

Genetics – Variation, Sexuality, and Evolution

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Genetics deals with variation and inheritance, and as such, forms the basis for understanding why fungi behave as they do. Ultimately, understanding for example, why one fungal species or even strain is pathogenic to perhaps only one species or variety of plant, how one fungus recognises another, why some fungi grow faster or decompose organic matter more rapidly than others, how the characteristics of fungi and the relationships between them and other organisms change with time, all comes down to understanding their genetics. Fungal ecologists, conservation biologists, plant and invertebrate pathologists, medical mycologists, biodeterioration specialists and those concerned with the exploitation of fungi in horticulture, and in the pharmaceutical, food and chemical industries, all need to understand basic fungal genetic concepts. Further, many fungi have traits that make them ideal for genetic and evolutionary biology research: many can be grown in pure culture under controlled laboratory conditions, minimising environmental variability that might conceal or confound genetic differences; many are haploid for much of their lives, allowing mutant alleles of genes to be readily detected phenotypically; many have high growth rates, short lifecycles, uninucleate haploid cells at some stage of the life cycle, large numbers of progeny from each cross, low chromosome numbers, and small genomes. The ascomycetes *Aspergillus nidulans*, *Neurospora crassa*, and *Saccharomyces cerevisiae* have many of these features, and are among the genetically best understood organisms.

In this chapter we define first what constitutes a fungal individual and population, and consider fungal species concepts. The rest of the chapter is divided into two main sections, the first concerned with the different lifecycles and sexual processes found within the diverse Kingdom Fungi. Then we review fungal variation, sources of variability, evolution, and formation of new species.

DEFINING INDIVIDUALS, POPULATIONS, AND SPECIES

There are three concepts essential for population genetics, ecology, and conservation – the individual, the population, and the species.

What Is an Individual? Definition, Recognition, and Consequences

Defining Individuals

For multicellular animals and plants, identification of what is an individual is often easy – one lion is an individual. Unicellular fungi and those with very limited mycelial growth can reasonably be regarded as individuals, but what constitutes an individual with larger mycelial fungi is harder to define, as with plants that form stolons or suckers and with clonal animals such as coral. The difficulty arises because individual compartments of a mycelium are capable of independent existence and production of new colonies, and colonies do not have a predetermined body shape and size (i.e. they have indeterminate growth). If large mycelia become fragmented, or asexual spores are produced, colonies are formed which are physically separated but genetically identical to each other. This occurs commonly in nature. Organisms related in this way are termed clones. Following the terminology of plant biologists there are thus two aspects to defining individuals: a whole group of genetically identical individuals (clones) may be defined as a **genet** and each asexually originating part of the genet can be termed a **ramet**.

Not only can mycelia fragment, but they can also join. If hyphae of two or more members of a clone come into contact with one another then they may fuse (pp. 102–104) in the same way that anastomoses are formed within a single colony (p. 56), resulting in coalescence of mycelia to form an individual functional unit. In the past it was thought that coalescence would occur whenever mycelium of the same species, irrespective of genetic origin, encountered each other; the resulting mycelium was thought to contain many different nuclei derived from merging of different individuals which could be ‘switched’ on and off according to environmental circumstances. The occurrence of somatic incompatibility (pp. 102–104), however, means that such mosaics are likely to be rare. Glomeromycota are an exception, with individual mycelia containing many different nuclei.

Genets and ramets vary considerably in their size and age. In the field, the sizes achieved depend on, among other factors, lifestyle and ecological strategies, age, size of resources colonised, and interaction with other organisms. Many fungi are restricted to the resources (e.g. leaf, twig, or branch) which they colonise and hence the size they can reach cannot be larger than the resource. Typically they would be much smaller because of the presence of other individuals of the same or different species. Some fungi tend to form mycelia that do not grow much more than a few cm radius, however large the resource or time given (e.g. *Penicillium* spp.). In contrast, some basidiomycetes can form extensive long-lived genets and ramets in soil and leaf litter. For most ectomycorrhizal (pp. 205–228) and saprotrophic leaf litter decaying species, genets are usually less than 10 m diameter, but species that form cords (pp. 59–60), rhizomorphs (pp. 59–60), and fairy rings can be larger. The largest organisms on the planet are probably *Armillaria* species with genets occupying up to 8 or so hectares on the floors of North American forest, with estimated ages of a thousand or more years. Little is known about the dynamics of ramets in the field, but their size, shape, distribution, and number will be constantly changing, especially during times of the year when growth is rapid ([Figure 4.1](#)).

Definition of an individual is important because it forms a baseline upon which numerical comparisons are often made, though use of biomass might be a less ambiguous metric. The actual definition of an individual depends on circumstances. The IUCN (International Union

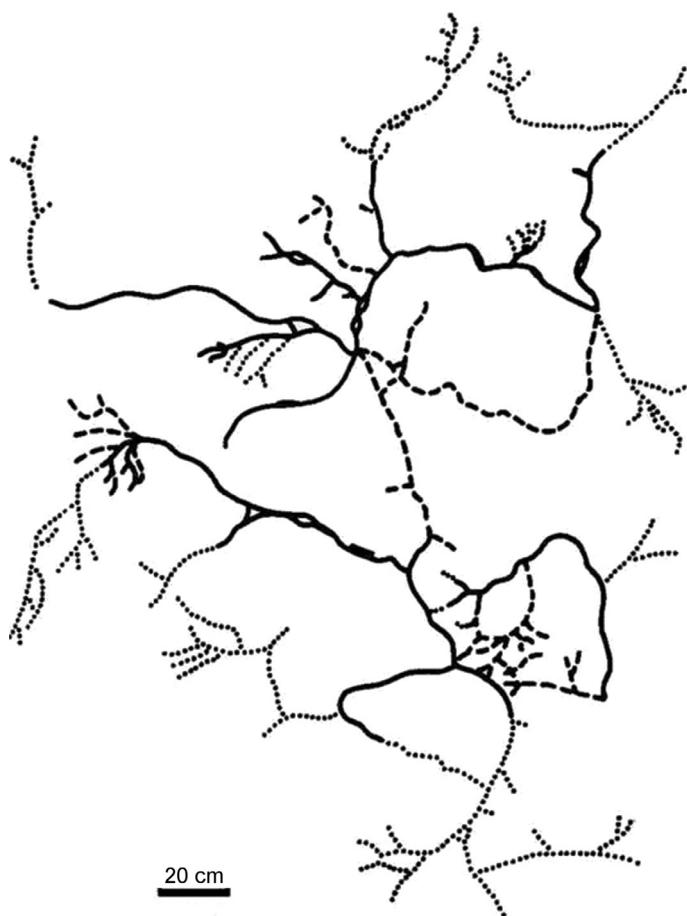


FIGURE 4.1 A map of a mycelial cord system of *Phanerochaete velutina* at the soil–litter interface in a temperate deciduous woodland, excavated by carefully removing the surrounding leaf litter, and then recovered with litter. The continuous and dashed lines indicate the system when it was first excavated. The dotted lines indicate new mycelial cords 13 months later, while the dashed lines indicate cords which were no longer present. Loss of one of the cords represented by a dashed line caused the original genet to be split into two ramets. *Source: Redrawn from Thompson, W., Rayner, A.D.M., 1993. Extent, development and functioning of mycelia cord systems in soil. Trans. Br. Mycol. Soc. 81, 333–345.*

of Conservation) defines an individual as the smallest entity capable of independent survival and reproduction (i.e. a ramet, not a genet). However, the cost and complex logistics of gathering genetic data on fungi over extensive geographical areas has led fungal conservationists to use the concept of functional individuals, rather than genets or ramets, for red-listing (pp. 392–394). In genetic studies the genet would usually be used to define an individual. Whichever appropriate definition is being used, it is essential that like is compared to like, when any quantitative comparisons of numbers of individuals are being made.

Roles and Consequences of Hyphal Fusion and Somatic Incompatibility

There are advantages of fusion: within a colony, fusion between hyphae can result in the reconnection of two streams that had previously branched (termed anastomosis). This allows the formation of networks (p. 56) and the ability to transport water, nutrients, and signals tangentially rather than just radially within a colony. Fusion of genetically identical colonies can allow pooling of resources and cooperation between different regions, be it following fusion of large mycelia of saprotrophic cord-forming basidiomycetes, or ectomycorrhizal mycelium, or of small colonies developing from asexually produced conidia (p. 103). Heterokaryon formation, when two genetically different hyphae of the same ascomycete species fuse, provides potential benefits of functional diploidy (p. 109) and the ability to generate mitotic genetic exchange in the absence of sexual reproduction during the parasexual cycle (pp. 122–123).

Fusion with non-self can, however, expose an individual and its genome to risks such as harmful nuclei, mitochondria, plasmids, viruses, retrotransposons, and other selfish genetic elements (pp. 132–134). Examples of these include: the presence of a gene in *Neurospora crassa* that enables nuclei containing it to replace other nuclei; in *Neurospora* and other fungi some mitochondria have genomes that render them defective in respiratory function but capable of normal replication; some strains of *Podospora anserina* have a plasmid that causes senescence; and there are viruses that can affect growth and fruit body development (pp. 357–358). Somatic incompatibility provides a defence against the spread of these.

Somatic Incompatibility

The ability to distinguish self from non-self is ubiquitous among all organisms, and is fundamental to distinguishing one individual from another. Filamentous fungi recognise self from non-self by incompatibility systems termed vegetative incompatibility, mycelial incompatibility, or somatic incompatibility (particularly in basidiomycetes). Within a species, recognition of non-self typically occurs following fusion of hyphae, and incompatibility usually results in death of the fusion cell. In basidiomycetes, recognition has a genetic basis and is regulated by one to three or, possibly more genes with multiallelic loci (termed vegetative compatibility (VC), *vic* or *het* loci). If two mycelia have different alleles at one or more *het* loci recognition as incompatible occurs. VC does not necessarily imply genetic uniqueness, and the relationship between the two depends on the number of loci and alleles involved. With basidiomycetes, most mycelia (usually dikaryons, p. 113) isolated from the natural environment tend to be incompatible when paired ([Figure 4.2](#)), implying that vegetative incompatibility groups usually correspond to genetic individuals, though this is not true for all species. With ascomycetes, VC types are commonly, but not always, associated with genetic individuals. Sometimes dominant VC types can spread over large distances, especially when a pathogen is invasive and a founder effect (p. 130) or selection of a more fitted VC type occurs, as in the spread of the Dutch elm disease fungus *Ophiostoma novo-ulmi*. With the basidiomycete *Serpula lacrymans*, low genetic variation in the founder populations (p. 130) has led to breakdown in the correlation between genetic uniqueness and VC groups.

More is known about hyphal recognition systems in ascomycetes than in basidiomycetes. In ascomycetes two types of genetic systems regulate vegetative incompatibility, termed allelic, and non-allelic, the former being most common. In both types, incompatibility occurs if there are differences at any of the *vic* or *het* loci. In allelic systems, incompatibility occurs

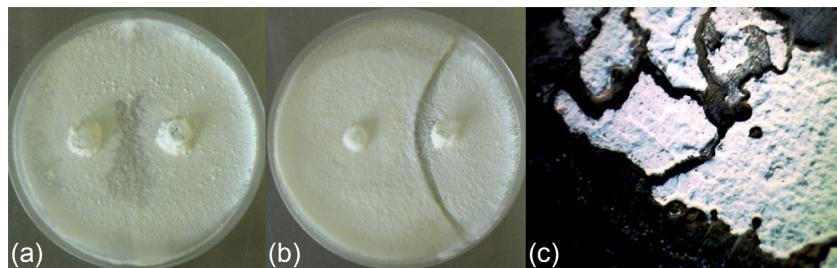


FIGURE 4.2 Vegetative incompatibility in wood decay fungi. When self-pairings are made in artificial culture, hyphae fuse and the two mycelia function as one because they are somatically compatible (a), but when mycelia from non-self pairings meet there is a clear rejection response (b), as seen here with *Trametes versicolor*. (c) Mycelia of *S. hirsutum* have grown out from individual decay columns in wood demarcated by interaction zone lines and are clearly separated from each other. Source: (c) Rayner A.D.M., Todd, N.K., 1979. Population and community structure and dynamics of fungi in decaying wood. *Adv. Bot. Res.* 7, 333–420.

if there are different alleles at the same loci, whereas in non-allelic systems, incompatibility occurs as a result of interaction between two genes at different loci. *Aspergillus nidulans* and *Neurospora crassa* only have allelic systems, but *Podospora anserina* has both allelic and non-allelic systems. The number of *het* loci identified and characterised varies between 7 and 11 depending on species (e.g. *Aspergillus nidulans*, *Ophiostoma novo-ulmi*, *Cryphonectria parasitica*, *Neurospora crassa*, and *Podospora anserina* have at least 8, 7, 7, 11, and 9 loci, respectively) (Table 4.1). The vegetative incompatibility systems of basidiomycetes probably have some of the same genetic characteristics but differ in others (e.g. mating genes do not appear to be involved in the basidiomycetes but sometimes they are in the ascomycetes) (Table 4.1).

With ascomycetes, as with basidiomycetes, recognition follows fusion. Prior to fusion, hyphal tip growth stops after contact with another hypha of the same species, the cell wall is broken down by hydrolytic enzymes at the point of contact, and a bridge is made between the two. Plasma membranes then fuse and cytoplasmic contents of the two compartments mix. Fusion between fungi identical at all *het* loci results in compatibility, and is frequently associated with changes in cytoplasmic flow (Figure 4.3). The mechanistic basis of self-fusion has been studied in most detail for fusion between conidial anastomosis tubes (CATs) during initiation of colonies of *Neurospora crassa*. CATs, produced by most ascomycetes, are short, specialised hyphae or cell protrusions that grow out from and link conidia or germlings to form interconnected networks (Figure 4.4). They have also been seen linking rust uredospores. The process of CAT fusion occurs in a continuum of six developmental stages: (1) induction, (2) chemotropism, (3) adhesion of cells, (4) cell wall breakdown and remodelling, (5) plasma membrane fusion, and (6) achievement of cytoplasmic continuity (Figure 4.4).

When there are differences at one or more *het* loci, rapid compartmentalization, death of the fusion cell, and often adjacent cells occurs (Figure 4.4). Within a few minutes of fusion, granules form within the cytoplasm of the fused compartment, septal pores (pp. 53–55) connecting with adjacent cells are blocked, cytoplasm becomes vacuolized, and these vacuoles burst, releasing proteases and other degradative enzymes. Destruction of the fusion cell is often complete within 30 min. Different species probably share the same cell death machinery as microscopic processes are similar, despite being mediated by different *het* loci.

