When the fungus is a necrotroph, as is *P. tritici-repentis*, the programmed plant cell death initiated by the toxin instead opens the plant to attack by the necrotroph—a heavy cost to maintaining resistance genes (46–48).

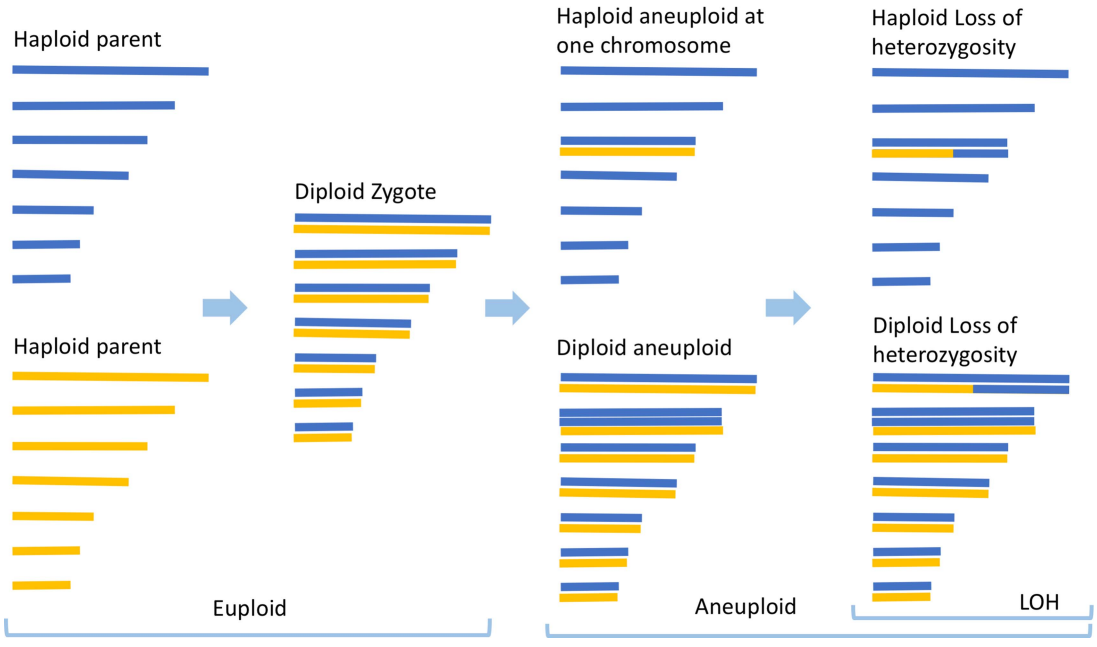
Among cases of fungal HGT, equally amazing as the ToxA story is the horizontal transfer of the multigene complexes that produce secondary products, or extrolites, e.g., polyketides such as aflatoxin or nonribosomal peptides such as penicillin. Although most polyketides may be transmitted vertically (49), it is clear that these complexes experience HGT, and it is equally clear that the dense clusters of genes in the complexes and their position near telomeres facilitate their transfer (50). It even has been hypothesized that the genes are aggregated to foster their later spread by HGT (51). Although it is clear that production of a specific extrolite is less likely to be transferred if the genes responsible for its synthesis are distributed throughout a genome, it is difficult to understand how selection could act on a potential future event. The more prosaic explanation for the clusters, i.e., that natural selection favors gene clusters because their regulation is better coordinated, likely provides the selective advantage needed to keep clusters intact, as in the case of the massive clusters found in species of the Ascomycota, *Penicillium*, involved in cheese making (52, 53).

Aneuploidy

The advent of massively parallel methods of DNA sequencing, and the high throughput sequencing they support, gave mycologists the ability to obtain genomes from populations of individuals. The strains of fungi that are resequenced to enable population genomics can come from members of a natural, interbreeding population, from strains collected from a clonal outbreak of a pathogen spread by asexually produced spores, or from a series of strains that have evolved inside a single patient. It is the last of these collections, made from a single patient infected with the pathogenic yeast *Candida albicans*, that let genomics demonstrate the importance of aneuploidy, the possession of more or fewer chromosomes than normal, as a source of fungal genetic diversity (Fig. 2) (54). In this study, *C. albicans* strains that had been isolated from candidiasis patients undergoing treatment with azole antifungal drugs had an extra, aneuploid chromosome made of two copies of the left arm of chromosome 5. In this diploid yeast, this aneuploidy doubled the copies of efflux pumps that can export the drug, a transcription factor positively regulating the pumps, and the target of the azole drug, an enzyme in the ergosterol synthesis pathway. Studies of another human pathogenic fungus, the Basidiomycota *Cryptococcus neoformans*, showed that a different type of clonal reproduction, unisexual mating, whereby meiotic basidiospores arise from the fusion of two mitotically produced nuclei, can also generate aneuploid progeny (55). In this latter research, the authors documented shortening of chromosomes among strains, as well as chromosome duplications, and found that unisexual progeny displayed significant phenotypic variation for traits involved in virulence. These traits included temperature sensitivity (but without loss of virulence in mice), increased resistance to antifungal azole drugs, and increased production of the virulence factor melanin. The authors also showed that unisexual mating was not essential for the generation of aneuploidy because the phenomenon was also seen in biparental, sexual progeny of two different *Cryptococcus* species (55).

Aneuploidy has also been shown to be involved in virulence in another animal pathogen, the Chytridiomycota that is responsible for amphibian decline, *Batrachochytrium dendrobatidis*. Amphibian decline has been correlated with the global pathogenic lineage (GPL) of strains obtained from infected animals. Aneuploidy is seen in both the GPL and, to a lesser extent, among individuals in the population from which the GPL emerged (56, 57). Data presented in both of these referenced studies show that passage of the fungus in the laboratory can result in loss and gain of aneuploid chromosomes and that genes known to code for virulence factors are hot spots for genomic change. More recently, comparison of the genomes of isolates from the same lineage, one original and the other having endured 6 years of

FIGURE 2 Diagrams showing aneuploidy and loss of heterozygosity (LOH) in haploid and diploid genomes. Each individual has seven distinct chromosomes colored to show heterozygosity.



laboratory transfers, also showed a correlation between reduced virulence and aneuploid loss of chromosomes (58).

Conditionally Dispensable Chromosomes

A different type of aneuploidy is seen in symbiotic Ascomycota, whether parasitic or mutualistic, that may have more chromosomes than do their nonsymbiont relatives. Unlike canonical aneuploidy, here the extra, conditionally dispensable chromosomes (CDCs) are different from, and in addition to, the chromosomes in the core genome (Fig. 3). Their presence varies among individuals in the species, and CDCs contain a disproportionate number of TEs. They may contain virulence factors not seen in the core genome, and they can be transmitted among species by hybridization, thereby conveying the ability to become parasites (59). A recent study with a well-assembled genome of *Fusarium poae* reported that the dispensable chromosomes lack an effective RIP, which explains their being riddled with TEs (40). This study also showed that CDCs act as a reservoir for duplicated genes and that the genes can then be transferred back to the core genome. Most of the fungi possessing dispensable chromosomes are plant symbionts, and one wonders if access to the abundant resources of a photosynthetic organism allows them to support an enlarged genome. Could this source of variation be a benefit of association with a domesticated plant, albeit an evolutionarily Faustian bargain given the danger of narrow host specialization? Alternatively, do fungi that have their pathogenicity factors in volatile regions of the genome respond more quickly to changes in host plant defenses and thereby avoid narrow host specialization?