TABLE 4.1 Characteristics of Vegetative Incompatibility and Related Genes in *Neurospora crassa* and *Podospora anserina*

NEUROSPORA CRASSA	
<i>matA-1</i>	Mating type gene transcription regulator
<i>mata-1</i>	Mating type gene transcription regulator
<i>het-c</i>	Allelic <i>het</i> gene; signal peptide with glycine-rich repeats
<i>het-6</i>	Allelic <i>het</i> gene; region of similarity to <i>tol</i> and to <i>P. anserina het-e</i>
<i>un-24</i>	Allelic <i>het</i> gene; large subunit of ribonucleotide reductase
<i>tol</i>	Suppressor; has a coiled-coil, leucine-rich repeat; some sequence similar to <i>het-6</i> and <i>P. anserina het-e</i>
<i>vib-1</i>	Suppressor; nuclear localisation sequence

PODOSPORA ANSERINA	
<i>het-c</i>	Non-allelic <i>het</i> gene against <i>het-d</i> ; glycolipid transfer protein (glycolipids are involved in interactions between cells)
<i>het-d</i>	Non-allelic <i>het</i> gene against <i>het-c</i> ; GTP-binding domain; similarity to <i>het-e</i> and to <i>N. crassa tol</i> and <i>het-6</i>
<i>het-e</i>	Non-allelic <i>het</i> gene against <i>het-c</i> ; GTP-binding domain; similarity to <i>het-d</i> and to <i>N. crassa tol</i> and <i>het-6</i>
<i>het-s</i>	Allelic <i>het</i> gene; prion-like protein
<i>idi-1, idi-3</i>	Vegetative incompatibility (VI)-related genes; signal peptide, induced by non-allelic (<i>het-c/e</i> and <i>het-r/v</i>) incompatibility
<i>idi-2</i>	VI-related gene; signal peptide, induced by <i>het-r/v</i>
<i>mod-A</i>	VI-related gene; modifier of <i>het-c/e</i> , <i>c/d</i> , and <i>r/v</i> incompatibility; SH3-binding domain, involved in signal transduction, cytoskeletal proteins, protein–protein interactions
<i>mod-D</i>	VI-related gene; modifier of <i>het-c/e</i> incompatibility; subunit of G protein, involved in signal transduction
<i>mod-E</i>	VI-related gene; modifier of <i>het-r/v</i> incompatibility; heat-shock protein
<i>pspA</i>	VI-related gene; vacuolar serine protease; induced by non-allelic (<i>het-c/e</i> and <i>het-r/v</i>) incompatibility

Note that names have been assigned independently in different fungi so, for example, *het-c* in *N. crassa* is not the same as in *P. anserina*.

Adapted from Moore *et al.*, 2002; Glass and Kaneko, 2003

What Is a Population?

A population comprises an assemblage of individuals of a species. Delimitation of the assemblage depends on the researcher and the questions being asked. The boundaries can range to include all of the individuals in a single organic resource, or in a forest, or in a geographic region, the ultimate outer boundary depending on the species limit set by hosts, tolerance of climate, etc. The boundaries set by a researcher may not be the borders for gene flow (pp. 128–130), as airborne spores can sometimes disperse over long distances. Another

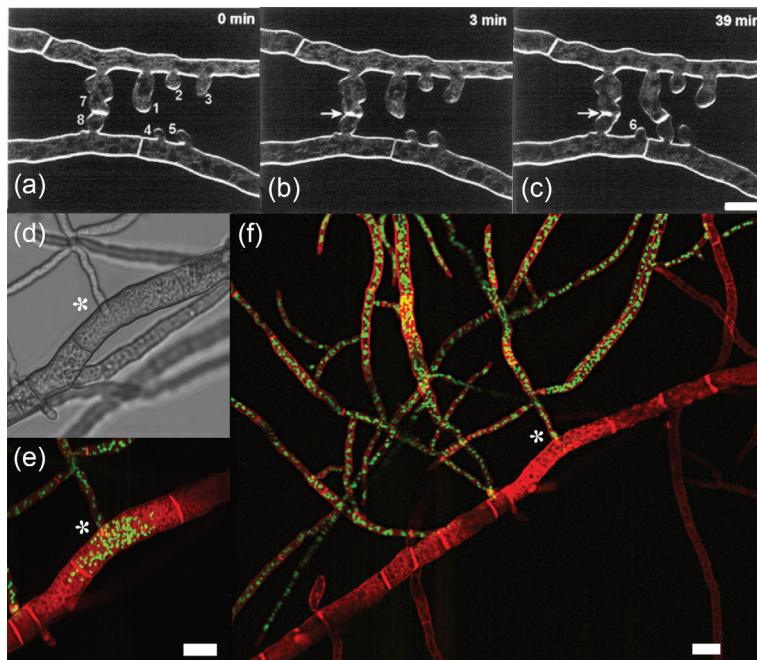


FIGURE 4.3 Vegetative compatibility and incompatibility. Stages of the hyphal fusion process (a–c) and non-self rejection responses (d–f) in *Neurospora crassa* viewed with confocal microscopy. (a–c) Show induction, homing, and fusion of hyphae in a mature colony. (a) Three branches (labelled 1, 2, and 3) from one hypha have started growing towards two short branches (4 and 5) on the opposite hypha. Two branches (7 and 8) have already fused. (b) A fusion pore has started to open between the two fused hyphal branches (arrowed), and in (c) it is completely open, allowing cytoplasmic continuity. (c) Branches 1 and 4 have fused, and another side branch (6) is developing. Bar = 10 µm. (d–f) Fusion and subsequent rejection of non-self hyphae. (d) A brightfield image showing a thin hypha of one strain fusing with the underside of a wide hypha of another strain (*). In (e) and (f) the same interacting hyphae have been labelled with a membrane selective red (grey in the print version) dye and one of the strains has been labelled with a nuclei selective green (light grey in the print version) H1-GFP. (e) A confocal image of (d) showing that nuclei (fluorescing green (light grey in the print version)) have migrated from the narrow hypha into the wider one. (f) One hour later incompatibility is evident. The compartment where fusion has occurred is stained intensely red (grey in the print version) due to increased permeability of the plasma membrane, and the nuclei have broken down, as evidenced by lack of green (light grey in the print version) fluorescence. Source: (a–c) Hickey, P.C., Jacobson, D.J., Read, N.D., Glass, N.L., 2002. Live-cell imaging of vegetative hyphal fusion in *Neurospora crassa*. *Fungal Genet. Biol.* 37, 109–119. (d–f) [Read and Roca, 2006](#).

concept is that of the metapopulation, which comprises local populations each with its own probability of going extinct. Uncolonised regions can be colonised from other populations within the metapopulation. So each log in a forest will contain its own population of a species, and when a new branch falls it will be colonised from the other populations in the forest; long-term survival of the species occurs at the metapopulation level.

Important characteristics of populations include their size and whether this is changing, their age structure, and their genetic variation. Fungal individuals are characterised by their **genotype**, and how this is actually expressed (i.e. how the traits are manifested) – **phenotype**, and populations comprise individuals having different genotypes and phenotypes. The

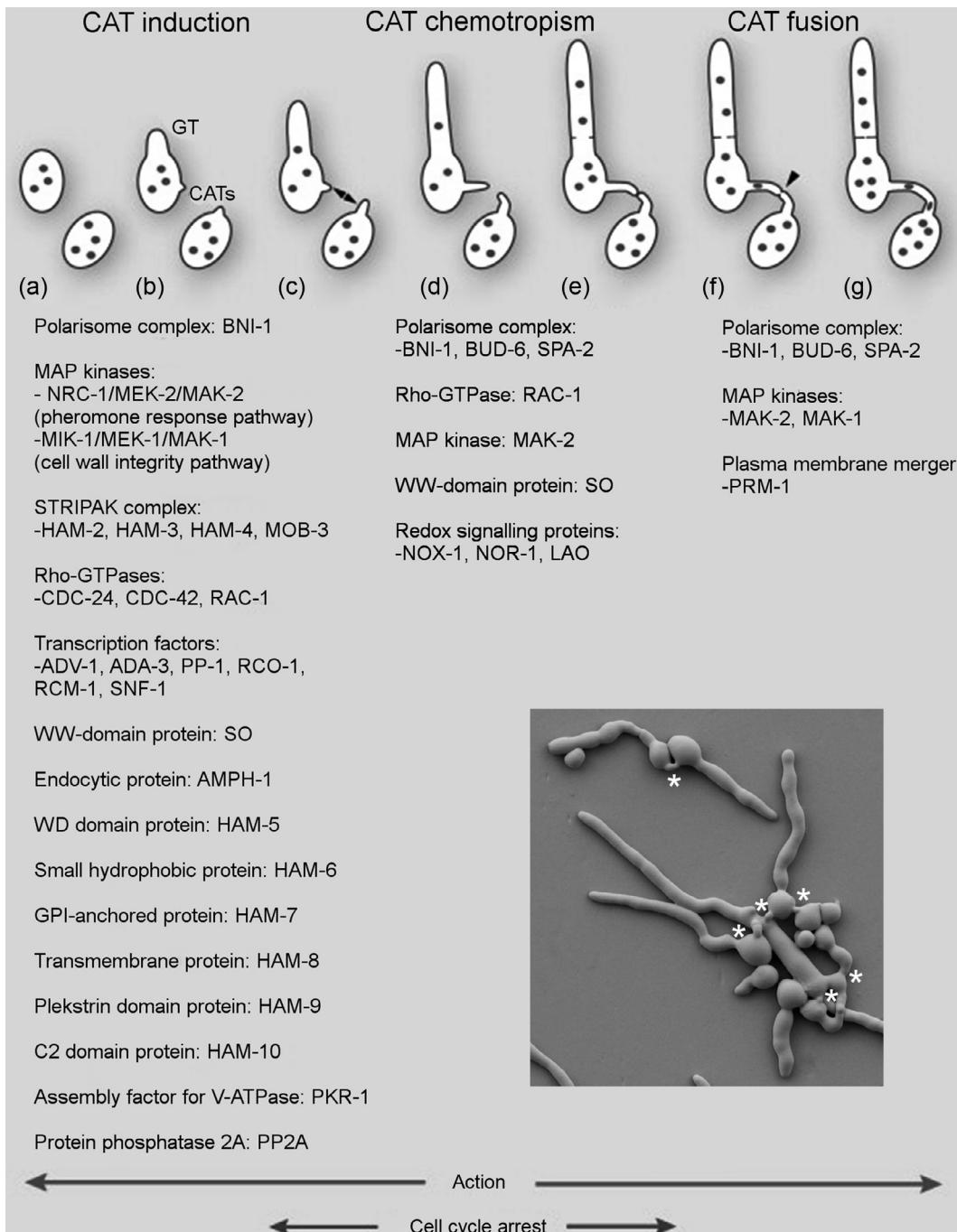


FIGURE 4.4 Self-recognition and the formation of conidial anastomosis tubes (CATs) in ascomycetes. The continuum of six developmental stages in CAT fusion is shown diagrammatically, together with the signalling networks and proteins involved at different stages. (a) Ungerminated macroconidia which contain 3–6 nuclei (circles). (b) Germ tubes (GT) grow out, and CATs form when conidia are close together, indicating the possibility that quorum sensing has a role in CAT induction. Actin cables accumulate within the cell at the sites

biological processes that affect populations are the focus of population biology, including the forces that shape the genetic composition of populations, such as mutation, recombination, drift and selection, and these are explained in the section 'Microevolution' (pp. 123–137).

What Is a Species?

The **species** is the fundamental unit of biological classification, but there are different ways of defining a species and practical difficulties in defining and delimiting species. Fungal species can be defined and recognised based on phenotype, reproductive isolation, and genetic isolation. Historically, as with plants and animals, fungi were first categorised by Linnaeus, based on morphological similarities (i.e. phenotype), – the **morphological species** concept. In the mid-1800s, Elias Fries laid the foundations for fungal classification based on reproductive morphological features, such as spore size, shape, and colour, and whether macroscopic basidiomycete fruit bodies had pores, gills, crusts, or the spores were enclosed. The main difficulty is in finding characters that define the boundaries of a species: the morphology of a fungus can be very variable depending on physiological state and environmental conditions; closely related species can have very different characters; and unrelated organisms can have evolved similar morphologies by different routes (convergent evolution). Within the Agaricomycetes, for example, the order Russulales contains species with seven different sexual reproductive fruit body types – gills, pores, teeth, clubs, crusts, epigaeous gasteromycete, and hypogeous gasteromycete; hydnoid fruit bodies (i.e. with teeth) are also found in the Polyporales, Thelephorales, Hymenochaetales, Gomphales, and Cantharellales (Figure 1.6). Other phenotypic characters have been used to augment morphological characters for fungi with simple morphology and those that have industrial importance. These include substrate utilisation in yeasts, and temperature and water potential for growth of *Penicillium*.

The **biological species** concept is most commonly used by macrobiologists, and of particular use to the geneticist. It is based on reproductive isolation, and defines the normal limits of genetic exchange. A biological species consists of all the populations that are able to mate successfully to produce viable offspring. Delimiting the species requires considerable study, since isolates of a fungus from different parts of the world must be brought together and mating tests carried out. Mating tests sometimes reveal that what was thought on morphological grounds to be one species is, in fact, two or sometimes more. However, while such mating tests may yield an unambiguous demarcation, sometimes matings between different isolates

FIGURE 4.4—CONT'D from which CATs subsequently emerge, and an apical cap of actin is present in the CAT tip. CATs and germ tubes both lack the Spitzenkörper (pp. 50–51) that is important in tip growth of other vegetative hyphae. (c) Genetically identical cells communicate with each other by releasing a chemoattractant from their tips (arrowed). (d) CATs become orientated along the gradient of chemoattractant resulting in growth towards each other. This is achieved by what has been termed the ping-pong mechanism: two CAT tips homing in on each other rapidly and repeatedly switch between two different signal proteins – MAK-2 and SO – at their tips. When SO is transiently present at the tip of one CAT, MAK-2 is present at the tip of the other and vice versa, acting alternately as a signal sender and signal receiver. (e) When tips make contact, they stop growing and adhere to each other. The cell walls are probably remodelled in the vicinity of the site of contact to prevent leakage when a pore forms between the two CATs. (f) A fusion pore is formed (arrowed) by localised cell wall breakdown and remodelling, and merger of the two plasma membranes. (g) The cytoplasm from both CATs, including all organelles, can then move between the two. Actin, but not microtubules, is required for the process of CAT fusion. The inset is a scanning EM showing a network of germinated conidia formed by fusion of CATs (position indicated by asterisks). *Source: Read et al., 2012.*

are only partially successful, yielding few progeny or ones of reduced vigour. Then it may be difficult to decide whether two isolates belong to the same biological species. A further difficulty is that this definition of a species is based on reproductive isolation, but reproductive isolation is only one step towards speciation (pp. 135–137). Intersterility is the stage at which speciation becomes irreversible, but it can occur at different times ranging between early and very late stages of speciation.

The biological species concept is difficult to employ with fungi that appear to have an entirely asexual lifecycle and with those with homothallic mating systems (pp. 119–120). Molecular methods, however, now make it possible to delimit species by determining the extent of genetic isolation or to put it another way, the limits within which genetic recombination has occurred. This **phylogenetic species** concept is based on analysis of the congruence of genealogies constructed from DNA sequences of appropriately polymorphic loci (e.g. the various parts of the ribosomal RNA operon (SSU, LSU, and 5.8S), as well as several protein coding genes (*rpb1*, *rpb2*, *efla*, and *tef1*), or of whole genomes. This approach is applicable to fungi that have no obvious mating as well as to those that do. Phylogenetic analysis often reveals three or four species (Figure 4.5) where mating reveals perhaps two

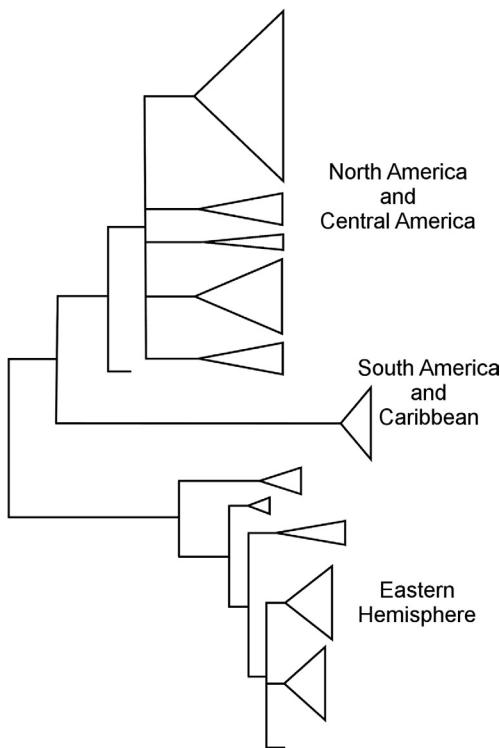


FIGURE 4.5 Phylogenetic species recognition in *Schizophyllum commune*. The intergenic spacer (IGS) of the nuclear ribosomal repeat was sequenced in 195 cultures of individuals from South, Central and North America, the Caribbean, Europe, Asia, and Australasia. Three clades (phylogenetic species) were revealed, one containing isolates from North and Central America, a second containing isolates from South America and the Caribbean, and a third with isolates from the Eastern Hemisphere. Based on morphology and mating, it had previously been thought that this was a single species. Source: Taylor *et al.*, 2000, based on information from James *et al.*, 2001.

or three, and morphology, only a single species. In a study on *Neurospora* in which two species – *Neurospora crassa* and *Neurospora discreta* – were distinguished on morphological grounds, seven were found based on ability to mate, and eight based on phylogenetic analysis. This is because with fungi, genetic isolation precedes reproductive isolation; both of these precede morphological divergence, and morphological differences are few – at least relative to most macroorganisms.

About 120,000 fungal species have been formally described so far, but there are likely to be over 5 million (Chapter 1), and new species are being continuously discovered. A newly discovered fungus is only accepted as a new species after it has been described and named in accordance with the International Code of Nomenclature for algae, fungi, and plants. A Latin binomial has to be provided, consisting of a generic name followed by a specific epithet. Often a new species is sufficiently similar to one already known to be assigned to an existing genus, but sometimes it is necessary to create a new genus to accommodate it. A description in English or Latin has to be provided, a type specimen has to be selected, and the specimen deposited in a fungarium (previously, herbarium). Thereafter, all subsequent specimens that sufficiently resemble the type specimen are considered to belong to the same species.

Taxonomists can differ greatly in what they regard as a sufficient resemblance. Some are 'splitters', assigning specimens with small differences to separate species, and others are 'lumpers', combining specimens with considerable differences in the same species. In the genus *Fusarium*, for example, some authorities have recognised scores of species and others less than a dozen. As knowledge increases, especially with the use of molecular approaches, species may be split and new names applied or even combined where the oldest name may be applied. This has to be done in accordance with the rules of the International Code for the new names to be correct. With the rise in sequencing of DNA from environmental samples, many species are being discovered for which no living material is available to fulfil the traditional rules for describing and naming species. New rules were recently agreed to accommodate these.

LIFE CYCLES AND THE SEXUAL PROCESS

The Roles and Consequences of Sex, Outcrossing, and Non-outcrossing

The role of sex is often regarded as the promotion of genetic variability through outcrossing. A species with a lot of genetic variability will be more likely to produce genotypes able to cope with changed environmental conditions than other species with little variability. A consequence of sex may, hence, be an increased capacity, through variation, to survive challenging environments or rapidly evolving parasites or competitors. Natural selection, however, favours features that increase the ecological fitness of an individual and its immediate progeny, not those that might be of value to the rest of the species, or remote posterity. So, the value of sex relates to the immediate products of sexual fusion or of meiosis. Sex has several different roles indicated below, and these may vary between species that have different life cycles, and as fungi are exposed to different environments.

The vast majority of mutations are deleterious rather than beneficial. Different cell lineages, however, will accumulate different unfavourable mutations. Outcrossing will hence result in a dikaryon or diploid in which the effect of mutations, provided that they are recessive,

will be masked by the complementary, correctly functioning alleles, and effects known as **complementation**. Also, the recombination that occurs when the haploid state is restored by meiosis will result in some progeny with fewer deleterious alleles than either of the haploid parental strains, counteracting a gradual tendency to accumulate unfavourable mutations.

Outcrossing may result in **heterozygote advantage**, in which the heterozygote performs better than either corresponding homozygote. This is well known in plants and animals, and has also been shown in yeast. Sex will also produce new variants, some potentially less susceptible to parasites than the parent strains. In fungi, the most common parasites are viruses (pp. 357–358), usually transmitted by vegetative hyphal fusion. The sexual process generates, through outcrossing and recombination, progeny with new VC genotypes which will not fuse with and acquire the viruses of the parental VC genotypes. In summary, sexual reproduction allows fungi to cope with changes in the environment they inhabit and threats posed by instability of their own genome.

Despite the many benefits of outcrossing there are also benefits to restricting it. In particular, restricted outcrossing keeps fit combinations of genes together, rather than being separated during recombination. In diploids, lack of outcrossing cleanses genomes of recessive mutations, some of which may have been deleterious; and it allows expression of phenotypes that are recessive but advantageous. Self-fertility provides reproductive assurance since sexual reproduction can occur in the absence of a mate. This is very advantageous when population sizes are small, for example, when there is dispersal limitation and very few migrants colonise a new habitat. So sex (i.e. nuclear fusion followed by meiosis) has a role in fungi, even when outbreeding will not result. Another possible role is in the repair of damage affecting both strands of the DNA molecule, for which a template from a homologous chromosome is needed. Meiosis would then allow copying from the template to occur during crossing over (gene conversion), as happens in the *Saccharomyces* species, amongst others.

While most fungi can reproduce sexually, some do not appear to do so, or at least only mate infrequently. Though there can sometimes be benefits of not outcrossing, as indicated in the previous paragraph, usually the benefits of genetic recombination, sex and outcrossing, are considerable. So how do fungal species with no known sexual processes survive? Molecular phylogeny (pp. 5–8) shows that mitosporic fungi belong to Ascomycota and Basidiomycota. This rather limited divergence from sexual forms indicates that they have not had a long evolutionary history. It seems likely that the loss of sexuality commonly results in short-term advantages but perhaps ultimate extinction. However, although many populations are clonal, molecular variation of mitosporic species shows that some genetic recombination occurs. Sexual reproduction of mitosporic fungi may sometimes occur in nature, as recently shown with *Aspergillus fumigatus* (p. 114), and another mechanism that may generate recombination is the **parasexual cycle** (pp. 122–123).

Glomeromycota, with the sexual process appearing to be completely lacking, is an outstanding exception to the idea that loss of sexuality may eventually lead to extinction. These fungi, which form arbuscular mycorrhizas (pp. 206–212), as well as being abundant today, occur in fossil material of the earliest land plants, and diverged from other fungal groups over 400 million years ago. The multinucleate spores of these fungi contain a population of genetically different nuclei. Live three-dimensional imaging and mathematical modelling showed that when spores of *Claroideoglomus etunicatum* are formed, rather than a single founder nucleus dividing, a stream of nuclei enter from nearby hyphae. This perhaps provides an alternative to

sex with natural selection operating to promote the survival of individuals with an optimally balanced population of genetically different nuclei. Also, these fungi, as valuable partners for their plant hosts, may receive some protection from the effects of hostile environments, but selection is most likely to operate on traits of the fungi in soil.

Types and Phases of Life Cycles

The sexual process in fungi, as in other eukaryotes, has three key steps: (1) **cell fusion** (plasmogamy) between two haploid cells, which are uninucleate in many fungi and genetically different, resulting in a cell with two different haploid nuclei; (2) **nuclear fusion** (karyogamy) of the two (typically) haploid nuclei giving a cell with a single (typically) diploid nucleus; and (3) **meiosis**, which results in four haploid cells. There is considerable variation amongst phyla in terms of which structures fuse during plasmogamy, and in which structures karyogamy and meiosis occur (Table 4.2). The timing of these events during the lives of fungi, and the intervals between these events, also varies considerably, resulting in a diversity of life cycles which can be categorised into five basic types (Figure 4.6), and are described below after considering the different types of plasmogamy.

TABLE 4.2 Distinguishing Features of the Lifecycles of Fungi and Fungus-Like Oomycetes that Reproduce Sexually

	Type of plasmogamy ^a	Site of karyogamy	Site of meiosis	Type of sexual spore
Fungus-like oomycetes	Gametangial copulation	Oospores	Gametangia	Oospore
Chytridiomycota	Gametes fuse	Zygote	Sporangium	Oospore
Zygomycetes	Gametangia fuse	Zygosporae	Zygosporae	Zygosporae
Ascomycota	Spermatisation (e.g. <i>Neurospora</i>)	Ascus initial ^b	Ascus ^b	Ascospore
	Gametangial copulation (e.g. <i>Arachnотis</i>)			
	Somatogamy (e.g. Free-living <i>Saccharomyces</i>)			
Basidiomycota	Somatogamy in the majority	Basidium initial ^c	Basidium ^c	Basidiospore
	Spermatisation between pycniospores and hyphae in Uredinales	Teliospores	Basidium	Basidiospore (haploid). Teliospore (dikaryotic then diploid); pycniospores/spermatia (haploid) (see Table 8.6)

^aSee text for explanation of types of plasmogamy.

^bSee Figure 1.14.

^cSee Figure 1.7.

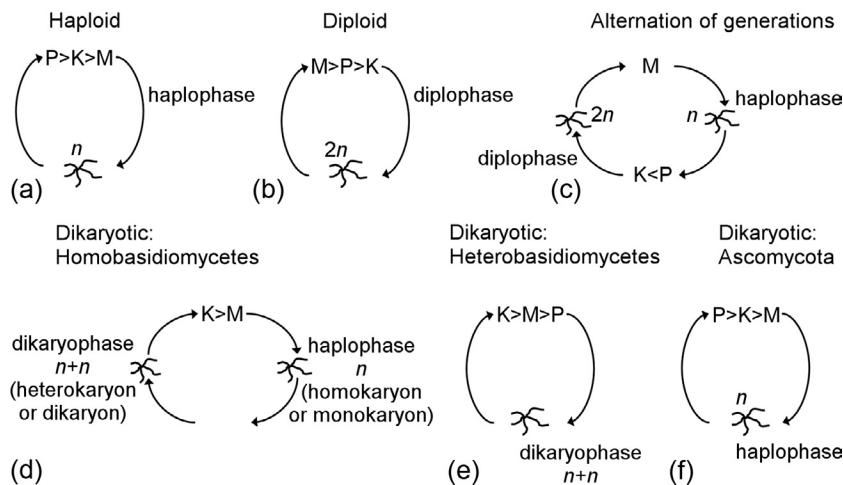


FIGURE 4.6 Fungal life cycles. There are five basic types, four of which are shown here: (a–c) haploid, diploid, and haploid/diploid respectively. The fifth is a completely asexual life cycle where nuclear fusion and recombination does not occur. The dikaryotic type could be considered as three subtypes (d, e, f). The life cycles differ depending on the extent of time between plasmogamy (P, cell fusion), karyogamy (K, fusion of nuclei) and meiosis (M).

There are five main types of plasmogamy, most commonly between cells which do not differ in morphology (isogamy), but sometimes where the cells are of different size (anisogamy) – as in fungus-like oomycetes, the larger being referred to as female and the smaller as male: (1) **Gametangial copulation** occurs in the fungus-like oomycetes, in which small male antheridia produce fertilisation tubes which grow chemotropically towards, form branches around, and then fuse with the larger female oogonia, allowing nuclei to pass through a fine penetration peg and fuse with oospores. In some Ascomycota, a thin tube (trichogyne) grows from the gametangium (termed an ascogonium) to the antheridium, and a nucleus passes along the trichogyne to the ascogonium. (2) **Fusion of gametes** – unicells, in which at least one, usually both, are motile. Commonly they are the same size (isogamous zoospores), but occasionally one is larger than the other (anisogamous), and in the chytrid genus *Monoblepharis*, the motile male gametes are released from an antheridium and penetrate a large static female oogonium (heterogamy or oogamy) to fertilise the oospores. (3) **Fusion of gametangia** in which first, typically, morphologically identical zygomycete zygomorphs grow together, swell to form progametangia, differentiate gametangia, and subsequently fuse to form a zygote, which develops into a thick-walled zygosporangium (Figure 1.22). (4) **Spermatisation** involves fusion between uninucleate non-motile cells and a ‘female’ gametangium. In *Neurospora*, for example, the trichogyne of the female curves around a conidium and the spore nucleus travels along the trichogyne into the ascogonium. In rusts (Basidiomycota), pycniospores are transferred to receptive hyphae. (5) **Somatogamy** involves fusion of structures which are morphologically no different from other vegetative parts, including between hyphae, and between yeast cells. All types of plasmogamy are under tight hormonal control.

There are five main types of lifecycle: haploid, diploid, haploid–diploid, dikaryotic, and asexual, characterised by the number of nuclei and ploidy of the nuclei and whether or not mating occurs (Figure 4.6):

Haploid lifecycle. For the majority of the life cycle the vegetative mycelium is haploid. When plasmogamy occurs, it is quickly followed by karyogamy and usually followed quickly by meiosis (e.g. in zygomycetes). In zygomycetes, meiosis occurs in zygospores and if they lie dormant, the spore remains a diploid, but mycelia that arise following germination are haploid.

Diploid lifecycle. The vegetative thallus is diploid. Following meiosis, plasmogamy, and karyogamy soon occur. For example, *Saccharomyces cerevisiae* can exist as a haploid, diploid, or polyploid, but in nature is mostly diploid. Though some *Candida* spp. are haploid (e.g. *Candida lusitaniae* and *Candida guilliermondii*), the human pathogen *Candida albicans* (pp. 306–307) is usually diploid in its vegetative state (but see *Asexual life cycle*, p. 114).

Haploid-diploid lifecycle. Vegetative growth occurs both in haploid and diploid phases, the transition from haploid to diploid occurring by plasmogamy, and the transition from diploid to haploid resulting from meiosis. The haploid and diploid phases are morphologically similar, but with diploid cells often larger than haploid ones (e.g. in the yeast cells of *Saccharomyces cerevisiae*).

Dikaryotic lifecycle. Dikaryotic (literally two nuclei) cells are characterised by the presence of two haploid nuclei, and this situation is characteristic of Basidiomycota and, in a sense, of many Ascomycota. Following plasmogamy, the two nuclei do not fuse immediately giving rise to a dikaryon. In Ascomycota only the ascogenous hyphae and crozier cells are dikaryotic (Figure 1.14). In most Basidiomycota, somatic cells are probably dikaryotic for much of the life cycle (Figure 1.17). When basidiospores (haploid) germinate, the mycelium typically has one nucleus per compartment. This situation continues until plasmogamy occurs with a sexually compatible (pp. 114–118) mycelium, and then each compartment has two nuclei.