Another type of unusual ploidy is demonstrated by hybridization among haploid, Ascomycota grass parasites in the genus *Epichloë*. These events, apparently not involving sexual processes, have produced diploid and triploid, asexual, mutualistic endophytes that are dispersed as hyphae in the seeds of their hosts. This unusual combination of aneuploidy and HGT, albeit among close relatives, has produced fungi that have a life history that is extremely different from their parental species, which are sexual, *Epichloë* species that transmit themselves horizontally via their ascospores and then aggressively parasitize and sterilize their grass hosts (60).

Loss of Heterozygosity

Where organisms are more than haploid, i.e., diploid, aneuploid, or polyploid, they are almost certainly heterozygous at many genes, itself an obvious source of genetic variation. These heterozygous organisms can lose heterozygosity through gene conversion or crossing over or other events that leave portions of homologous chromosomes genetically identical (61) (Fig. 2). At first it might seem that loss of heterozygosity would necessarily reduce phenotypic variation, but phenotypic diversity can be increased if dominant alleles are lost and release the activity of the recessive allele. Such a case could also occur through aneuploidy, where loss of one copy of a chromosome would expose recessive alleles on

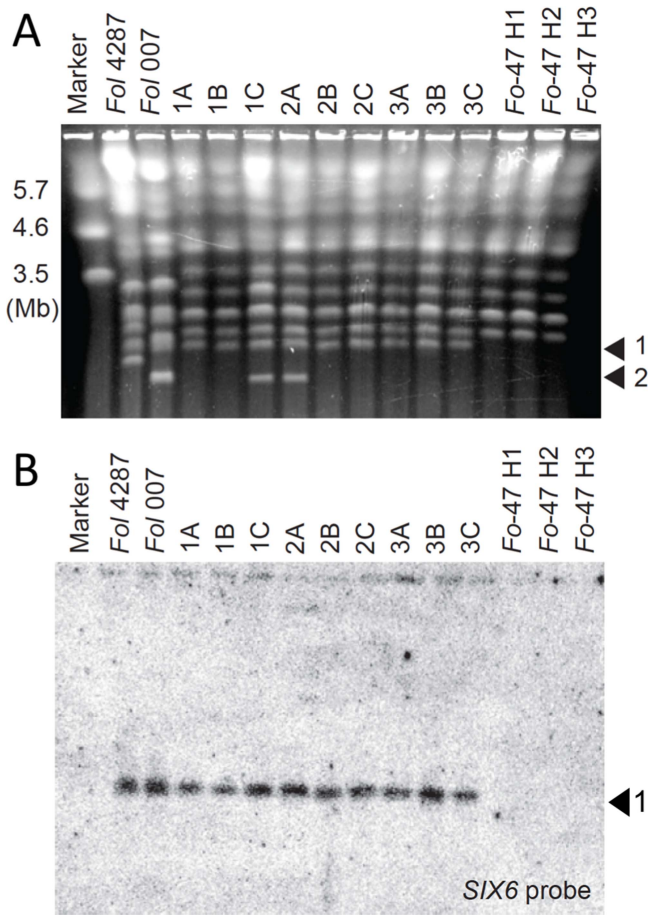


FIGURE 3 Contour-clamped homogeneous electric field gel karyotype of *Fusarium oxysporum* chromosomes showing conditionally dispensable chromosomes (CDCs) and their transmission between strains. **(A)** Donor strain *Fol*007 (left) harbors CDCs 1 and 2 (arrows), and recipient strain *Fo*-47 (right) lacks them. Strains 1A-3C (middle lanes) are derived from simple coincubation of *Fol*007 and *Fo*-47. These strains have the *Fo*-47 karyotype and have gained CDCs 1 or 2 (arrows), or both, from *Fol*007. **(B)** Southern hybridization of the contour-clamped homogeneous electric field gel to a probe with DNA from CDC 1 (*SIX6*), confirming the presence of CDC 1 in donor strain *Fol*007 and progeny strains 1A-3C, which possess the karyotype of the recipient strain *Fo*-47. Adapted from reference 148.

the remaining, now haploid, copy. An insightful example of this type of loss of heterozygosity was reported in *C. albicans* strains that are resistant to azole antifungal drugs due to the activity of multidrug efflux pumps. Here, it was mutations in the gene coding for a transcription factor on chromosome 3 (which regulates the aforementioned pumps on chromosome 5) that were responsible for the constitutive expression of the pump genes. *C. albicans* being diploid, and the mutant transcription factor genes being recessive, the phenotype was seen only when the dominant wild-type allele was lost. Selection by the azole drug favored strains of *C. albicans* that both had the mutation in one of the copies of the transcription factor and had lost the wild-type allele through aneuploidy (62).

One of the tenets of population biology, Haldane's sieve, states that adaptive alleles are more likely to be dominant because newly mutated alleles are rare and cannot experience positive selection unless they are dominant and support a heterozygous phenotype. However, adaptation due to recessive alleles being released from dominance by loss of heterozygosity was recently demonstrated in an experimental study employing *S. cerevisiae* strains under selection for resistance to the antifungal drug nystatin (63). Diploid strains were created that were either homozygous for a recessive allele that conferred resistance to nystatin or heterozygous with a wild-type, susceptible allele. When grown without the drug, fitness was not strictly correlated with hetero- or homozygosity. When, however, the strains experienced nystatin stress, the strains homozygous for the resistant allele outperformed heterozygotes unless loss of heterozygosity removed the dominant, susceptible allele from heterozygotes, showing how recessive alleles can be adaptive. Loss of heterozygosity has also played an important role in the evolution of the GPL of *B. dendrobatidis* by generating variation among individuals of the GPL through recombination associated with mitosis, alone or in combination with meiosis (64). Here, is it possible that the loss of heterozygosity has released recessive alleles that promote virulence in this amphibian pathogen?

Genome Rearrangement

Rearrangement of the genome is also a source of fungal genetic variation, and one that provides a mechanism for gene family expansion and contraction through gene duplication, deletion, or disruption (65). In the Ascomycota class Dothideomycetes, comparison of rearrangements among the genomes of 18 representative taxa showed that most rearrangements were within chromosomes (66), an example of mesosynteny (67). The most intensive study of fungal genome rearrangements has focused on those associated with an absence of recombination, specifically in mating loci and mating chromosomes. In these regions, recombination in the mating locus would produce progeny unable to mate, a phenotype readily eliminated by natural selection. Studies of barriers to recombination associated with rearrangements have been enabled by well-assembled genomes in the Ascomycota *Neurospora tetrasperma* (68, 69) or in the Basidiomycota *Cryptococcus* (70, 71) and *Microbotryum* (72–74). An interesting but unanswered question is whether the rearrangements seen in these mating regions suppress recombination or whether recombination is suppressed by some other means, thereby allowing rearrangements to accumulate. Expect much more research on the impact of genetic rearrangements in the near future (75) as it becomes economically feasible to practice population genomics with the superbly assembled genomes provided by the new generation of long-read, single-molecule DNA sequencers.