While the majority of basidiomycetes have a single nucleus per compartment prior to mating, some have several or many (e.g. in the genera *Coniophora*, *Stereum*, and *Phanerochaete*). These are obviously not monokaryons (mono means one), and are referred to as coenocytic homokaryons (i.e. many nuclei of the same origin, which are not necessarily identical). Following plasmogamy, each compartment contains nuclei from both mycelia, and these mycelia are referred to as heterokaryons (different nuclei). Monokaryons can also be referred to as homokaryons, and dikaryons as heterokaryons.

Dikaryons (or heterokaryons) are also formed in the so-called **Buller phenomenon**: dikaryotic (or heterokaryotic) or diploid mycelia can provide one nucleus (or more in species with many nuclei per compartment) to unmated monokaryotic (or homokaryotic) or haploid strains, termed di-mon (or he-ho) mating. For example, heterokaryon–homokaryon (he-ho) matings occur in *Stereum hirsutum*, dikaryon–homokaryon (di-mon) matings in *Schizophyllum commune*, and diploid and haploid strains of *Armillaria gallica*. Sometimes homokaryotic sectors arise in a heterokaryotic mycelium, allowing the possibility of remating with an appropriate homokaryon. Reassortment of nuclei in this way has been seen in *Heterobasidion annosum* in somatic incompatibility (pp. 102–104) interaction zones in the lab, and in dense populations in wood in the field.

How long mycelia remain as homokaryons has rarely been studied; some basidiomycetes may only have haploid nuclei for hours or days before successful mating/plasmogamy transforms them to dikaryons. Rare basidiomycetes may not encounter a mating compatible mycelium for many weeks or years, and may even live their whole life as a monokaryon. Even common basidiomycetes, such as *Trametes versicolor* can sometimes persist as homokaryons in nature for several years.

When a dikaryon has formed, this state (i.e. one nucleus from each of the two original mycelia in each cell/compartment) is maintained at cell division by a mechanism involving formation of clamp connections (Figure 1.8). Eventually, the mycelium may form sexual fruit bodies, the two nuclei fusing to form a diploid in the cells (basidium initials) that quickly become basidia (Figure 1.7; p. 11). So the diploid is confined to a single cell type in the life cycle. Meiosis then occurs in the basidia and each basidiospore produced contains a haploid nucleus, which subsequently germinates to give a haploid mycelium.

The rusts (Uredinales) and the smuts (Ustilaginales) are obligate, biotrophic plant pathogenic basidiomycetes with complex life-cycles involving several spore types (Table 8.6). The sexual stage is pathogenic, and mating typically occurs in association with the host. The smut fungus, *Ustilago maydis*, for example, exists as a non-pathogenic budding yeast. When two mating type compatible cells meet and fuse, dikaryotic hyphae are produced that are able to infect the host plant, maize (pp. 16–18).

Asexual lifecycle. In some fungi the sexual process has not been seen. In Ascomycota and in Basidiomycota, species which appear only to reproduce asexually are termed **mitosporic** fungi. However, lack of evidence for sexual reproduction in the lab does not necessarily mean that it does not occur in nature. *Aspergillus fumigatus*, for example, was thought for a long time to be asexual, but is now known to have a sexual cycle – under certain nutrient and environmental conditions (sealed plates of oatmeal agar, incubated for 6 months in the dark at 30 °C) it produced cleistothecia (p. 20) containing ascospores. For several *Aspergillus* species hitherto considered to be asexual, whole genome sequences have revealed the presence of suites of genes associated with parts of the sexual cycle in other ascomycetes, including for mating and pheromone response, meiosis, and development of fruit bodies. Population genetic studies have also shown evidence of genetic recombination (linkage disequilibrium) which indicates past sexual activity.

Many plant and animal pathogens are considered to be asexual, but this is likely due to the fact that only one clone has the set of genes needed to infect the appropriate plant or animal. Although *Magnaporthe oryzae* is, for example, considered to have an asexual life cycle, there appears to be a sexual population in India, and the global distribution of clones and clonal lineages probably reflects rare ‘escapes’ from the sexual population. The human pathogen *Candida albicans* was, until recently, thought to be completely asexual, always being diploid, however, mating between cells with opposite mating types (see below) does occur. However, as well as this heterothallism (see below), homothallism also occurs.

Mating Systems

Fungi have mating systems or breeding systems that determine whether or not individuals of the same species can mate. Some fungi are self-fertile but many fungi have genetic systems that prevent mating between very genetically similar individuals, (i.e. self-sterile) so that genetic diversity will be increased. With basidiomycetes, since somatic (vegetative) incompatibility (pp. 102–104) often prevents non-self mycelium from fusing to form a stable connection containing nuclei from both, this rejection mechanism has to be overcome before successful mating can occur. With ascomycetes, somatic (vegetative) incompatibility is suppressed during mating, provided that mating occurs between female reproductive structures (protoperithecia) and a male cell, and does not require hyphal fusion (heterokaryon formation) prior to

perithecia formation. Compatible matings are determined by mating-type (MAT) factors. The MAT loci have a complex genetic structure. In *Coprinus cinereus*, for example, there are four sites each having two closely linked loci with multiple alleles. However, in population genetics, these complex loci can usually be treated as if they were simply multiple alleles at two loci, A and B, i.e. $A_1\dots A_n$ and $B_1\dots B_n$, or in the case of some basidiomycetes one locus $A_1\dots A_n$.

Successful mating is achieved if alleles at the mating type loci differ. Since mating occurs between different genetic strains, the fungi that operate these systems are termed **heterothallic** (Greek, *hetro*=different, *thallos*=young shoot), and the controlling systems are termed **homogenic incompatibility** (Greek, *homo*=same) or equally appropriately **heterogenic compatibility**, since mating fails if strains are of identical **mating type**; the sexual process only occurs if two strains which differ in mating type interact.

Several kinds of mating systems, as described below, have been recognised in the fungi. Many fungi have two mating types, effectively two sexes. Morphological differences are often not evident and they are referred to as, for example, + and −, A and B, A and a, or A and α. Some fungi or fungus-like eukaryotes produce visibly different structures involved in plasmogamy, as described above, and these are often referred to as male and female structures. However, it is inappropriate to equate two sexes with male and female as mycelia arising from a single haploid spore are often able to produce both male and female structures, but fertilisation will only occur as a result of an encounter with the opposite mating type. For example, a haploid mycelium of *Neurospora crassa* can produce female protoperithecia and conidia which are potentially male gametes. It is only in the fungus-like oomycetes that sexual differentiation is involved in the promotion of outcrossing. The mating systems of some fungi are more complex, with more sexes, and these are normally referred to with letters and numerical subscripts.

Population biologists studying diploid plants and animals use terms such as parents, progeny, outbreeding, self-fertilisation, etc., but these terms have slightly different meanings when applied to fungi, because in the vast majority, the vegetative thallus is haploid, at least for a while. So the vegetative thallus can be treated as 'self' rather than as a gamete. A major distinction is between fungi that operate **outcrossing** strategies, that promote genetic exchange, and **non-outcrossing** strategies where genetic exchange is promoted less (Figure 4.7). The term outcrossing encompasses situations where mating occurs between different haploid genotypes irrespective of whether they derived from the same spore source (e.g. the same basidiocarp or ascocarp) or from a different spore source. On the other hand, the term **inbreeding** does denote origin from the same spore source, and outbreeding denotes origin from different spore sources.

Mating Systems that Promote Outcrossing

Breeding Systems with Two Mating Types: Dimixis

In those fungi studied to date, the two mating types differ with respect to the allele present at a single mating type locus. Mating is only successful between haploid cells or mycelium that differs at the mating type locus. The system ensures that mating does not take place between the genetically identical progeny of a single haploid cell. A diploid cell that arises from mating will carry both mating types, and when meiosis occurs the resulting haploid progeny will be half of one mating type and half of the other. So there is 50% chance that the encounter

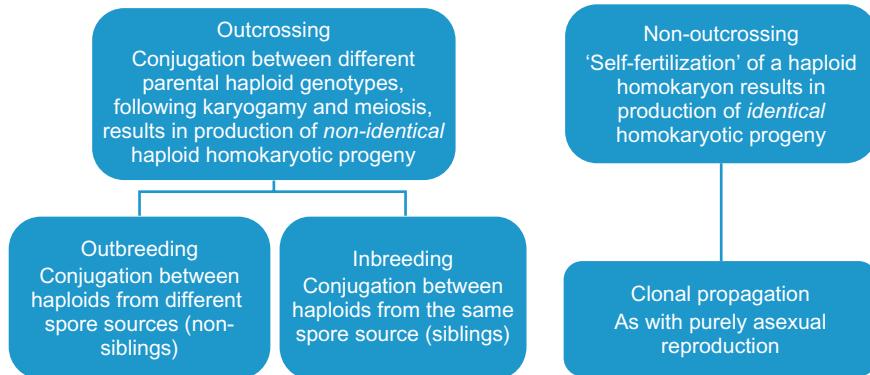


FIGURE 4.7 Terminology for describing breeding strategies in fungi with a haploid phase (i.e. a yeast/mycelium with nuclei derived from a single haploid nucleus). Source: Rayner, A.D.M., Boddy, L., 1988. *Fungal Decomposition of Wood: Its Biology and Ecology*. John Wiley, Chichester.

between two individuals derived from a single diploid will result in mating (the **inbreeding potential**). Since species with this breeding system only have two mating types in the whole population, there is also a 50% chance of an encounter between two unrelated individuals resulting in mating (**outbreeding potential**) (Table 4.3). Thus, although selfing is prevented, the likelihood of mating between close relatives is not reduced.

Breeding Systems with Many Mating Types (Diaphoromixis): Unifactorial (Bipolar) Incompatibility

Some Basidiomycota have a single mating type locus (and are hence 'unifactorial') but within the whole population have a large number of mating type alleles. Mating occurs between any haploid mycelia that differ in mating types (i.e. have different alleles of the mating type locus). So the diploid basidium nucleus is heterozygous for mating type (e.g. A_1A_2). Hence, two of the haploid spores borne on a basidium will be of one mating type (e.g. A_1) and two of the other (e.g. A_2). This is why this type of system is sometimes termed **bipolar**. Since any diploid strain yields two mating types, the inbreeding potential, as with breeding systems having only two mating types, is 50%. That is, if the mycelia from a large number of basidiospores from the same fruit body are paired in all combinations, 50% of the pairings will mate successfully. Since, however, a large number of mating types can occur in a population, almost all encounters with non-sibling strains can be compatible and the outbreeding potential can approach 100% (Table 4.3), so there is a bias towards outbreeding.

Breeding Systems with Many Mating Types (Diaphoromixis): Bifactorial (Tetrapolar) Incompatibility Systems

Many Basidiomycota have mating systems with two unlinked mating type factors, designated *AB*. Alleles at both the *A* and the *B* loci must differ for successful mating. The *A* and *B* genes control different parts of the mating process. In *Coprinopsis cinerea* and *Schizophyllum commune*, the *A* genes control the development of clamp connections

TABLE 4.3 Characteristics and Examples of Fungi Having Different Mating Systems that Promote Outcrossing

System	Examples	Number of mating type loci	Number of different alleles at each locus in the whole population	Designation of mating types	Inbreeding potential (%) ^a	Outbreeding potential (%) ^b
Two mating types	Most zygomycetes; <i>Neurospora crassa</i> (Ascomycota); <i>Saccharomyces cerevisiae</i> and <i>Schizosaccharomyces pombe</i> (Ascomycota, yeast)	1	1	$\pm, Aa, a\alpha$	50	50
Unifactorial (bipolar) incompatibility	<i>Coprinus disseminatus</i> and <i>Stereum gausapatum</i> (Basidiomycota)	1	Many	$A_1 \dots A_n$	50	Nearly 100
Bifactorial (tetrapolar) incompatibility	<i>Coprinopsis cinerea</i> and <i>Schizophyllum commune</i> (Basidiomycota)	2	Many	$A_1 B_1 \dots A_n B_n$	25	Nearly 100
Modified tetrapolar incompatibility	<i>Ustilago maydis</i> (Basidiomycota, smut)	2	2 at locus <i>a</i> , many at locus <i>b</i>	$A_1 B_1 \dots A_2 B_n$	25	50

^aThe probability that a randomly encountered sibling (product of the same diploid parent) will be compatible.

^bThe probability that a randomly encountered unrelated individual will be compatible.

(Figure 1.8), which maintain nuclei from both parents in each compartment. The *B* genes regulate exchange of nuclei between both partners, migration of nuclei from one mycelium through the other and vice versa, and encode for pheromone and receptor systems. Differences at both loci are necessary for successful mating; differences at only one of the loci result in semicompatibility.

If one of the nuclei in a fungus has mating factors $A_1 B_1$ and the other $A_2 B_2$, basidiospores can possess nuclei that have the same mating factors as either of the parents (i.e. $A_1 B_1$ or $A_2 B_2$), and $A_1 B_2$ and $A_2 B_1$. Such systems with four possible combinations of mating factors are termed **tetrapolar**. When haploid mycelia derived from basidiospores from the same fruit body are paired in all combinations, only 25% of crosses will mate successfully because only 25% of the crosses will have different alleles at both mating loci (Table 4.4), so there is a greater outbreeding bias than with bipolar systems. In a population that contains many *A* and *B* factors, the outbreeding potential will approach 100%. Recombination between the subunits of the *A* or the *B* factors can occur during meiosis, yielding a new mating type compatible with the parent types. The frequency of such recombination between mating factors varies greatly between species and strains, and is also influenced by environmental conditions. Where it is high, new mating types will arise with high frequency, so the inbreeding potential will greatly increase.

TABLE 4.4 Mating among the Haploid Progeny from the Fruit Body of a Basidiomycete with Tetrapolar Incompatibility

Mating type alleles	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	–	–	–	+
A_1B_2	–	–	+	–
A_2B_1	–	+	–	–
A_2B_2	+	–	–	–

The basidium nuclei have the mating genotype $A_1B_1, A_1B_2, A_2B_1, A_2B_2$. Meiosis gives rise to haploid progeny with four possible mating type genotypes – $A_1B_1, A_1B_2, A_2B_1, A_2B_2$. Mating is only completely successfully if encounters are between mycelia that differ with respect to both mating type factors. The chequerboard shows that only 25% of the possible encounters satisfy this condition. The inbreeding potential is thus 25%.

Modified Tetrapolar Incompatibility

In some Basidiomycota there are many B factors but only two A factors. This resembles typical tetrapolar incompatibility in giving an inbreeding potential of 25%, but resembles incompatibility systems with two mating types in having an outbreeding potential of 50% (Table 4.3).

The Evolution of Mating Type Genes and Their Function in Fungal Life Cycles

Mating type genes are central to the operation of the fungal life cycles described above. While emphasis has traditionally been placed on differences in breeding systems between phyla, more recent analysis of these sex-determining regions of the genome in zygomycetes, ascomycetes, and basidiomycetes has revealed some underlying homologies of the genes involved. Mating type loci of fungi operate like sex chromosomes in more complex eukaryotes, to control compatibility between sexual partners, enable sexually compatible haploid cells to recognise and attract each other, and to prepare the cell for sexual development after fertilisation.

Genetically controlled mating compatibility – heterothallism – was first discovered in the basal lineage zygomycetes, in *Phycomyces blakesleeanus*, named after Albert Blakeslee, the American discoverer of fungal heterothallism. The mating type locus encodes a type of transcription factor belonging to the High Mobility Group (HMG). The plus and minus strains of *Phycomyces blakesleeanus* each carry a different HMG. The function of these regions in controlling sexual development was shown when sexual spores were found in a mutant haploid strain with a diploid mating type region. The two HMG's segregate with mating type in crosses. One of them consists of a longer DNA sequence than the other; the sequenced genome revealed it to have been expanded by repetitive elements of DNA. Such expansions are known to suppress recombination at the regions of the DNA where they occur, and might explain why this mating type region has persisted intact through evolution of more complex systems. Such suppression of recombination by repetitive elements in mating type loci may drive the expansion of sex-determining regions, not only in fungi but in other eukaryote lineages as well, even perhaps leading to the evolution of sex chromosomes like our own.

In heterothallic ascomycetes there is typically one mating type locus with two forms, and the study of these in mating of the model yeast *Saccharomyces cerevisiae* provides one of the most complete analyses of developmental gene expression in eukaryotes. The so-called *MAT* loci determine haploid and diploid cell identity. They function to ensure outbreeding

by encoding sex-specific expression of many genes required in mating. In both *MAT α* and *MAT α* haploid cells, sex pheromones, and their receptors are expressed under the control of transcription factors encoded at the *MAT* loci. The transcription factors involved in mating of *Saccharomyces cerevisiae* include two alternative homologous sequences ('homeodomains') HD1 and HD2, one in each mating type. *MAT α* cells express a DNA-binding protein (alpha-box protein) that activates transcription of its alpha sex pheromone, as well as receptors to sense the pheromone secreted by compatible cells of the *MAT α* mating type. The *MAT α* cells have a different transcription factor, encoded by an HMG domain, which activates transcription of a-pheromone and α -pheromone receptors, setting up reciprocal pheromone attraction between neighbouring compatible haploid cells. Pheromone binding at mating then triggers the expression of all the genes involved in mating, via an intracellular mitogen activated protein (MAP)-kinase cascade. After mating, the mating type genes help to establish and maintain the characteristics of the diploid cell.

In basidiomycetes, some of these genes are conserved. The *A* locus contains genes for both HD1 and HD2 homeodomain transcription factors, and the *B* locus contains genes for pheromones and pheromone receptors. The heterodimer of HD1 and HD2 protein can be formed by any pair of protein products of allelic gene pairs, provided these are not identical. At the *B* locus, pheromone and receptor genes have been gathered together. Basidiomycota are the only lineage known to have evolved tetrapolar outbreeding systems. Within the phylum, a variety of derived sexual cycles has evolved from tetrapolar ancestry. In some species, bipolarity is secondarily generated, either by fusion of the two loci or by loss of *B* mating type function (although not by loss of the *B* genes, because these still function in the regulation of development, albeit in mating-type independent fashion). Anamorphic fungi in some ascomycetes and basidiomycetes with no known sexual phase in their life cycles contain sex-encoding regions, with the practical implication that reactivating this latent potential for sexual recombination might provide a useful breeding strategy for industrial strain development. Simplification of mating systems to reduce or obviate the need for mating seems also to have resulted from adaptation to niches including, for example, *Cryptococcus* the human pathogen (Chapter 9), and *Ustilago* the smut pathogen of plants (Chapter 8). However, tetrapolar mating remains widespread in other lineages including Agaricomycetes.

Systems Restricting Outcrossing

Self-fertility

Some fungi are self-fertile, that is the sexual process can occur between genetically identical cells. Self-fertility is also referred to as **homothallism** (Greek, *homo* = same). Homothallism is termed **primary** if there is no evidence of a heterothallic ancestor. If it is clear that an earlier heterothallism has been circumvented, the term **secondary homothallism** is used. For example, *Neurospora tetrasperma* (like *Neurospora crassa*) has two mating type alleles, but instead of an ascus containing eight uninucleate ascospores, only four are produced, each with two nuclei, one of each mating type. The resulting vegetative mycelia and protoperithecia also contain nuclei of both mating types, so fertilisation by conidia of a different strain is not required for ascus production. Basidia of the cultivated mushroom, *Agaricus bisporus*, have two binucleate spores per basidium instead of the four uninucleate spores characteristic of most Basidiomycota. A unifactorial incompatibility system occurs but the two nuclei in a

basidiospore are commonly of different mating type, hence, the mycelium that arises from a single basidiospore is usually able to produce fertile fruit bodies. This could be an advantage for fungi in situations where encountering a compatible mate is unlikely, for example following long-distance spread and colonisation of new habitats. Occasionally, a spore of *Agaricus bisporus* only has one nucleus. In *Moniliophthora perniciosa* (the witches' broom pathogen of cocoa (*Theobroma cacao*)), there is usually one nucleus per spore on each basidium, but in the same basidiocarp, up to about 8% of spores can be binucleate, and occasionally trinucleate. So, in Ascomycota and Basidiomycota, there is probably a continuum from full outcrossing to secondary homothallism.

While some species are completely self-fertile, other species have some populations that are non-outcrossing while others are outcrossing. For example, the wood-decay basidiomycete *Stereum sanguinolentum* has many (>30) distinct clonal subpopulations in Northwestern Europe, but outcrossing populations in Austria and North America. As well as non-outcrossing sexual systems, clonal subpopulations can be generated by the production of asexual spores, especially by Ascomycota, and extensive clones can develop even in populations with sexual outcrossing mechanisms.

Strains of the yeast *Saccharomyces cerevisiae* have two mating types, *a* and *α*. Some strains appear to be homothallic, with mating occurring among the progeny of a single haploid cell. The apparent homothallism is the result of a switch from *a* to *α* or of *α* to *a* mating type which can occur at cell division (Figure 4.8a). The molecular basis of mating type switching is the replacement of the genetic factor of the mating type (*MAT*) locus by a copy of the alternative factor resident at a silenced locus (i.e. a locus at which the gene is not expressed), there being one silenced locus for each mating type (*HML* homologous to *MATα* and *HMR* homologous to *MATA*). Recombination between *MATA* and *HMR(a)* or between *MATA* and *HML(α)* results in a switch in mating type.

Homothallic and heterothallic strains differ in genotype at a locus which initiates mating type switching, possessing the alleles *HO* or mutant *ho*, respectively. *HO* encodes an endonuclease that induces a DNA double strand break within the *MAT* locus, which provides a substrate to initiate the recombination event with the opposite silent locus. At each mitotic division of a free living haploid yeast cell, *HO* induction occurs within the original mother cell allowing mating with the adjacent, genetically identical daughter cell (Figure 4.8b). Outbreeding is also possible between cells that are of opposite mating type from a meiotic event or if it encounters a cell having a different mating type in the environment (Figure 4.8b).

Mating type switching also occurs in the homothallic fission yeast *Schizosaccharomyces pombe*. *Schizosaccharomyces pombe* is distantly related to *Saccharomyces cerevisiae*, and mating type switching has apparently evolved independently in the two species. It may also occur in apparently homothallic filamentous fungi, but it would be less easy to detect.

Self-fertility does not prevent outcrossing but can reduce the likelihood of it occurring, as encounters are more likely to take place between cells of common parentage than between unrelated cells. The absence of the requirement for encountering mycelia/cells with a complementary mating type may have short-term advantages, especially if no other spore type is produced, or if meiospores (spores produced following meiosis) have some special role. It is not clear how far homothallism restricts recombination, as molecular variation in nature has been studied only occasionally. Recombination has been found in populations of secondarily homothallic species and clonal populations (e.g. *Neurospora crassa*).

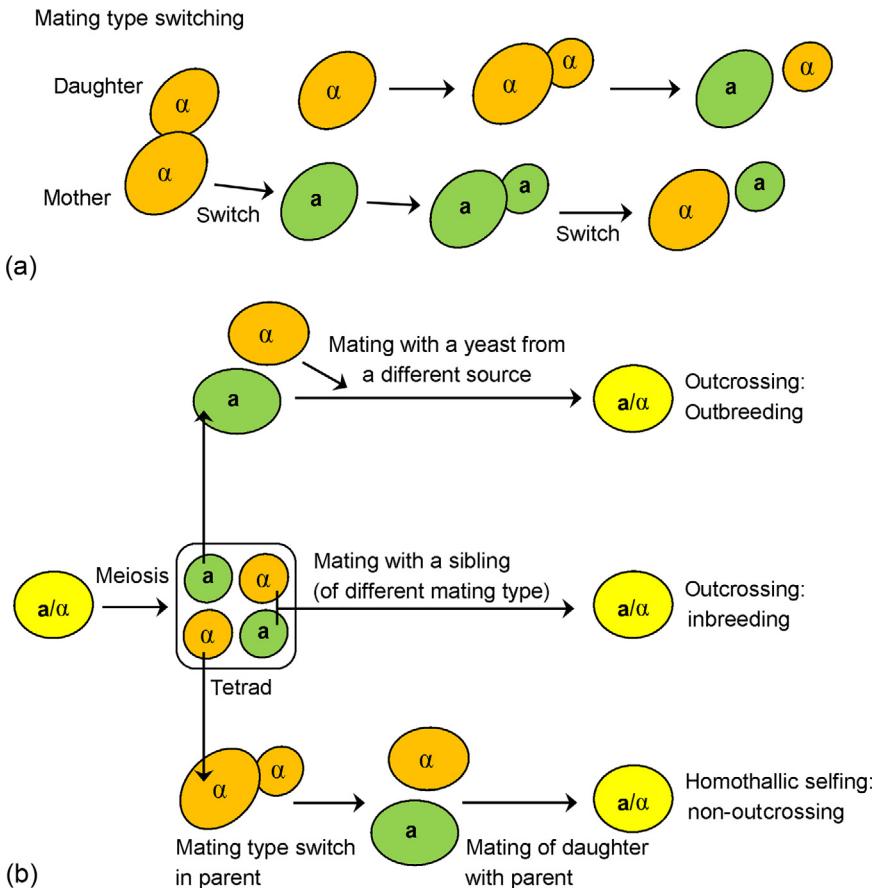


FIGURE 4.8 Mating and mating type switching in ascomycete yeast. The complexity allows multiple options for inbreeding and outbreeding. (a) Mating type switching occurs in haploid *Saccharomyces cerevisiae*. The *MAT* locus switches form (i.e. from **a** and **α** mating type and vice versa, in the G1 phase of every cell cycle, allowing self-mating (b). (b) Outcrossing is also possible between cells that are of opposite mating type, either from a meiotic event (inbreeding from mating with siblings) or if a yeast encounters a cell having a different mating type in the environment (outbreeding). Intratetrad mating leads to a slower loss of heterozygosity, in subsequent diploid generations, than homothallic selfing, which fixes loci instantly.

Fertility Barriers

Though, as discussed above (p. 114), many fungi are self-sterile and have systems that promote outcrossing, fertility barriers can limit the extent of outcrossing. Two main types are genetic disharmony and heterogenic incompatibility. If, through geographical separation, there is little gene flow between two populations for a long time, genetic differences between the populations may arise (i.e. **genetic disharmony**). Such differences could lead to the inefficient performance or failure of some step in the complex interactions between the participants that occur in mating. As a result, mating could fail completely or only a few progeny may be produced. Furthermore, efficient metabolism and normal development are dependent not

only on the action of individual genes but on harmonious interaction throughout the genome. Hence, even if mating is successful, an unsatisfactory interaction between the genes from dissimilar parents may result in hybrids with poor viability or which are sterile, and unable to compete with either parent strain. The second type of fertility barrier – **heterogenic incompatibility** – occurs when mating fails as a result of one or a few genetic differences. Heterogenic incompatibility resulting in failure of vegetative mycelia to fuse has already been considered (pp. 102–103). In the ascomycete *Podospora anserina*, some of the interactions which prevent vegetative cell fusions also act to prevent mating. In some fungi, some mechanisms of heterogenic incompatibility may act solely in the sexual phase. The possible role of heterogenic incompatibility is returned to when considering the parasexual cycle (below).

Amixis

With amixis, all of the usual morphological features of the sexual process are present, but nuclear fusion and meiosis do not occur. This is equivalent to loss of sexuality and obligate inbreeding. It can be distinguished from homothallism only by detailed cytological study. Amixis has been little studied in fungi but some examples are known: *Podospora arizonicensis* (Ascomycota) produces spores that look like normal meiospores but have been produced by mitotic divisions. In the Chinese paddy straw mushroom, *Volvariella volvacea*, homokaryotic mycelium produces basidiocarps; two identical, haploid nuclei enter each basidium, fuse and undergo meiosis, producing four haploid nuclei, one being present in each of the four basidiospores. The progeny are genetically identical to the parent but it remains to be seen whether the process of meiosis could cause epigenetic changes (p. 134), as occur during meiosis in animals and other eukaryotes.

The Parasexual Cycle

Somatic (vegetative) incompatibility (pp. 102–103) usually prevents widespread heterokaryosis between non-self mycelia. However, VC is not a complete barrier to formation of heterokaryons between different individuals. Occasionally two haploid nuclei in a vegetative mycelium may fuse to give a somatic diploid nucleus. If the mycelium is heterokaryotic then the fusion may be between genetically unlike nuclei to give a heterozygous diploid nucleus. This is the start of the **parasexual** cycle (Figure 4.9). Such diploidization is rare, occurring perhaps once in a population of a million nuclei. Mitotic crossing over in these diploid nuclei may occur and can generate diversity. This is a rare event compared with meiotic crossing over, occurring perhaps once per 500 mitoses. Errors in mitosis are quite common, and often the mitosis of a diploid nucleus results in aneuploidy. Such abnormalities in chromosome number (aneuploidy, see p. 124) commonly lead to poor growth, and further change in chromosome number results in restoration of the haploid state. Before the advent of molecular biology, parasexual recombination was used to produce new strains of asexual fungi of commercial importance (e.g. to produce strains of *Penicillium chrysogenum* with increased penicillin production). It has also been exploited in the lab for linkage mapping in asexual fungi. Parasexual recombination is probably largely confined to laboratory culture, and does not appear to be an important phenomenon in nature, though there is some evidence that *Cryphonectria parasitica* in North America has recently recombined by a parasexual process. Fungi that are diploid for most of their lifecycle can also undergo a parasexual cycle. In *Candida albicans*, for example,

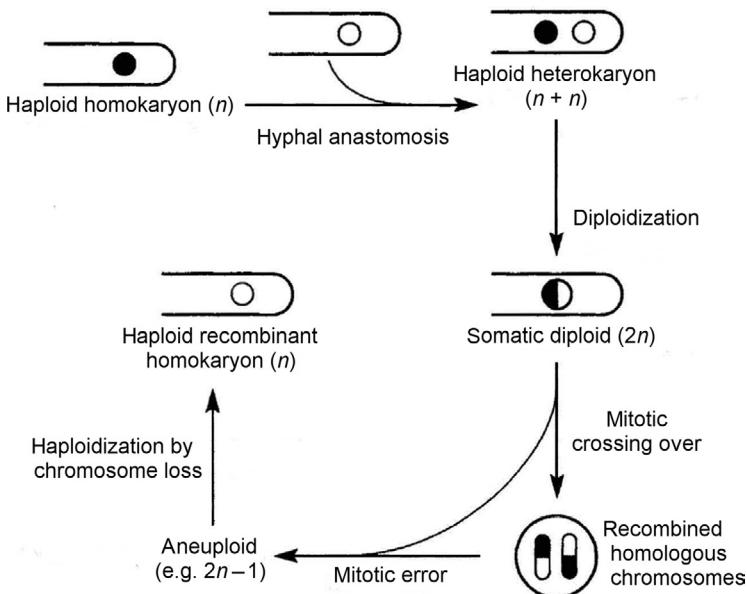


FIGURE 4.9 The parasexual cycle. Hyphal fusion between two genetically different homokaryons produces a heterokaryon. If the haploid nuclei fuse they produce a heterozygous diploid nucleus. Rather than undergoing meiosis, they divide mitotically. Recombination between homologous chromosomes (mitotic crossing over) can sometimes occur. Sometimes, mitotic errors can yield aneuploids, with abnormal chromosome numbers (e.g. $2n - 1$) and commonly poor growth. The haploid state is restored by loss of chromosomes. Since the chromosomes that are lost can have come from either of the original homokaryons, a recombinant can result, even if mitotic crossing over has not occurred.

diploid cells fuse to form tetraploid ($4n$) cells, which undergo mitosis and random loss of chromosomes that returns them to the diploid state without meiosis occurring.