Mutation and Recombination

Until this point, we have avoided mention of the obvious contribution of sexual recombination to fungal genetic variation, although mutation, mating, and recombination, which provide the ability to generate progeny with genotypes different from parents and siblings, are at the heart of genetic variation. So important is this contribution that large natural populations, as must be the norm for many fungi, are thought to have all possible mutations in their genomes as a result of a combination of recent, ongoing mutation and standing variation (76). A recent study of polymorphism in North American and Russian populations of the Basidiomycota *Schizophylum commune* showed that this fungus is the most genetically diverse Eukaryote yet discovered, due to a rapid mutation rate and likely a large population size (77). As diverse as *S. commune* is, it is not alone and is in the close company of roundworms and choanoflagellates (78). Despite all of the costs associated with mating and recombination compared to clonal reproduction, i.e., from finding a mate, to loss of well-adapted genotypes, to having to share half of the genomes of progeny with a partner, sex and recombination are omnipresent in fungi (79) because they accelerate adaptation to changing environments, as shown by careful experimentation employing *S. cerevisiae* (80). Variation, however, cannot be limitless because some genetic combinations must be deleterious and selected against under conditions encountered in nature. Evidence for these natural

limits is seen when phenotypes of parents and their progeny from laboratory crosses are compared; the variation in the progeny typically exceeds that of the parents or the natural population from which they were sampled. This transgressive variation exhibited by the progeny is often seen in the progeny of artificial crosses for complex traits, for example, the fungal phenotypes of aggressiveness in a plant pathogenic fungus (81) or growth rate in a wood decay fungus (82). Sexual reproduction deserves more mention, and it will be taken up again, below, in the section on associating genetic and phenotypic variation.

Epigenetics

Epigenetics is another important source of variation in fungi, not only among individuals but also among hyphal segments in a single individual (83), and its true importance is likely not yet appreciated because no population study of epigenetic variation has been published. As with studies of rearrangements, studies of epigenetic variation are to be expected in the near future because the aforementioned new generation of long-read, single-molecule DNA sequencers also have the ability to assess methylation of nucleotides (84).

Endosymbiotic Bacteria

Endofungal, bacterial symbionts, although not part of the fungal genome *per se*, are also a source of genetic variation for fungi, and one whose importance is emerging. These endosymbionts had been discovered in arbuscular mycorrhizal fungi by microscopy (85), but the extent of their presence was not appreciated until genomic studies of fungi revealed bacterial DNA among the expected fungal sequences (86). Recent studies show a fitness advantage to fungi possessing the endobacteria (87) and a lack of genome degeneration in the endobacteria themselves (88, 89). Nor are endosymbiotic bacteria restricted to arbuscular mycorrhizal fungi; one well-studied endobacterium is in the Mucoromycota *Rhizopus microsporus*, where it produces a toxin essential for the ability of this fungus to parasitize rice (90).

Fungal Viruses

Less explored is the role of fungal viruses as sources of genetic variation in fungi. The best-studied case is a virus that reduces virulence and reproductive capacity in the tree pathogen *Cryphonectria parasitica* (91). An equally compelling example is a virus that, along with its host Ascomycota, *Curvularia protuberata*, confers improved thermotolerance in this endophytic fungus and its host plant, *Dichanthelium lanuginosum*, allowing them to live in geothermal areas of Yellowstone National Park (92). Although it is clear that fungi have strong defenses against virus (93), a recent search of RNA and virus particles isolated from five well-known plant-pathogenic fungi using a metatranscriptomic approach found 66 previously undescribed mycoviruses (94). Surely, many additional virus sequences are lurking unrecognized in the unsequenced pool of fungal genetic material. The nucleic acid diversity found in viruses and endofungal bacteria, plus that found in another cytoplasmic feature, the mitochondrion, can contribute to phenotype directly and by interacting with nuclear diversity. The role of cytoplasmic elements in heritability of a fitness phenotype, growth rate, was recently explored in yeast (95) and might also prove valuable in studies of fungi where mating can create progeny with identical nuclei but different mitochondria, for example, Basidiomycota (96) or dikaryotic Ascomycota (97).

USING NATURAL GENETIC AND PHENOTYPIC VARIATION TO ASSOCIATE GENES WITH PHENOTYPES

Having discussed different sources of genetic variation for fungi, it is time to ask, Is there any practical value to this knowledge—that is, value beyond satisfying the insatiable curiosity of mycologists and, of course, providing the genetic variation that enables fungi to adapt to the ever-changing environment? A simple answer to this question is that the same genetic variation that facilitates the adaptation of fungi can be used to solve a problem faced by many modern biologists, i.e., finding the genetic features responsible for a phenotype of interest or, more generally, associating phenotypic variation with genetic variation.

The Standard Laboratory Strain

It is still the case that molecular, cell, and developmental mycologists use a few strains of their study organism to facilitate comparison of research conducted in different laboratories and at different times. For example, neurosporologists favor *Neurospora crassa* OR74A (also known as FGSC 2489), saccharomycetologists favor S288C, cryptococcologists favor H99, candidologists favor SC5314, aspergillologists favor A4, and so on. Alas, in doing so, they miss out on the alleles and traits found in natural strains and they work with an individual that has become adapted to the laboratory environment and is no longer representative of the wild population from which it was cultivated. The key

question for cell and developmental mycologists is, Would moving to multiple wild strains to better associate genetic and phenotypic variation be worth the loss of comparability found in a standard strain?

First, it must be acknowledged that mycologists who work with standard strains go to a lot of effort to develop genetic variation in the standard strain by inducing mutations. With the forward-genetic approach, which begins with a phenotype of interest and artificially induces mutations in thousands of strains, the mycologist then has to screen thousands of mutants and, having found the desired mutant phenotype, associate the mutant gene with the mutant phenotype. If, instead of a phenotype, the researcher begins with an interest in a particular gene, then the process is reversed and the gene is first mutated or deleted, and the search begins for a phenotype, a process that can seem never-ending given that a particular phenotype may be encountered only under very specific and unknown conditions. For both forward and reverse genetics, the sequencing of the standard strain is a boon that facilitates either identifying the gene or confirming that it, alone, has been mutated, but it is also a boon that reinforces the community focus on standard strains.