VARIATION, MICROEVOLUTION, AND SPECIATION

Variation Within Fungal Species in Nature and the Laboratory

As with plants and animals, including humans, there is a great deal of variation within fungal species. Such variation can affect all aspects of the biology of a fungus. Strains isolated from nature often differ in morphology and physiology. *Aspergillus nidulans* strains, for example, differ in colony growth form, in the amount of sexual and asexual sporulation, in extension rate and quantity of metabolic products. Many different VC groups occur in *Aspergillus nidulans* and in other ascomycetes (pp. 102–103). Natural populations of basidiomycetes (e.g. *Schizophyllum commune*) have many different mating type alleles (pp. 114–118). Strains of plant pathogens vary in their ability to attack different strains/varieties of their host species (Chapter 8), which is of considerable practical importance in plant breeding and agriculture. Strains of a pathogen species that can be distinguished from other members of the same species by their ability to be pathogenic on a specific host species, host variety, or

group of hosts (i.e. have the same combination of virulence genes), are termed variously **pathotype, biotype, or race**.

Strains may also differ in chromosome number. In some species, there are strains that are polyploid, with haploid chromosome numbers that are a multiple (e.g. 2X, 4X) of the basic haploid number (X) (e.g., in the chytrid *Allomyces*, the yeast *Saccharomyces* and in most fungal groups). Alternatively, a strain may be aneuploid, with one or a few chromosomes duplicated or lost (e.g. X+1, X-1, X-2) (e.g. in *Neurospora crassa* and *Aspergillus nidulans*). Also, many pathogenic fungi possess complements of accessory (also called dispensable or 'B') chromosomes, enriched in gene duplications, and transposable elements (TEs) (pp. 132–134), that may play a role in pathogenicity or fungicide resistance. *Nectria haematococca* accessory chromosome 14, for example, has a cluster of genes that encode detoxification of a phytoalexin (p. 256).

Genetic variation may be continuous or discontinuous. **Discontinuous variation** refers to the situation where a feature is either present or absent. A dispensable chromosome, for example, is either present or lacking, and a strain either belongs to a particular mating type or it does not. Genes of plant pathogens encoding avirulence or host-specific toxins often exhibit presence/absence polymorphisms. Discontinuous variation occurs where a single character has a decisive effect. **Continuous variation** refers to the situation where a feature varies in magnitude between strains. Thus, strains may show a wide range of growth rates, some differing only slightly, but with a large difference between the fastest and slowest growing strains. Continuous variation is seen where the magnitude of a feature is influenced by many genes, each with a relatively small effect. However, it has recently been shown that sometimes different alleles can provide a continuous (quantitative) phenotype.

In the laboratory, even the same isolate of some species (e.g. *Fusarium*) are highly variable in culture, morphological changes occurring with subculture. Others (e.g. *Aspergillus*) are regarded as stable. So some species exhibit greater phenotypic plasticity than others. Phenotypic differences can reflect the drastically different conditions in culture compared with nature. Many *Fusarium* species, for instance, live in very dilute solutions in the water-conducting vessels of plants, but in laboratory culture, nutrient concentrations are typically much higher. Hence, with *Fusarium* there may be intense selection favouring any variant capable of coping with the unnatural conditions in culture more efficiently. Subculture by means of pieces of agar bearing mycelium can propagate mutated cells rather than the original. Also, as it can give a culture arising from the hyphae present rather than spores, the ability to sporulate is sometimes rapidly lost, as there is then no selection against mutants defective in sporulation. Plant pathogenic fungi often lose the ability to sporulate and lose virulence in culture, but the loss in phenotype can often be traced back to methylation (epigenetic modification – see pp. 134–135) rather than to mutations. Thus both prolonged growth in artificial conditions and repeated subculture can lead to genetic or epigenetic change. It is thus desirable to maintain stocks under conditions that permit prolonged preservation in a dormant state, such as liquid nitrogen refrigeration, or to use freshly isolated material.

Microevolution

Variation in fungal populations has been discussed above. Here we consider how this variation is generated, and how it changes over time. The main sources of genetic variability result from mutation, recombination, transposons, and horizontal gene transfer (HGT), all

of which alter the composition of an organism's DNA, which encodes most of an organism's heritable characteristics. However, other mechanisms are also responsible for heritable phenotype; epigenetic changes alter phenotype by causing changes to DNA, but not to the nucleotide sequences.

Over time, allele frequencies at or below the species level change – a process termed **microevolution**. An allele may diminish in frequency, and even disappear from a population. On the other hand, it could increase in frequency and sometimes replace all alternative alleles in a process called a **selective sweep**. These changes in allele frequencies can occur due to mutation rate, selection, gene flow, and genetic drift, and are explained first below, followed by consideration of recombination, TEs, HGT, and epigenetics.

Mutation

Mutation is a permanent change to the nucleotide sequence of a genome. It can be a point mutation at a single locus, or a deletion, insertion, or rearrangement of a larger section of DNA. It only weakly affects allele frequencies, but is a major factor for introducing new alleles. Gene mutation is the ultimate source of genetic variability. Genes vary in their mutation frequency, but one mutation per million copies of a gene per generation can be taken as an average value. The genome size of different fungi varies, but taking a genome of 10,000 genes as typical, in each generation one cell in a hundred could in some respect carry a new mutation. Most of the compartments/cells in a fungal colony are capable of giving rise to further vegetative cells or to spores, so the number of cells in a fungal population that can undergo mutation and leave progeny can be very large. Furthermore, many fungi are haploid for a large part of their life cycle, so mutations will be expressed immediately, and if beneficial will spread through a population by natural selection (next section).

An example of the effectiveness of mutation in producing variation is provided by yellow rust (*Puccinia striiformis*). Most populations in Europe and North America are clonal, yet new races still emerge quickly, as a result of mutation. The same is true for several other rusts and powdery mildew fungi (Chapter 8).

Large populations often have more alleles than small populations, because there are more mutations upon which selection can operate. Also, large populations experience less genetic drift (p. 130), and are less likely to lose alleles. So with regard to plant pathogens in agroecosystems, it is important to keep population sizes low so that there are fewer mutations in avirulence genes or genes encoding fungicide resistance. With regard to breeding plants for resistance to pathogens, if two (or more) genes are introduced simultaneously into a host genotype, then the pathogen will need two (or more) simultaneous mutations from avirulence to virulence to overcome resistance to infection. If the mutation rate is 10^{-6} , then the probability of two genes mutating simultaneously would be 10^{-12} , and the probability of three genes mutating 10^{-18} . The mildew *Blumeria graminis* f. sp. *hordei*, produces around 10^{13} spores $\text{ha}^{-1} \text{ d}^{-1}$, so about 10 double mutants would be produced each day in 1 ha of barley, and one triple mutant in 10^5 ha. Often 10^6 ha of crop are planted, but since the vast majority of spores fail to land in a suitable environment, the rare mutants have little chance to establish. Hence plant breeders are now keen to employ **resistance gene pyramids** composed of novel genes that have not yet been defeated.

In summary, by creating new alleles, mutation is an important first step of evolution. Recombination (pp. 130–132) can also create new alleles by intragenic recombination, seen

for example, in genes encoding fungicide resistance and host specific toxins in some plant pathogenic fungi. Over an extremely long time, mutation can result in significant changes in allele frequencies. However, if mutation was the only factor acting on populations, then the rate of evolution would often not be observable.

Natural Selection

Darwin was impressed by the success of plant and animal breeders in producing, by means of **artificial selection**, new varieties with features that were considered desirable. This involves selecting individuals showing the required features to a greater extent than other individuals, and breeding from them, the process being repeated over many generations. Disease-resistant crop plants are obtained in this way, by breeding from the most resistant plants. Darwin also realised that in natural populations many individuals did not survive to reproduce. He proposed that any features that favoured survival would be **naturally selected**, and tend to spread through a population, ultimately leading to evolutionary change. Natural selection of favourable variations is now generally accepted as being the major, and probably the only, basis for the acquisition of features that result in a population becoming better adapted to its environment, the process of **adaptive evolution**.

Natural selection is largely responsible for adaptive genetic changes in a population, including stabilising, directional (in which variation decreases) and disruptive changes (in which variation increases) (Figure 4.10). Most commonly it is a conservative force, eliminating deviants and maintaining the status quo. This is because a population is normally well adapted to the environment in which it occurs, and genetic change, whether from mutation, recombination, or gene flow, is more likely to result in a decrease rather than in an increase in fitness. Natural selection acting to eliminate variants that differ markedly from the average is termed **stabilising selection**. For example, heterokaryotic isolates of the basidiomycete *Schizophyllum commune* obtained from fruit bodies, and thus representing mycelium that has survived natural selection, have a limited range of radial extension rates. Heterokaryons produced in the laboratory by mating homokaryons have a much wider range of extension rates but with approximately the same mean. Hence in the natural population, individuals with extension rates much lower or much higher than the mean have been eliminated by stabilising selection. Hybridisation and artificial selection can give variants differing widely from the forms occurring in nature, indicating the widespread operation of stabilising selection.

Natural selection can also act to produce change – termed **directional selection**, both to characters showing continuous variation and those determined by single genes. Directional selection operating on a continuously varying feature will favour individuals at one extreme (Figure 4.10). Acting on features determined by single genes, it will either diminish or increase the frequency of an allele, in extreme instances bringing about the extinction of the allele or causing it to be ‘driven to fixation’, completely replacing alternative alleles. The appearance in the course of a few decades of several pathotypes in an Australian population of *Puccinia graminis* f. sp. *tritici* of clonal origin, is an example of directional selection occurring. Mutation pressure could not have affected the spread of the pathotype mutants in so short a time. In the higher termite/*Termitomyces* mutualism, the termites feed on nutrient-rich fungus nodules – immature fruit bodies containing asexual spores, some of which survive passage through the gut and act as inocula when new food is brought into the nest (p. 332). Since only a few spores successfully form mycelia, this leads to reduced variation within a nest. Since genotypes that

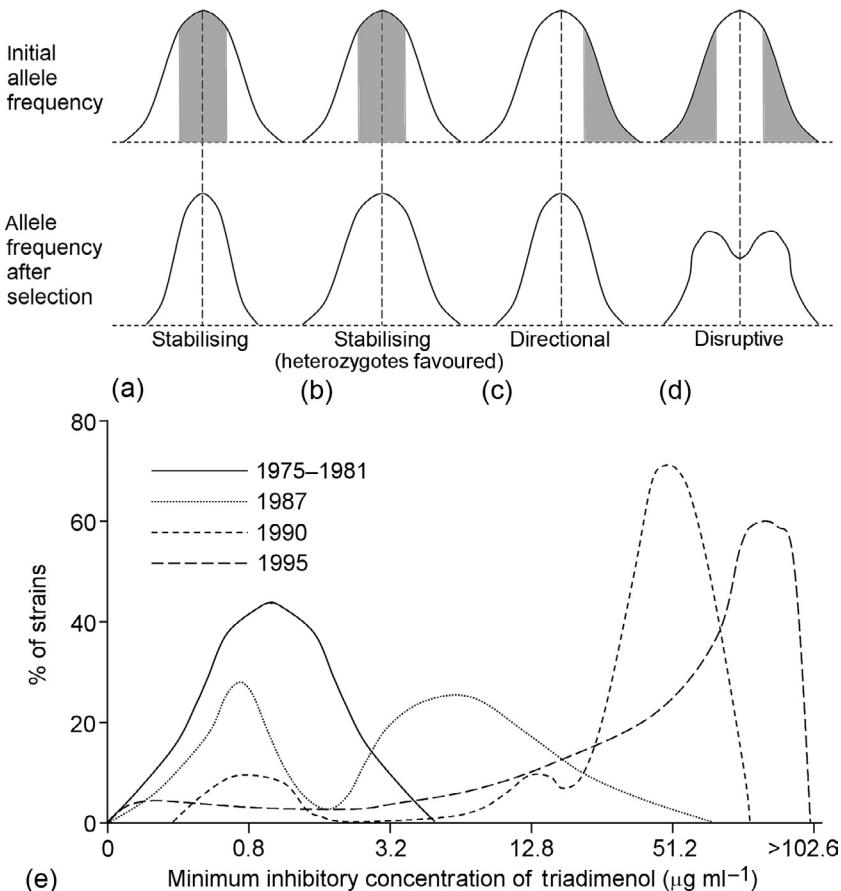


FIGURE 4.10 Natural selection. Selection can result in (a, b) stabilising allele frequencies in populations or in (c) directional or (d) disruptive changes. The upper row indicates the initial allele distribution, with the stippled areas representing favoured alleles. The bottom row shows the distribution of allele frequency following one or a few generations of selection. (e) Directional selection in loss of sensitivity of *Rhynchosporium secalis* populations, on barley in the UK, to the fungicide Propiconazole. The mean minimum inhibitory concentration increased by about 10-fold in 3 years. Source: Brent, K.J., Hollomon, D.W., 1998. Fungicide resistance: the assessment of risk. FRAC Monogr. 2, 1–53.

produce many nodules are most likely to be inoculated onto fresh organic matter, the termites effectively select for high nodule production. If a mutant arises that produces few nodules, it will be underrepresented in inoculation of fresh organic matter and hence ‘automatically’ selected against.

Disruptive (or diversifying) selection favours individuals at the two extremes of a spectrum of variability and acts against intermediate forms. It occurs where there are two possible environments for exploitation, and success results from being highly effective in one rather than moderately effective in both (Figure 4.10). It accounts for the origin within a pathogenic species of forms specialised for different hosts, such as *Puccinia graminis* f. sp. *tritici*, which attacks wheat, and *Puccinia graminis* f. sp. *avenae* which attacks oats. The two forms can still

hybridise and retain a limited ability to infect the other form's host, implying a relatively recent origin by disruptive selection from a less specialised common ancestor.