Quantitative Trait Loci

A traditional way of increasing genetic and phenotypic variation in a study without resorting to mutagenesis is to mate the standard strain with a partner that shows genetic and phenotypic differences and then use the progeny to associate phenotypic and genetic variation. As mentioned above, the progeny will display variation in the form of combinations of alleles and resulting phenotypes that exceed those seen in nature. The genes or loci responsible for complex, quantitative traits can then be mapped in this quantitative trait locus (QTL) approach using markers developed to cover the genome. This approach was used with the filamentous fungus *Neurospora crassa* to find QTL associated with female mate choice and to show that the reinforced barriers to sexual reproduction behind mate choice had evolved by natural selection (98). However, as is often the case with QTL studies, the authors were not able to find specific genes responsible for the trait. This problem arises because each QTL can comprise tens to hundreds of genes, owing to the relatively few recombination events in a meiosis, even when hundreds of independent meioses are involved in generating a progeny population. With QTL analysis, full genomes are not a panacea, because it is not a paucity of markers that is the problem but, rather, the large regions in linkage.

Two means of decreasing the size of the linked regions are typically employed. One, an advanced intercross, simply increases the number of matings and meioses used to make the progeny for the mapping population. Although it is labor-intensive to make the cross, this method has been used successfully with *S. cerevisiae* to associate genes with the phenotype of stress resistance (99). The other approach, bulked (or bulk) segregant analysis, increases the number of progeny and, when phenotyping the progeny, sorts those with the most extreme phenotypes into two groups, e.g., those that are the most tolerant and least tolerant to a stressful environment. Bulked segregant analysis is even more efficient when the phenotypic screen recovers only survivors, for example, growth in the presence of an antifungal drug. Individuals with the extreme phenotypes, or the survivors, are sequenced en masse, and the reads are aligned. In these alignments, nearly all genes show the variation present in both parents, except for genes strongly associated with the phenotype; here, the allele of one or the other parent will predominate. Bulked segregant analysis has been used successfully with fungi to find genes involved in sugar metabolism in *Saccharomyces* (100), salt tolerance in the Ascomycota *Lachancia* (101), and, in *Neurospora*, temperature-responsive cell cycle regulation (102) and germling communication and fusion (103).

Recognizing Populations of Fungi

The genetic and phenotypic variation in a study can be further increased by studying many individuals in a wild population and then using an approach developed for studies of human genetics, genome-wide association studies (GWAS), or reverse ecology, an approach developed to mine populations of genomes, among them *S. cerevisiae*, to find genes with a history of strong natural selection (104). As noted above, with QTL analysis, even the hundreds of independent meioses resulting from a cross of fungi cannot break long, linked regions of the genome. In contrast, the old history of mating and recombination in a wild fungal population makes for very short linked regions and remarkable precision in associating genes and phenotype. For example, a recent GWAS in *Neurospora* found that single nucleotide polymorphisms (SNPs) in the 3′ untranslated region of a gene, but not SNPs in the coding region, were associated with the phenotype, a hypothesis validated by subsequent gene deletion experiments (105). GWA requires that populations first be accurately recognized (Fig. 4). If GWA is mistakenly practiced on a mixture of even two populations, the strongest phenotypic differences, which are most likely to be studied, will be between the popu-

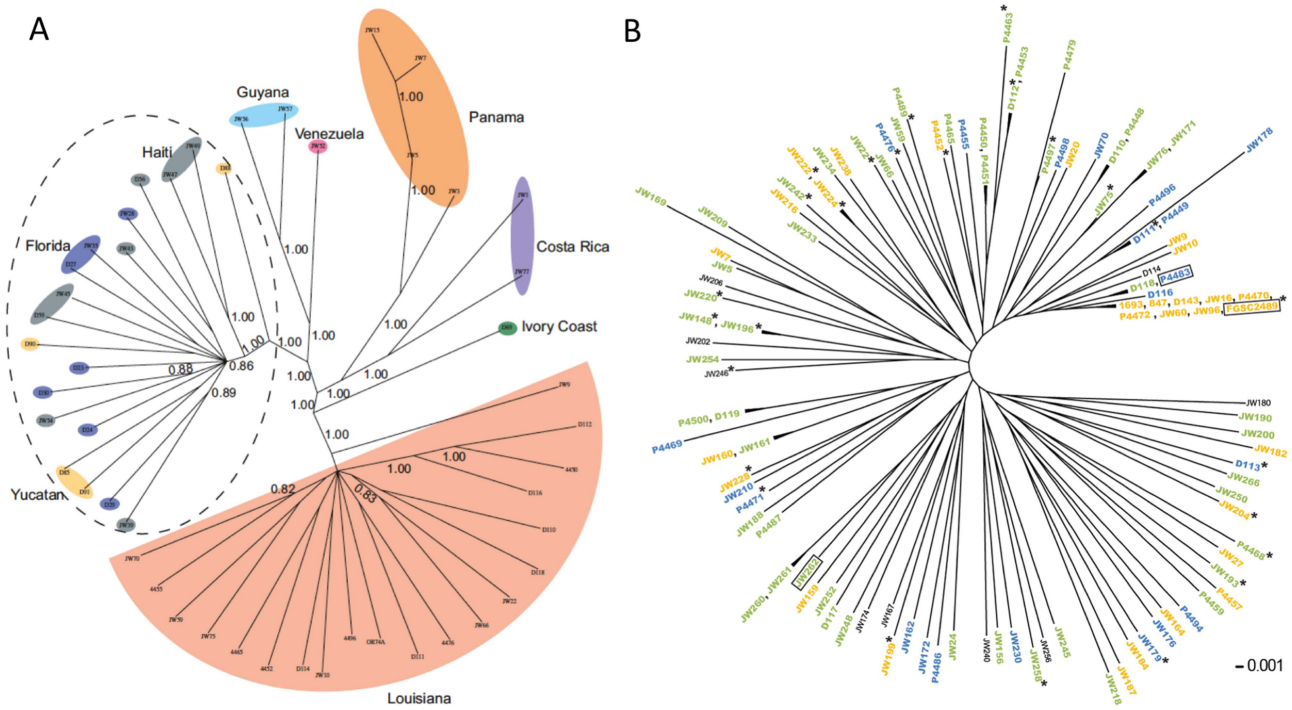


FIGURE 4 Population structure. **(A)** Bayesian phylogenetic analysis of SNPs from transcriptomes of 50 *Neurospora crassa* individuals from around the Gulf of Mexico showing that individuals thought to form one population actually are found in seven populations. Adapted from reference 112. **(B)** Bayesian phylogenetic analysis of SNPs from transcriptomes of 112 *N. crassa* individuals from the same geographic area as the Louisiana population in A showing no population subdivision. Note the many individuals with the same genotype as the laboratory strain, FGSC 2489, indicative of mistakes made in transferring isolates. Adapted from reference 103.

lations and will associate with every fixed, genetic difference between populations. At the outset, one cannot know the extent of a fungal population, so the genomes of a representative sample of individuals, selected to maximize geographic and phenotypic diversity, must be sequenced, and the variation in the DNA sequence, i.e., the SNPs, compared to identify the populations. Well-diverged populations can be recognized by using the SNPs to construct a phylogenetic tree or network. Population structure can also be discovered using clustering approaches. Phylogenetic trees have the advantage that statistical support for branches can be assessed; clustering methods have the advantage that admixture can be detected. The process of recognizing populations is iterative and may require additional collection if what was thought to be a single, interbreeding population proves to be a complex of several populations.

Genome-Wide Association Studies (GWAS)

GWAS in humans require thousands to tens of thousands of individuals to accurately associate genes with complex traits, e.g., heart disease. However, with fungi, studies employing as few as 23 individuals have found genes associated with traits as complex as pathogenesis or signaling between individuals in a species of fungi, although these genes enjoyed a balance of alleles in the population and a strong effect on the phenotype. A pioneering GWAS of virulence in the Basidiomycota pathogen of coniferous trees, *Heterobasidion*, used just 23 individuals to find a dozen SNPs associated with virulence, and 4 of them were in genes already known to affect virulence in other fungi (106). Cell-to-cell communication and fusion between germinating conidia provided another complex trait where GWAS successfully associated a gene coding for a calcium sensor with the communication trait using just 24 *Neurospora* individuals, albeit 24 individuals preselected from a larger, preliminary screening (105). In this *Neurospora* study, the one significant association, to a calcium sensor, led to another two genes whose proteins were known to associate with the sensor and, when a higher likelihood of false positives was accepted, another six genes associated with the trait. In this study, the nine hypothesized associations were validated (i.e., could not be disproved)

by gene deletion experiments (105). Among recent studies with plant-pathogenic Ascomycota (reviewed recently [107]), GWAS found a new virulence factor in *P. nodorum* (108), the gene coding for a small secreted protein virulence factor in *Zymoseptoria tritici* (109), and genes for virulence, toxin production, and sensitivity to azole antifungal drugs in *Fusarium graminearum* (110).

Hybridization

The ideal natural population for GWAs would be an old, natural hybrid population with twice the variation of the species contributing to the hybrids and the short genomic regions in physical linkage found in freely interbreeding groups. There is evidence that these populations do occur in fungi, as shown by a study of a parasite of wild grass in Iran, *Zymoseptoria pseudotritici*, a relative of the well-known wheat pathogen, *Z. tritici* (111). Comparison of genomes from five *Z. pseudotritici* individuals showed, in half of the genome, twice the genetic variation seen in *Z. tritici* and, in another half, no variation. In the genome half where variation was found, it was of just two types, and within each type there was no variation. The authors inferred that *Z. pseudotritici* originated from a population of F1 hybrids between two, as yet undiscovered, *Zymoseptoria* species, that the hybrids were not able to mate with either parent population, and that severe bottlenecks caused the loss of parental variation in the young F1 population. The example of *Z. pseudotritici* adds homoploid hybrid speciation (speciation originating by hybridization with no increase in chromosome number) to the ways that fungal species can originate and, in this case, it was estimated to have occurred as recently as 200 years or 380 to 550 sexual generations ago. *Z. pseudotritici* sports dispensable chromosomes, like *Z. tritici*, which may indicate that such chromosomes are not restricted to fungi that parasitize agricultural crops. The *Z. pseudotritici* results found hybridization without introgression, but hybridization with introgression has also been discovered in fungi and provides a way for mycologists to discover genes important to adaptation, as will be discussed in the next section.

Gene Flow between Populations

Where population genomic studies of fungi have discovered more than one population or species, they have often also found evidence of gene flow into the populations or among the species. The explanation for this gene flow is that mating between members of the different populations or species leads to hybrids, and these hybrids can then mate with individuals in either of the parental populations, promoting introgression of genes from one population into the other. The regions that persist in the recipient population are those whose genes confer a selective advantage to the recipient and, if the advantage is strong enough, sweep through the recipient population. These regions can be detected in populations of fungi with sequenced genomes because they show either exceptional divergence or exceptional similarity compared to the surrounding genome, as described below.

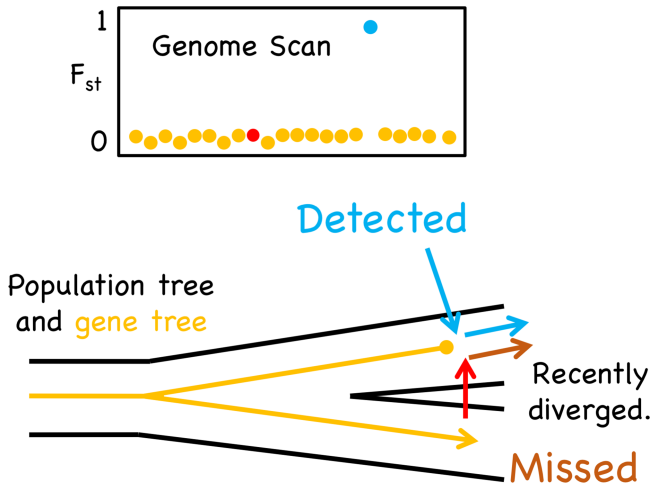


FIGURE 5 Hybridization and introgression in weakly diverged populations. Hybridization and introgression can be detected in genome scans of closely related populations when the genes are introduced from a more diverged population. (**Top**) Genome scan by Fst (a measure of relative genetic divergence) showing that nearly all genes have low divergence (yellow dots and one red dot), but one gene shows exceptionally large divergence (blue dot). (**Bottom**) Population tree with one gene tree highlighted in yellow showing that well-diverged genes entering from older, more diverged populations (blue dots and arrows) will be detected by comparison with the low divergence in the rest of the genome. However, genes exchanged between the populations will be missed (red dots and arrows) due to their low divergence being indistinguishable from the rest of the genome.

When the population divergence is recent and the genomes of both populations are aligned, regions of exceptional divergence are easily recognized against the background of very similar regions (Fig. 5). Where the population divergence is older, regions of exceptional similarity stand out, but regions of exceptional divergence also may be detected (Fig. 6). The genomes resequenced from the populations need not be complete; they can even be transcriptomes, as long as one well-sequenced genome is available to align the others. To search for regions of exceptional divergence or similarity, measures of genetic diversity are calculated for short windows of aligned sequence and the process is repeated

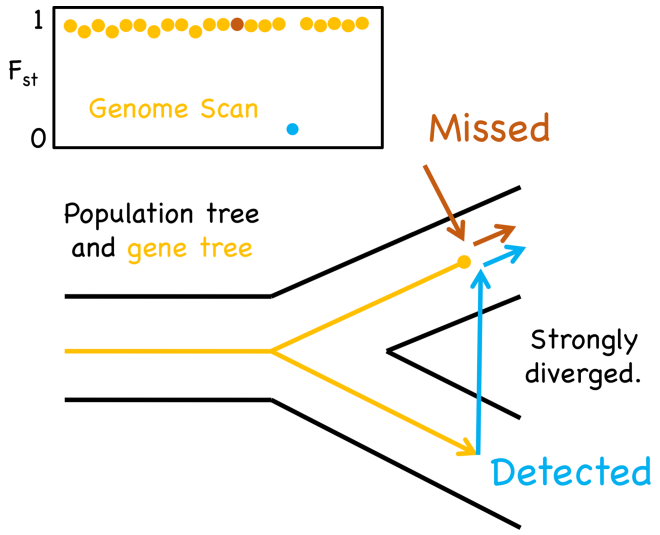


FIGURE 6 Hybridization and introgression in strongly diverged populations or species. Hybridization and introgression can be detected in genome scans of distantly related populations or species when the gene flow is between the two well-diverged groups. (**Top**) Genome scan by Fst (a measure of relative genetic divergence) showing that nearly all genes have high divergence (yellow dots and one red dot), but one gene shows exceptionally low divergence (blue dot). (**Bottom**) Population tree with one gene tree highlighted in yellow showing that genes exchanged between the populations will be detected (blue dots and arrows) due to their lack of divergence compared to the high divergence of the rest of the genome. However, genes entering from populations from other well-diverged lineages (red dots and arrows) will show divergence similar to the rest of the genome and be missed.