Though natural selection may drive an allele to fixation or extinction, it may act to maintain a balance with two or more alleles occurring at fairly high frequency. Natural selection operating in this way is referred to as **balancing selection**. It can occur where, in the diploid or dikaryotic phase, the heterozygote is fitter than either homozygote (heterozygous advantage – p. 110). Balancing selection can also take the form of **frequency dependent selection**, in which selection increases the frequency of an allele if it is rare and decreases its frequency if it is abundant. A rare mating type allele, for example, will increase in frequency, since individuals that possess it will be able to mate with most other individuals. If, however, the allele becomes abundant, then individuals possessing it will be very likely to encounter individuals of identical mating type, with resulting failure of mating. Frequency dependent selection will also occur with the gene-for-gene relationship that determines pathogenicity (p. 256). A rare virulence allele will increase in frequency since there will have been little selection for host resistance. If, however, it becomes abundant, then the corresponding gene for host resistance will also become abundant, and selection will operate against individuals carrying the now nearly useless virulence allele.

Natural selection may act differently in haploid and diploid/heterokaryotic fungi. In haploid fungi, a gene will be expressed either throughout or at some stage in the life cycle, and natural selection can operate to influence the frequency of its alleles. In diploid fungi, a recessive allele will have no effect on phenotype, and thus will be partly sheltered from natural selection; if deleterious, it will decrease in frequency more slowly than it would if partly or completely dominant or present in a haploid fungus. Its extinction is likely to be long delayed, since when low frequencies are reached it will rarely be present in the homozygous state in which it would be exposed to selection. In heterokaryotic basidiomycetes, the arguments for haploid fungi are relevant to the homokaryon phase, and the arguments for diploid fungi are relevant to the heterokaryon phase. Hence, fungi that are diploid or heterokaryotic through most of their life cycles are likely to carry many currently deleterious recessive alleles which, in a different genome or environment, might prove advantageous.

Gene Flow

For the geneticist a population consists of individuals that readily exchange genes. The transfer of genes between populations that are spatially separated, and are hence less likely to exchange genes, is termed **gene flow**. Extent of gene flow can be ascertained by examining allele frequencies within and across populations. Potential gene flow can be assessed by quantifying spore recruitment by spore trapping. Species specific spore trapping methods, such as using a homokaryotic mycelium as bait for basidiospores of compatible mating type (pp. 114–118), usually show that spores reflect the local established population, and are rarely found > 20 km from known locations. Nonetheless, spores sometimes travel long distances. For example, the rare basidiomycete *Peniophora aurantiaca* was detected in Göteborg, Sweden, over 1000 km from its closest known occurrence. Also, there are examples of some plant pathogens that have moved from one continent to another as airborne spores (pp. 263–264).

The frequency of alleles in a population can be affected by gene flow from other populations (i.e. migration). The magnitude of the effect will depend on the numbers of immigrants compared with numbers in the native population, and the extent to which the immigrants differ from the natives in allele frequency. It is likely that allele frequencies in small populations

adjacent to large ones will be influenced strongly by gene flow. Between distant populations, gene flow is likely to be sporadic, but may be facilitated by intervening populations acting as stepping stones. The effect of gene flow will be to reduce genetic differences between populations, and hence may delay or prevent the divergence of populations in different geographical areas into separate species. A level of gene flow as low as one migrant per generation is sufficient to prevent population divergence. This is true even in large populations as although one migrant has a diluted effect, genetic drift (p. 130) is also smaller.

The presence, extent, or absence of gene flow varies amongst fungi. With *Neurospora crassa*, individuals collected from what was thought to be one population spanning the Caribbean Islands to the southern United States turned out to be at least two distinct populations, which diverged approximately 0.4 mya but are not yet fully differentiated into species, still sharing 90% Single nucleotide polymorphisms (SNPs). Genomic 'islands' of differentiation include genes related to temperature and circadian rhythm. The subtropical Louisiana population has a higher fitness at low temperature (10 °C), with several of the genes within the distinct genomic islands having functions related to response to low temperature. Southern Louisiana has an average yearly minimum temperature 9 °C lower than the Caribbean, so divergence is probably due to local adaptation to temperature. Another difference was in the circadian oscillator frequency gene, which suggests that the difference in latitude may be another important environmental factor differentiating the populations. The populations are not completely isolated, and there is still gene flow, but the presence of these genomic islands of differentiation, implies the environment exerts a strong enough selection pressure to keep the two populations differentiated despite the ongoing gene flow. In contrast to *Neurospora crassa*, genetic differences in the cosmopolitan basidiomycete *Schizophyllum commune* are mainly in allele frequencies, with genetic distance correlating with geographic distance and a lack of sharp boundaries between populations.

Gene flow by long-distance spore dispersal is implied, with even a limited intercontinental gene flow. Genetic differences between populations are not always correlated with geographic distance, but with the distance that a fungus has 'travelled' to get to a new location. The distance travelled is not always equivalent to the geographical distance, for example, if there is a geographical feature that has been circumvented rather than crossed over, the travelled distance is greater than the geographical distance.

Gene flow can be important even over long distances. Almost every naturally occurring *Neurospora* colony that is sampled is genetically distinct, indicating that the colonies have arisen from single ascospores, the products of meiosis, and not from conidia. It is hence reasonable to infer that the main role of conidia, which compared with ascospores are produced in massive amounts, is to fertilise protoperithecia. Studies on the mitochondrial DNA of *Neurospora crassa* show regional differences. Studies on the nuclear genome, however, show that regional diversity is no greater than that within populations. It seems, therefore, that gene flow resulting from the dispersal of conidia has helped maintain homogeneity of the nuclear genome throughout the species. In fertilisation, however, the conidia do not contribute mitochondria, allowing geographically distinct populations of mitochondrial DNA to evolve.

The wheat stem rust provides an example of the potential importance of gene flow as a source of genetic novelty in a population. Major changes occurred in 1954 in the ability of *Puccinia graminis* sp. *tritici* in Australia to attack different host varieties. Study of rust collections, maintained from that period, indicates that a change in isozyme pattern also occurred.

The change was due to a pathogen strain hitherto lacking in Australia but found in African populations. The implication was that an introduction from Africa occurred, either by long-distance spore dispersal or inadvertently by humans – the latter possibility being an increasing hazard.

Genetic Drift and the Founder Effect

Genetic drift is the change in frequencies of alleles in a population due to chance. If a population is small then chance could determine whether a neutral allele becomes extinct or increases in frequency to fixation. If a population is very small then such random genetic drift could determine the fate of an allele even in the presence of moderately strong natural selection. In nature, however, it may be unusual for a population to stay small long enough for drift to occur – the population could become extinct, grow, or merge with another population. Tendencies to genetic drift will be opposed by gene flow. Hence if a fungus is abundant and widespread with copious spores capable of long distance dispersal, gene flow is likely to counteract any tendency to genetic drift. There is evidence for this in the cosmopolitan and abundant fungi *Neurospora crassa*, *Puccinia graminis* f. sp. *tritici*, and *Schizophyllum commune*.

There are, however, ways in which random events could determine the genetic structure of a population and the course of microevolution. One or a few individuals will not cover the genetic diversity in the population; many alleles present in the whole population will be absent from such a small set of individuals. A small set of individuals could occur as the result of a catastrophe almost destroying a population or by the dispersal of one or a few individuals to a new environment. The population resulting from such a **founder effect** will be genetically different from the one from which it originated. Many fungi live in environments that are highly favourable but transient, and will hence be liable to colonisation from one or a few spores when they arise, and population crashes when they disappear. Founder effects are likely to occur with such fungi and, if the fungi are not highly abundant, may not subsequently be overwhelmed by gene flow.

Australia provides lots of examples of single founder events: *Puccinia striiformis* – cause of yellow (stripe) rust of wheat – was introduced into Australia in 1979 (p. 263), as a single race from Europe, but mutations have now resulted in new pathotypes which differ from those in Europe. Similarly, *Cryphonectria parasitica* – cause of chestnut blight (pp. 287–289) – in North America has much less genetic diversity than in Asia, probably reflecting a founder effect. The dry-rot fungus, *Serpula lacrymans*, originated in northeast Asia, where it has most genetic variations. However, there is very little genetic variation in the founder populations across the globe ([Figure 4.11](#)). In some areas the indoor genetic populations are unique (e.g. Japan), representing a single founder event, whereas elsewhere (e.g. Australia), there is slightly more variation representing founder events from Japan and from Europe.

Recombination

Although mutation can result in new genotypes that become established in a population, such genotypes may, as indicated above (pp. 125–126), differ in only a few ways from the strains from which they were derived. On the other hand, the mating of two strains that differ at many loci will result, through recombination at meiosis, in a wide variety of new genotypes. Continuing with the example, from a previous section (p. 125), of *Blumeria graminis* f. sp. *hordei*, infecting three fields with different barley cultivars, one with resistance gene *R1*,

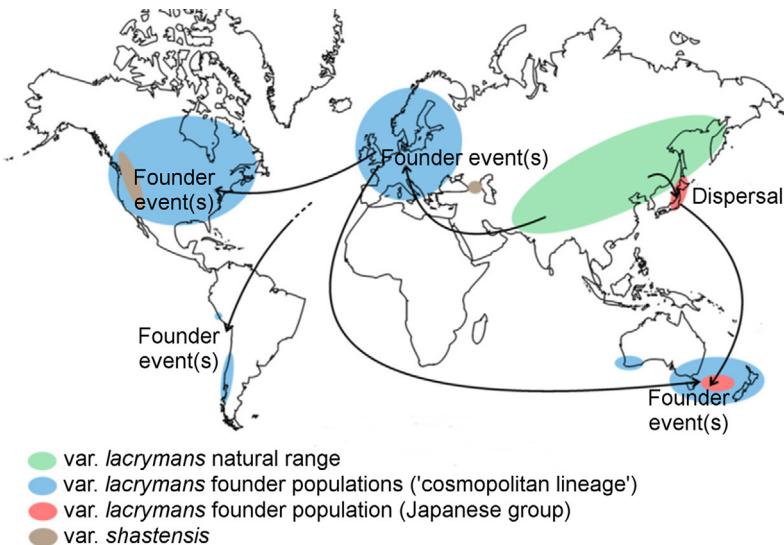


FIGURE 4.11 Worldwide spread of the dry rot fungus, *Serpula lacrymans*, from its origins in northeast Asia. The Japanese indoor population represents a single founder event. From there it was spread to southeast Australia and New Zealand. A genetically highly homogeneous population is present in Europe, the Americas and Australia, and New Zealand. It was probably first spread to Europe from Asia in infected wood, and from there to the other areas in a similar way, perhaps in wooden ships. Source: Kauserud, H., Knudsen, H., Höglberg, N., Skrede, I., 2012. Evolutionary origin, worldwide dispersal, and population genetics of the dry rot fungus *Serpula lacrymans*. *Fungal Biol. Rev.* 26, 84–93.

a second with *R2*, and a third with no resistance, if two mutants for virulence *vr1* and *vr2* arise separately in the non-resistant cultivar, those spores with *vr1* could successfully establish in the *R1* cultivar, and those with *vr2* in the *R2* cultivar. After epidemics in *R1* and *R2*, the spores could move back into the non-resistant crop, where sexual reproduction between the two could occur. If the genes are unlinked, recombination would result in 25% of the progeny having both *vr1* and *vr2* alleles, which would allow infection of cultivars with both *R1* and *R2* genes.

A classic example of genetic effects of the sexual process in natural populations is provided by early studies on the amount of variability of *Puccinia graminis* f. sp. *tritici* populations east and west of the Rocky Mountains in the United States. The sexual stage in the rust's life cycle occurs on barberry (*Berberis*). This was eradicated east of the Rocky Mountains in the late 1930s, preventing sexual recombination in the eastern populations since that time. West of the Rocky Mountains barberry, and hence sexual recombination in rust populations, persist. The eastern rust populations have relatively few pathotypes and isozyme patterns, and each pathotype is associated with a single isozyme pattern, different from the isozyme patterns associated with other pathotypes, showing that genetic recombination has not occurred. West of the Rocky Mountains pathotypes and isozyme variants are numerous, and the association between pathotype and isozyme pattern is random, indicating that genetic recombination has occurred. Hence the sexual process has generated virulence and isozyme diversity, and has broken any constant associations between pathotype and isozyme pattern. Another example is provided by *Puccinia striiformis* f. sp. *tritici*. Populations in Europe and Australia are clonal

with limited molecular diversity, but in China there is considerable phenotypic and genotypic diversity and evidence of genetic recombination.

Whether populations are clonal, freely recombining or with limited recombination can be established by molecular methods, or by means of classical population genetics. However, the latter, as applied to fungi in nature, is so laborious that relatively little information has been obtained in this way. Nonetheless the procedure by which the presence or absence of recombination can be inferred from data on molecular variation can be illustrated by considering genes and alleles. If two genes (*a* and *b*) each have two alleles (1 and 2), there are four possible genotypes, a_1b_1 , a_1b_2 , a_2b_1 , and a_2b_2 . If only two genotypes are found in a population, for example a_1b_1 and a_2b_2 , then recombination between the genes is not occurring, due either to the genes being closely linked on the same chromosome or because mating and genetic recombination is absent in the population. If all four genotypes are equally frequent, then it is likely that unrestricted recombination is taking place. Since there is the possibility that two genes may be linked, it is in practice necessary to consider a larger number of genes, and in a natural population most genes will have not two but many alleles. The presence of recombination (i.e. signature of sex) can be deduced from the pattern of variation in a population. If all three of the following are found after clone-correcting the dataset, it is almost certainly a sexual population: (1) high genotype diversity, usually assessed with molecular genetic markers or DNA fingerprints; (2) random associations among the alleles found at individual, neutral loci; and (3) both mating types present at equal frequencies (if an ascomycete).

Such molecular studies are now providing valuable information on recombination within and between populations in nature. For example, Californian populations of the human pathogen *Coccidioides immitis* (p. 303), a fungus for which a sexual stage is unknown, has been shown to have essentially unrestricted genetic recombination. However, another human pathogen that lacks a sexual stage, *Candida albicans* (p. 302, pp. 305–307), is clonal with little recombination, which is generated via a parasexual cycle (pp. 122–123). A sexual stage, as indicated earlier in this chapter, results in recombining populations in both heterothallic and homothallic fungi, but if mitospores are also produced, clonal as well as recombining populations, are likely to arise, as in *Puccinia graminis* f. sp. *tritici* mentioned above.

Transposable Elements

TEs, also known as **transposons** or more colloquially as ‘jumping genes’, are DNA sequences that can move from one location in a genome to another. Indeed, TEs are sometimes spread to other species by HGT (p. 134) TEs can cause changes in genome architecture and gene function, via recombination and expansion, resulting in rearrangement of chromosomes and new gene neighbourhoods. Transposition often results in duplication of a DNA sequence and the insertion of one copy at a different site in the genome termed **Duplicative transposition**. Comparative phylogenomics has shown that gene duplication occurs frequently in fungi. It allows genes to acquire new functions by mutation (pp. 125–126), since one copy remains to maintain the original function, leaving the second copy free to mutate without compromising the organism’s fitness.