as this virtual window is moved along the entire genome. Measures of divergence that have been used for these sliding windows include measures of relative divergence (relative between populations), e.g., Fst, and those of absolute divergence (within regions of aligned genomes), e.g., Dxy (112). Dxy is simply the number of nucleotide substitutions per site between populations and Fst is the proportion of genetic variance found in one population compared to that found in all populations. Use of several measures is recommended because each can be confounded by different patterns of nucleotide variation (113, 114).

There are limits to this approach. In the case of recently diverged populations, the scant variation between the populations is not sufficient to permit discovery of any regions that have moved between the population, although such exchange must be frequent (Fig. 5). Instead, it is regions that have invaded the populations from more diverged populations, likely unknown, that can be found (Fig. 5). Conversely, when comparing well-diverged populations, regions that move into one or the other population from evolutionarily more distant populations may not appear to be more diverged than the rest of the well-diverged genomes (Fig. 6). In this case, it is the regions that move between the populations that are easily detected, because they show little or no divergence (Fig. 6).

Yeast Population Genomics

As might be expected, the first fungal report of population genomics was with *S. cerevisiae* (115), finding populations in the species that correlated with geography and evidence of hybridization, likely associated with domestication of this socially important fungus. When 99 Asian individuals were added to the analysis, the amount of genetic variation doubled and new populations were discovered, some of them in areas undisturbed by human activity (116). Surprisingly, neither GWAS nor reverse ecology has been practiced with *S. cerevisiae*, although the potential for such studies seems immense (117). One reason that such studies in yeast would be rewarding is that hypothesis testing is possible on a remarkably large scale, as shown in a recent study of sugar use in *S. cerevisiae* and its close relative, *Saccharomyces bayanus* (118). *S. cerevisiae* is a picky eater, preferring glucose when fed mixtures of glucose and galactose, whereas *S. bayanus* consumes both indiscriminately. To test the hypothesis that the phenotype of sugar use was under control of the seven-gene galactose pathway, the researchers swapped promoters of all seven genes, en masse, from *S. cerevisiae* into *S. bayanus*, largely recapitulating in *S. bayanus* the *S. cerevisiae* phenotype. As the authors note, "genetic mapping of complex phenotypes is within reach" (118).

It is *Saccharomyces paradoxus*, the sister species to *S. cerevisiae*, in which population genomics has been researched, enabled by studies of its populations (119) and more than 100 individuals (120). In two North American *S. paradoxus* populations, study of adaptation to the environmental parameters of temperature and frequency of freeze-thaw cycles found that the more northerly population was better adapted than the more southerly one to growth at extreme low and high temperatures and to growth during freeze-thaw cycles (121). The authors attributed the adaptation to a higher mean temperature in the south that resulted in fewer days with temperatures both above and below freezing. Subsequent genome sequencing discovered a third population sympatric with the southerly end of the southern population that originated from hybridization between the northern and southern populations (122). Polyploidy

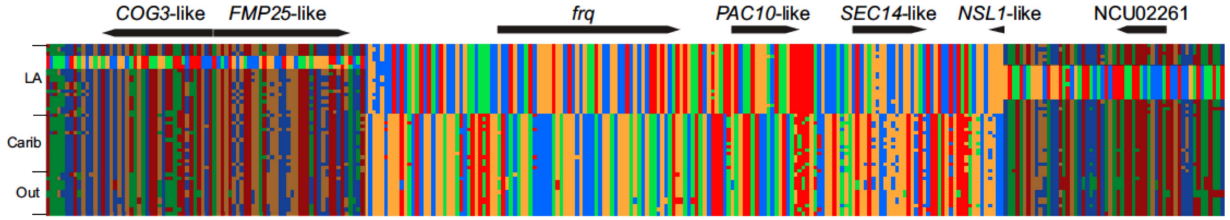
was not involved, as it was with the genome duplication associated with the origin of the *S. cerevisiae* clade; rather, homoploidy was involved, and members of this population have the tolerance for extreme temperatures found in the northern population and the sensitivity to freeze-thaw cycles found in the southern one. Based on genetic similarity, the third population arose by introgression of northern genes into individuals from the original southern population such that the third population has between 2 and 6% northern genes. Interestingly, the third population shows the same reduced fertility in crosses to either parent, perhaps due to reinforced barriers to mating that can arise in sympatry, as was noted above for *Neurospo*ra (98).

Reverse Ecology: Ascomycota Populations

Among filamentous Ascomycota, *N. crassa* has been a model for population studies due to a remarkable collection of wild isolates (123) and a well-studied laboratory strain that has been the subject of both a genome sequencing project (2) and a comprehensive gene deletion project (124). An *N. crassa* population bordering the Gulf of Mexico that included the laboratory strain was chosen for GWA (125). When 50 of the individuals had been sequenced, phylogenetic analysis revealed not one but seven populations (112) (Fig. 4). No population had enough individuals for a GWAS, but fortunately, two of them had at least 20 members, one in Louisiana and one further south in the Caribbean. These two populations differ by 2° to 10° of latitude and a difference in average winter temperature of 9°C. When the aligned genomes were scanned to detect regions of exceptional divergence, using measures of both relative and absolute divergence, more than 30 such regions qualified, but only 2 were identified by all three approaches. Among the genes in these two regions were two known to protect against cold temperatures: a cold shock RNA helicase and a prefoldin chaperone that, in yeast, protects actin from cold temperatures (Fig. 7). The resulting hypothesis, that the Louisiana population had adapted to life at low temperature, was not disproved by measuring the growth of 10 isolates from each population at low (10°C) and normal (25°C) temperature.

A second test was devised using a similar growth approach and the aforementioned gene deletion collection (124). For all eight genes found in the two regions, a gene deletion strain was obtained from the gene deletion collection, and these strains were screened at 10°C. Most gene deletion strains showed no loss of cold tolerance (an important control for the possibility that simply deleting genes would affect cold tolerance), but this was not so for strains deleted for the RNA helicase or the prefoldin. The same result was again found in progeny of crosses between these two deletion strains and wild type; i.e., inheritance of the wild-type allele conferred adaptation to low temperature that was lost if it was deleted. The authors called this approach to discovering adaptation reverse ecology (104), because it is the reverse of most studies of adaptation. Normally, adaptation studies begin with knowledge of a phenotype adapted to an environmental pressure, e.g., the darkening of coat color in mice that have adapted from life on light-colored desert soils to life on dark-colored lava flows (126). In reverse ecology, one begins with genomes, identifies genes subject to natural selection (in the case of *N. crassa*, selection associated with hybridization, introgression, and selective sweeps), and with the knowledge of the genes and their function combined

FIGURE 7 Regions of extreme divergence between populations of *N. crassa*. Rows are aligned genomes of Louisiana (LA), Caribbean (Carib), and other populations (out) seen in Fig. 4. Columns are nucleotide positions in four colors for the four bases. Highlighted is the region of high divergence between the Louisiana population and the Caribbean and other populations. The genome variation in this region is consistent with a history in the Louisiana population of hybridization and introgression. Low variation among Louisiana individuals in this region is consistent with a recent selective sweep. Variation in the length of introgressed regions in the Louisiana population may indicate that the sweep is still in progress. Among the six genes in the region of divergence is *PAC10*-like, which codes for a prefoldin that chaperones cold-sensitive proteins. Adapted from reference 112.



with knowledge of variation in the physical environment, infers the environmental factor (with *N. crassa*, growth at low temperature).

Reverse Ecology: Basidiomycota Populations

Basidiomycota have also been the subject of reverse ecology, e.g., the dikaryotic, ectomycorrhizal mushroom *Suillus brevipes* (127). This basidiomycete is symbiotic with pines, and 11 individuals were cultivated from fruiting bodies found in coastal California and another 17 from fruiting bodies in the Sierra Nevada of California. Using a guide sequence from the montane population, the 28 genomes were aligned, and half of the 1.2 million SNPs were used to make a phylogeny that found two populations, one coastal and one montane. These two populations are extremely similar, with only 0.01% of the SNPs being reciprocally fixed in the two populations. The same populations were recognized by a population structure approach (128), which showed no admixture. Despite the extremely similar genomes, Bayesian population analysis (129) estimated that the two populations had been diverged for 25,000 generations, with undetectable gene flow. Scans of the aligned sequences using metrics of relative and absolute divergence found two regions of the genome that, together, contained half of all reciprocally fixed SNPs. Of the four genes found in these two regions, one (*Nha1-like*) is invariable and homozygous in the coastal population, suggestive of a hard, selective sweep. It encodes a protein homologous to a Na+/H+ exchange protein found in yeast membranes that is involved with salt tolerance. It is tempting to speculate that the coastal population must contend with more salt than the montane population, but this speculation has not yet been tested. More recently, the *Suillus* study was expanded to include individuals from throughout western North America. With more geographic coverage, the study found evidence of admixture in Canadian populations situated between two, less diverse populations, one in Colorado and the other in Minnesota (130). The authors used evidence for selective sweeps and analyses associating genetic variation with environmental parameters (temperature and moisture) to identify proteins that may be involved in adaptation, e.g., membrane transporters, nucleic acid helicases, and the aforementioned Na+/H+ exchange protein. As often is the case with nonmodel fungi, hypotheses about the involvement of these genes in adaptation await testing.

Reverse Ecology: Ascomycota Species

Our discussion of fungal reverse ecology began with closely related, fungal populations, but the first such study was with well-diverged species in the Ascomycota human pathogens *C. immitis* and *C. posadasii*, agents of the human disease coccidioidomycosis (131). These fungi were studied just as high throughput sequencing was becoming possible, and most of the genomes were sequenced by the traditional Sanger method. The substantial investment was made possible by prior studies of species and populations of these fungi, which identified at least five populations spread over two species (132, 133), a concrete example showing that evolutionary studies can have an impact in medicine. With the aim of comparing at least two individuals from each population, 20 genome sequences were obtained and aligned. Between the two species, 43% of all SNPs were reciprocally fixed, a much higher proportion than the 9% fixed differences between the Louisiana and Caribbean *N. crassa* populations or the 0.01% between the coastal and montane *Suillus* populations. As with *Neurospora* and *Suillus*, regions of the *Coccidioides* genomes were found with exceptional measures of relative divergence, in this case, exceptionally low Fst, which indicated that as much as 7% of the genome of each species had a history of hybridization and introgression between the two species (131). One region of introgression from *C. posadasii* into the Southern California population of *C. immitis* was amplified and sequenced from an additional six individuals. When aligned, the introgressed regions in all individuals shared a common edge marked by a metalloproteinase gene, Mep4, which is related to a known virulence factor, Mep1 (134). The assumption that selection for Mep4 drove the introgression was challenged when a subsequent study failed to find a difference in Mep4 transcription in the saprobic and parasitic *Coccidioides* growth phases, especially when the study found a 20-fold difference in a small (99 amino acid) previously undiscovered gene lying between Mep4 and the edge of the introgressed region (135). Again, the hypothesis that this small gene drove introgression awaits testing.

Reverse Ecology: Basidiomycota Species

A more recent example of reverse ecology with well-diverged populations or species comes from the Basidiomycota *Cryptococcus gattii*, a haploid capable of causing disease in otherwise healthy humans (136). This example is interesting because it shows that it is possible to find regions of exceptionally large divergence even between well-diverged populations or species if one uses a comparator with a broad scale, e.g., Dxy, rather than one with a restricted scale such as Fst. *C. gattii* has four distinct, species-level (137) groups, VGI-VGIV, with

VGII containing the strains responsible for outbreaks of cryptococcosis in otherwise healthy hosts in Vancouver, Canada, and the U.S. Pacific Northwest. Genomes were sequenced from 53 individuals representing all four VGs, with a focus on the outbreak population. From a Dxy sliding-window scan of the aligned VGII sequences, two adjacent regions were found within a 15-kb region of one scaffold that showed exceptionally high percentages of polymorphic sites (136). Phylogenetic analyses of the first of these regions suggested that it originated by hybridization and introgression from a different VG, VGI. In one of the VGII individuals, an introgressed region is also aneuploid and homozygous, suggesting duplication after introgression. This introgressed region contains three genes that, by homology with *S. cerevisiae*, appear to be involved in actin polymerization, dissociation of Rad51 D-loops, and metal ion transport plus vacuole protein targeting. It will be very interesting to see if deletions or allele swaps of any of these three genes have an effect on virulence, although the adaptation implied by the introgression could be related to any biological or physical environmental parameter. It seems likely that the hybridization occurred by sexual reproduction, consistent with the discovery of a diverse, outbreeding population of VGII in South America that also embraces the genetic variation displayed by strains found in northwestern North America, Australasia, and Southeast Asia (136, 138, 139).