As with other eukaryotes, there are two classes of TEs: class I (termed **retrotransposons**) transpose by synthesising cDNA copy based on an RNA intermediate using reverse transcriptase; class II (**DNA transposons**) transpose the DNA directly (Table 4.5). The copy numbers of fungi are much lower than in other eukaryotes, only a small proportion of the genome

TABLE 4.5 Examples of Transposons in Fungi

Type	Characteristics	Fungus	Transposon name
Class I: LTR retrotransposons	Have long terminal repeats (LTRs) flanking the polyprotein genes. They usually encode two open reading frames (ORFs).	<i>Aspergillus fumigatus</i>	<i>Afut1</i>
	Have <i>Gag</i> (encode for viral coat proteins) and <i>pol</i> genes (encode for integrase, protease, reverse transcriptase and RNase)	<i>Botrytis cinerea</i>	<i>Boty</i>
		<i>Fusarium oxysporum</i>	<i>Foret-1, Skippy</i>
Class I: LINE (long interspersed nuclear elements) retrotransposons	Have poly-A-tails but no LTRs, <i>gag</i> or <i>pol</i> genes	<i>Fusarium oxysporum</i>	<i>Palm</i>
		<i>Neurospora crassa</i>	<i>Tad1-1</i>
Class I: SINE (short interspersed nuclear elements) retrotransposons	Derive from RNA polymerase transcripts; no special structural features; no <i>gag</i> or <i>pol</i> genes. They are transactivated (increased rate of gene expression) by reverse transcriptases provided by retrotransposons or LINE-like elements	<i>Erysiphe graminis</i> f. sp. <i>hordei</i>	<i>EGH24-1, Eg-R1</i>
		<i>Nectria haematococca</i>	<i>Nrs1</i>
Class II (also called DNA transposons)	<i>Fot1/Pogi-like</i>	<i>Aspergillus nidulans</i>	<i>F2P08</i>
		<i>Botrytis cinerea</i>	<i>Flipper</i>
		<i>Fusarium oxysporum</i>	<i>Fot1, Fot2</i>
	<i>Tc1/mariner</i> superfamily	<i>Aspergillus niger</i>	<i>Ant1</i>
		<i>Fusarium oxysporum</i>	<i>Impala</i>
		<i>Phanerochaete chrysosporium</i>	<i>Pce1</i>

Two other class I transposons have recently been introduced DIRS and Penelope-like (PLE).

Source: [Kempken and Kück, 1998](#)

being repetitive DNA (e.g. 1.1% and 7.3 %, respectively) in the plant pathogens *Ustilago maydis* and *Magnaporthe oryzae*, and 5% in the human pathogen *Cryptococcus neoformans*. Perhaps the most studied fungal transposon is *Ty* ('transposon yeast'), a retrotransposon, which occurs in *Saccharomyces cerevisiae*. A terminal portion of the *Ty* sequence is known as δ , and facilitates insertion. *Ty* acts as a retrovirus. It is transcribed into RNA and encapsidated. Part of the RNA sequence encodes a reverse transcriptase which copies the RNA into a DNA sequence which then inserts into the yeast genome. *Saccharomyces cerevisiae* commonly carries about 35 copies of *Ty-1*, one form of *Ty*, about 6 copies of *Ty-917*, a mutant form, and about 100 solo δ sequences.

The genomes of plant pathogenic fungi contain a larger fraction of TEs than saprotrophs. They often form clusters with rapidly evolving genes involved in host-pathogen interactions (e.g. host specificity genes in *Fusarium* spp., *Magnaporthe oryzae*, and *Verticillium* spp.), and oomycete *Phytophthora infestans* effector genes. In contrast, TEs are not clustered in *Blumeria graminis*, but rather are distributed throughout the genome. Evolution of new lineages able to

colonise new hosts is associated with TEs in the above examples, and in the case of *Blumeria graminis* increase in TEs and genome size coincided with a change in trophic behaviour to obligate biotrophy.

The expression and mobility of TEs are mostly silenced epigenetically (below) but this may be disrupted under physiological stress, including that posed by climate change and invading new habitatshosts, leading to rapid restructuring of the genome and altered gene expression, with associated changes in morphology and physiology, and speciation (pp. 135–137). For example, the fungus-like oomycete *Phytophthora ramorum* (the cause of sudden oak death), which comprises only a few clonal lineages in California, has different phenotypes in live oak (*Quercus agrifolia*) and bay laurel (*Umbellularia californica*) despite having identical genotypes; the physiological stress caused by colonising the oak is thought to result in disruption of epigenetic silencing of TEs. Comparison of symbiotic and non-symbiotic species of Agaricomycetes has shown that TEs are usually more common in symbiotic fungi than close relatives that are saprotrophs. Moreover, genes that have been identified as having crucial roles in the development of symbiotic tissues appear to be associated with TE's and also lack homologues in other organisms. Both of these observations suggest the possibility that TEs have played a part in the genetic changes that underlie a move from saprotrophic to biotrophic nutrition.

Horizontal Gene Transfer

Comparing whole genomes has led to greatly increased understanding of gene evolution, including the discovery of the importance of HGT in which genes have been directly transferred across fungal lineages, without sexual recombination. Not only has there been transfer between fungal species, but genes have also been acquired from bacteria and plants. Examples are given in Chapter 6 (p. 201).

Epigenetics

DNA sequences encode most of an organism's heritable characteristics, but other mechanisms are also responsible for heritable phenotype. Functionally relevant modifications to the genome can occur which do not involve changes to the nucleotide sequences – termed epigenetic. These changes are reversible and inheritable. Epigenetic phenomena include changes in DNA methylation, histone modification, centromere location, and RNA silencing systems.

DNA methylation occurs in many fungi (e.g. *Neurospora crassa*), but not all (e.g. *Aspergillus nidulans*). Most filamentous fungi have DNA methyl transferases (DMTs) which form 5-methylcytosine by methylating C5 of cytosine. DNA methylation is a genome defence that blocks transcription of TEs (pp. 132–134) and other 'selfish DNA' – gene silencing. Other genes can be silenced too. Repeat induced point mutation (RIP) is a process that has evolved uniquely in fungi as a defence against TEs. During the RIP process transposons, and other duplicated sequences, are identified and point mutations are introduced by conversion of C:G pairs to T:A pairs in both sequence copies. These mutations, and sometimes also additional DNA methylation, lead to gene silencing. RIP was seen first in *Neurospora crassa*, and was the first eukaryotic gene silencing mechanism discovered, but has now been demonstrated to occur in many other fungi (e.g. *Aspergillus nidulans*, *Aspergillus oryzae*, *Colletotrichum cereale*, *Ophiostoma ulmi*, and *Penicillium chrysogenum*).

While the effects mentioned so far relate to DNA, modification of core histones (including H2A, H2B, H3, and H4) and replacement of canonical histones with variants can occur after

translation, resulting in epigenetic phenomena. Gene silencing can also result from RNA-based silencing mechanisms: small interfering RNA (siRNA) can alter expression from invading viruses or selfish elements; the plant pathogen *Botrytis cinerea* produces siRNA that silences genes in host plants allowing it to colonise (pp. 274–276); normal gene expression can be regulated by micro RNA (miRNA) in dedicated pathways.

Speciation

Speciation is the situation where new species arise from preexisting species. It is more or less impossible to know exactly when a new species has arisen, not least because there is a lack of agreement about how different two taxa must be to qualify as being different species. There is, however, abundant evidence of speciation in progress or recently accomplished. This evidence comes from the comparison of closely similar taxa, and from their attempted or successful hybridisation.

Speciation is, most commonly, a splitting of an existing species into two, although one of these species may subsequently replace the other. For example, *Rhynchosporium* on barley (*Hordeum vulgare*; causing ‘scald’), rye (*Secale cereale*) and wild *Agropyron* species is actually three different species that evolved independently and diverged by host specialisation. Each has a unique haplotype (based on sequences of four independent loci and pathogenicity tests). There is no gene flow between populations on rye and barley (evidenced by population genetic data).

New species can arise in different ways: through adaptation, genomic change, or hybridisation with other species. Three main modes of speciation are recognised for eukaryotes: (1) **allopatric** or **geographical** speciation which occurs where populations have become geographically isolated. Genetic differences accumulate in the isolated populations, and these eventually become reproductively isolated. (2) **Sympatric** speciation where gene flow between populations in the same area ceases. (3) **Abrupt** speciation which arises mainly when polyploidy or chromosomal rearrangement causes reproductive isolation. These categories are not quite as useful for fungi as they might superficially appear, as although fungi can be distributed widely they occupy microhabitats; significant separation might be as little as different locations within the same fallen tree trunk or, for a rust fungus, wheat (*Triticum*) and oak (*Quercus*) plants in the same area. Nonetheless there are many examples, revealed by multiple gene genealogy, of cryptic species that have non-overlapping geographical ranges, suggesting that they have evolved by allopatric speciation. For example, the morphological species *Neurospora crassa* has one phylogenetic species located in the Congo (Africa), another in the rest of Africa (but not Congo) and the Caribbean, and a third in India. There are at least nine species within the morphological species *Fusarium graminearum*, a cause of scab on wheat and barley, four endemic to South America, one in Central America, one in Australia and one in India. Providing evidence of sympatric speciation is also difficult as it is almost always impossible to exclude a past period of allopatry.

Given the above difficulties, we will just discuss factors that predispose to speciation and factors that result in reproductive isolation. Though reproductive isolation is the criterion upon which the biological species concept is based (p. 107), it is just one of the many stages of speciation. It can occur at early or late stages in the speciation process, and can be a critical stage, as in sympatric speciation, or simply a by-product of genetic divergence, as in allopatric speciation. It is clearly not essential to all modes of speciation as asexual fungi can also speciate.

Speciation in Fungi with a Sexual Phase

Genetic divergence can occur by non-selective means such as drift (p. 130), or when the selective forces acting on the two populations differ. Gene flow (pp. 128–130) between populations will oppose and limit genetic divergence, but gene flow will be of little importance if the two populations are distant from each other and dispersal is inefficient. Then, increasing divergence is likely to result in **genetic disharmony** (p. 121) between the populations, which may affect the mechanisms involved in mating, so that if the two populations come into contact again, mating may fail, or if progeny do result, they may be few or of low viability.

Fungal populations may come to differ from each other in chromosome number, through **polyploidy or aneuploidy** (p. 124). Differences in chromosome number between strains usually reduces the chances of hybridisation and genetic exchange, because of chromosome pairing difficulties at meiosis, though some fungi (e.g. *Zymoseptoria tritici*) have a high level of sexual reproduction among strains that have highly imbalanced numbers of chromosomes. Thus, differences in chromosome number will often increase the likelihood of strains diverging into separate species. On the other hand, many species are not completely intersterile, allowing the possibility of hybridisation. If **hybridisation** is successful, the resulting hybrid may differ from both parental types in morphology and chromosome number and may also prove to be competitive. Chromosome counts and hybridisation experiments indicate that the chytridiomycete *Allomyces javanicus* originated as a hybrid between two species with considerable morphological differences, *Allomyces arbuscula* and *Allomyces macrogynus*. Many emerging plant pathogens are hybrids (pp. 285–290). The rust *Melampsora × columbiana* emerged in 1997 from hybridisation between *Melampsora medusa* and *Melampsora occidentalis*, which are respectively pathogens of *Populus deltoides* and *Populus trichocarpa*. It was a homoploid (i.e. had the same number of chromosomes as its parents). Allopolyploids (i.e. with different numbers of chromosomes to the parents) are instantly reproductively isolated, but homoploids are not. *Melampsora × columbiana* emerged as a new species pathogenic on a *Populus* hybrid resistant to the two parents.

Chromosomal rearrangements can theoretically result in speciation – **chromosomal speciation**. However, showing that rearrangements are a cause rather than a consequence of speciation is difficult. Until recently it has been almost impossible to make detailed analyses of fungal chromosomes, but population genomics projects are likely to expand understanding of this area dramatically in the coming decade.

Reproductive isolation which, as already mentioned, can be a cause or consequence of speciation, can result from two types of barriers – pre and postmating. Premating isolation can result from several different barriers: (1) specialisation for a certain host; (2) specialisation of biotic vectors can prevent contact between populations (e.g. *Botanophila* flies preferentially select the endophyte *Epichloë typhina* rather than *Epichloë clarkia*); (3) differences in time of reproduction; (4) a high rate of selfing (e.g. in the anther smut *Microbotryum violaceum*); (5) assortative mating where individuals are able to discriminate between the same and different species, especially in Agaricomycetes. Postmating isolation barriers result from sterility or non-viability of progeny derived from matings between species (e.g. crosses between *Neurospora* species result in abnormal or only a few progeny, or only a few viable ascospores). Epigenetic mechanisms (pp. 134–135) may also contribute to postmating isolation.

The inability of two populations to mate does not necessarily entirely prevent gene flow between them, as both may be able to mate with a third population. For example, Californian isolates of the basidiomycete *Serpula himantoides* are unable to mate with Swedish isolates, but both are able to mate with Indian isolates. Where populations adapted to different habitats are in close proximity, mating between them could result in progeny ill-adapted to either environment, or, in progeny of low viability due to genetic disharmony, if considerable divergence has already occurred. Under such circumstances there will be selection for the evolution of mating barriers to prevent the production of progeny of low fitness. One or a few mutations can suffice to bring about such **heterogenic incompatibility**. It is hence likely that barriers to mating can arise both fortuitously and by natural selection. Gene flow can also be limited, and divergence promoted, by self-fertility (pp. 119–120) and amixis (p. 122).

Speciation in Asexual Fungi

Speciation in asexual fungi, if there is such a thing as a truly asexual fungus (p. 114), will occur in ways similar to that in acknowledged sexual species. However, there is no sexual recombination to break down successful combinations of multiple alleles. The selective pressure on one gene will have an effect on the whole genome. The human pathogen *Penicillium marneffei* (p. 308) can spread long distances by aerially dispersed spores, however, it is highly endemic. DNA multilocus typing has shown that different clones are found in different environments, implying that adaptation to these environments constrains the fungus' ability to disperse successfully. Similarly, many species in the plant pathogenic *Magnaporthe grisea* complex are host specific, with different recently evolved lineages in cutgrass, millet, rice, and torpedo grass. The rice pathogen *Magnaporthe oryzae* (pp. 265–267) arose recently (about 7000 years ago) probably when rice was domesticated.

However, molecular methods have shown extensive genetic recombination in a few apparently asexual fungi, such as *Coccidioides immitis* (p. 303), though this could be due to hitherto undetected mating. Nonetheless, in fully asexual species genetic recombination can occur via the parasexual cycle (if it is not simply a laboratory artefact – pp. 122–123), but will be limited by vegetative incompatibility. Hence a mitosporic fungal species defined on traditional morphological criteria is likely to consist of a number of biological species, with some clonal and some showing genetic recombination, as has been found in the banana pathogen *Fusarium oxysporum* f. sp. *cubense*. It seems likely that in mitosporic fungi, when genetic exchange cannot occur due to vegetative incompatibility, the resulting clones constitute biological species, and through subsequent evolution may develop into morphologically distinguishable species. The different VC groups are genetically distinct lineages, for example in *Aspergillus flavus*.

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