Reverse Ecology with Uncultivatable Fungi

Earlier, it was noted that population genomic studies are facilitated when the fungal individuals can be cultivated. A recent study of an obligate plant parasite, one that has never been cultivated, shows that cultivation is not essential. Comparison of genomes of 46 individuals of the obligate plant pathogen that causes powdery mildew, *Blumeria graminis*, that had been collected from three crops—wheat (*B. graminis* f. sp. *tritici*), rye (*B. graminis* f. sp. *secalis*), and the hybrid plant triticale (*B. graminis* f. sp. *triticale*)—revealed a fascinating evolutionary history (140) that recalls the aforementioned study of Zymoseptoria (111). Based on genome comparisons, *B. graminis* f. sp. *triticale* is a recent hybrid of the other two powdery mildews, and one that arose in the past 50 years, coincident with the introduction of the hybrid plant, triticale, to agriculture. *B. graminis* f. sp. *triticale* has regions of DNA from both parents, but most came from *B. graminis* f. sp. *tritici*, indicating that the initial hybridization was followed by introgression of the F1 into the *B. graminis* f. sp. *tritici* population. Based on the amount of genetic variation seen in *B. graminis* f. sp. *triticale*, hybridization involving several parents as well as their F1 progeny likely contributed to its origin. Given that one parent, *B. graminis* f. sp. *secalis*, can cause some disease symptoms on triticale, whereas the other parent, *B. graminis* f. sp. *tritici*, cannot, it is assumed that *B. graminis* f. sp. *secalis* effectors are important to the ability of *B. graminis* f. sp. *triticale* to infect triticale. Unexpectedly, transcription studies have not implicated any of the known *B. graminis* f. sp. *secalis* effectors found in *B. graminis* f. sp. *triticale*. Again, this is a system with great potential for gene disruption studies to identify the genetic basis of *B. graminis* f. sp. *triticale* infection of triticale.

Hybridization: Caught in the Act

The population genomic studies presented so far show evidence of hybridization and introgression, but none have caught populations in the act of hybridization. The most recent hybrid found so far comes from a recent study of *Neurospora discreta* (141), a close relative of *N. crassa*. One phylogenetic species in the *N. discreta* complex, PS4, has an exceptionally large latitudinal range in North America, from New Mexico to Alaska, and also is found in Europe and Asia. When genomes were sequenced and analyzed for more than 50 individuals from this species, they showed three, well-diverged lineages (with 35 to 45% of SNPs reciprocally fixed between populations): Alaska and Europe (AK-EU), California and Washington state (CA-WA), and New Mexico and Washington state (NM-WA). Among the Alaskan individuals was one possessing, evenly distributed through its chromosomes, 12% of the genome of the apparently allopatric, NM-WA lineage (Fig. 8). Mysteriously, at one collecting site in Washington, where the CA-WA and NM-WA lineages are clearly sympatric, there was no evidence of hybridization. Clearly, this is a case where more individuals should be sampled and studied.

LAST WORD

Every technical advance in examining fungal genomes has added to our knowledge of the type and extent of genetic variation available to fungi. There is every reason to think that this trend will continue as fully assembled genomes of more and more individuals become available to mycologists, for example, the aforementioned population genomic studies of genome rearrangement and epigenetics that are expected to emerge from genomes obtained from single molecule, real-time sequencing.

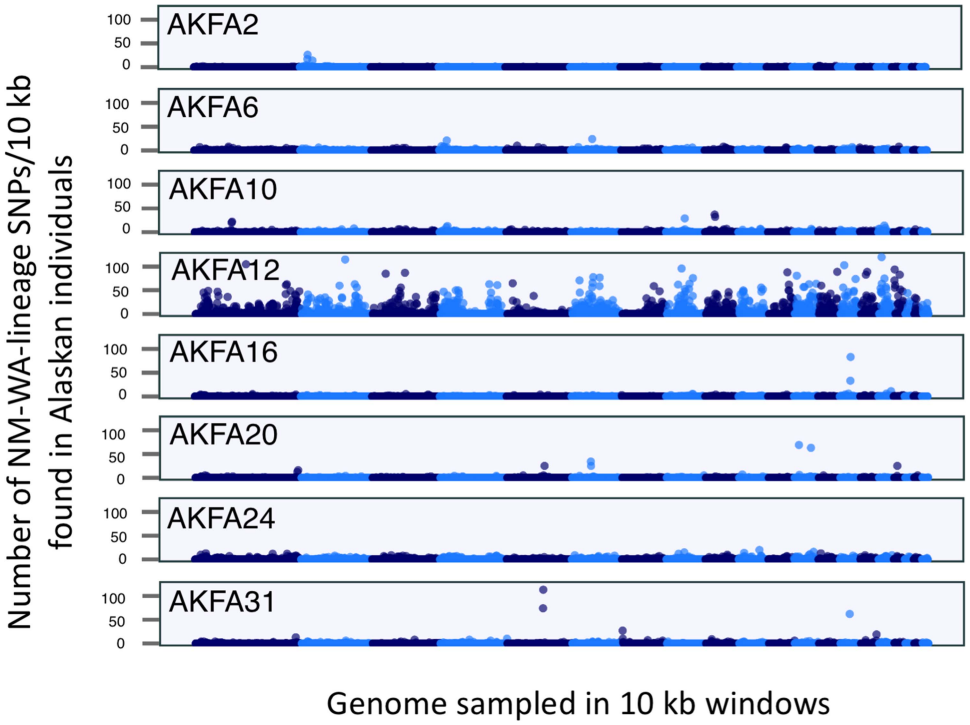


FIGURE 8 Evidence of recent hybridization. Genome scans for introgressed DNA in the 20 largest contigs of eight *Neurospora discreta* individuals from the Alaska-European lineage. Numbers of SNPs introgressed from the New Mexico-Washington (NM-WA) lineage are shown on the *y* axis. Alaskan strain AKFA12 stands out as having 12% of its genome introgressed from the NM-WA lineage, as expected from a few matings between a hybrid individual and members of the Alaskan population. Adapted from reference 141.

Among the many outputs of genomic research, none outstrips hypothesis generation. An outcome of this abundance is a bottleneck for the completion of any scientific endeavor: hypothesis testing. Here, again, technological advances may prove important. For example, the successful use of CRISPR-associated protein-9 nuclease to transform *S. cerevisiae* (142), filamentous fungi in the Ascomycota genera, *Aspergillus* (143), *Trichoderma* (144), and *Pyricularia* (= *Magnaporthe*) (145), as well as the Basidiomycota genera *Ustilago* (146) and *Cryptococcus* (147), indicates that hypothesis testing in all types of fungi may become, if not routine, then at least possible.

A unifying feature of genetic diversity in fungal genomes, with the exception of HGT between phylogenetically distant lineages, is that every means of generating diversity is operating all the time, such that fungal genomes are remarkably fluid and variable. That there is any constancy at all to fungal genomes is a strong testament to the power of purifying natural selection. Is there something special about fungi in terms of their life cycles or population sizes that makes them unusual in terms of generating genetic variation and enduring particularly strong natural selection? Or is it simply that the data exist for fungi because they threaten our health and food supply? Other Eukaryotic microbes have life cycle features and population sizes similar to those of fungi, as do some animals, e.g., corals. It may be that fungi are not unique in terms of genetic diversity and strength of natural selection and that the same observation will be made for plants and animals when population genomics becomes affordable for these kingdoms.

ACKNOWLEDGMENTS

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