L-Fungal genetics, molecular genetics, and genomics

Structure and organization of the fungal genome

The genome of an organism includes all the genetic information, not just the genes encoded by the nucleus. In fungi the genome often includes four separate components: the chromosomal genes, the mitochondrial genes, plasmids (and mobile genetic elements), and fungal virus genes, which are truly resident genetic elements. Each of these can contribute significantly to the phenotype of fungi.

Chromosomes and chromosomal genes

Most fungi are haploid, but the Oomycota are diploid, a few fungi can alternate between haploid and diploid somatic phases, and some yeasts (e.g. *Candida albicans*) are permanently diploid. Some fungi and fungus-like organisms have polyploid series (e.g. *Allomyces* spp. and *Phytophthora* spp., including *P. infestans*). This can be shown by staining the hyphae with a fluorochrome such as DAPI, which intercalates (inserts) in the A-T-rich regions of DNA, and then measuring the fluorescence of individual nuclei under a microscope. Fluorescence increases in a step- wise manner with each increase in the number of chromosome sets.

The chromosomes of nearly all fungi are small and highly condensed. They are difficult to count by conventional microscopy of stained cells because the nuclear membrane persists during most of the mitotic cycle. However, counts have now been obtained for several fungi by a combination of cytology, linkage analysis (which enables genes to be assigned to particular chromosomes), and pulse-field electrophoresis of extracted chromosomes. As shown in Table 9.1, the haploid chromosome count of most fungi and fungus-like organisms seems to lie between 6 and 16, but can be as low as 3 or as high as 40.

Table 9.1 Reported chromosome counts in some representative fungi.

Fungi	*Chromosome count*
Oomycota	
Phytophthora spp. (many)	9–10
Achlya spp.	3, 6, 8
Saprolegnia spp.	8–12
Pythium	commonly 10 or 20
Chytridiomycota	
Allomyces arbuscula	16
A. javanicus	14 (variable in hybrids and polyploids)
Ascomycota	
Schizosaccharomyces pombe	3
Neurospora crassa	7
Saccharomyces cerevisiae	16
Emericella (Aspergillus) nidulans	8
Coccidioides posadasii	4
Trichophyton rubrum	4
Magnaporthe grisea	7
Basidiomycota	
Filobasidiella neoformans	11
Schizophyllum commune	11
Coprinus cinereus	13
Puccinia kraussianna	30–40

The nuclear genome size of fungi is small in comparison with many other eukaryotes. For example, the genome size of *Saccharomyces cerevisiae* is just over 12 Mb (12 megabase pairs, or 12 million base pairs), and that of *Schizophyllum commune* (Basidiomycota) is about 37 Mb. Some reported genome sizes of other fungi are given in Table 9.2. The values for *S. cerevisiae* and *Schizophyllum commune* are only about three and eight times larger than the genome of *Escherichia coli* (4 Mb) and much smaller than the genome of the fruit fly *Drosophila* (165 Mb) or humans (about 3000 Mb).

Fungi transcribe a substantial amount of the nuclear DNA into messenger RNA – an estimated 33% in *S. commune* and 50 – 60% in *S. cerevisiae*. Compared with other eukaryotes, therefore, they have relatively little noncoding (redundant) DNA. Fungi resemble other eukaryotes in that their protein-encoding genes contain noncoding DNA sequences termed introns. The introns are

transcribed into mRNA but are excised before the mRNA is translated into proteins. However, the introns of fungi are very short (often about 50–200 base pairs) compared with those of higher eukaryotes (often 10,000 base pairs or more), and *S. cerevisiae* is unusual because it has very few introns.

Table 9.2 Some reported (approximate) genome sizes of fungi and fungus-like organisms.

Fungi/fungus-like organisms	*Genome size (Mb)*
Aspergillus fumigatus (potential human pathogen)	30
A. niger (industrially important: citric acid, enzyme production)	30
Candida albicans (human commensal and potential pathogen)	16
Filobasidiella (*Cryptococcus*) *neoformans* (human pathogen)	21
Emericella (*Aspergillus*) *nidulans* (experimental model fungus)	28
Neurospora crassa (experimental model fungus)	40
Phanerochaete chrysosporium (wood-decay basidiomycota)	40
Phytophthora infestans (plant pathogen; Oomycota)	240
Phytophthora sojae (pathogen of soybean; Oomycota)	62
Pneumocystis jiroveci (pathogen of immunocompromised humans)	7.7
Saccharomyces cerevisiae (brewing and breadmaking yeast)	12
Schizosaccharomyces pombe (experimental model; fission yeast)	14

Mitochondrial genes: normal functions and involvement in aging

Mitochondria contain a small circular molecule of DNA. The size of the mitochondrial genome varies, from as little as 6.6 kb (kilobase pairs) in humans to more than 1 Mb in plants. Fungal mitochondrial genomes are often in the range of 19 – 121 kb; for example 70 kb in *S. cerevisiae*, and 50 kb in *Schizophyllum commune*. Any variations are due mainly to the amount of noncoding material, because all mitochondrial DNAs code for the same things: some components of the electron- transport chain (including cytochrome c and ATPase subunits), some structural RNAs of the mitochondrial ribosomes, and a range of mitochondrial transfer-RNAs. Both the nuclear and the mitochondrial genes are needed to produce complete, functional mitochondria.

The mitochondrial DNA of fungi has received special attention in relation to aging, because in several filamentous fungi (*Podospora, Neurospora, Aspergillus*) a single mutation in a single mitochondrion can lead to senescence of the whole colony, when the mutant gene causes the gradual displacement of wild-type mitochondrial DNA.

Plasmids and transposable elements

Plasmids usually are closed-circular molecules of DNA with the ability to replicate autonomously in a cell. However, they can also be linear DNA molecules if the ends are "capped" (like chromosomes) to prevent their degradation by nucleases. Plasmids or plasmid-like DNAs have been found in several fungi. The most notable example is the "two-micron" plasmid of *S. cerevisiae*, so-called because of its 2 μm length as seen in electron micrographs. This plasmid is a closed circular molecule of 6.3 kb, and it is unusual because it is found in the nucleus, where it can be present in up to 100 copies. It has no known function, but in the past it was used to construct "vectors" for gene cloning in yeast.

Most other plasmids of fungi are found in the mitochondria. The best characterized are the linear DNA plasmids of *Neurospora crassa* and *N. intermedia*. They show a degree of base sequence homology to the mitochondrial genome, suggesting that they are defective, excised segments of the mitochondrial genes. However, some other mitochondrial plasmids of *Neurospora* are closed circular molecules with little or no homology to the mitochondrial genome. They have a variable "unit" length of about 3–5 kb (in different cases) and the units can join head-to-tail to form larger repeats. None of these fungal plasmids has any known function, so they are not like bacterial plasmids that code for antibiotic resistance, pathogenicity, or the ability to degrade pesticides, etc.

Transposons (transposable elements) are short regions of DNA that remain in the chromosome but encode enzymes for their own replication. They produce RNA copies of themselves, and they encode the enzyme, reverse transcriptase, which synthesizes new copies of DNA from this RNA template, similar to the action of retroviruses such as HIV. The new copies of DNA can then insert at various points in the same or other chromosomes, leading to alterations in gene expression. Transposons seem to be rare in filamentous fungi, but there are several types in *S. cerevisiae*. The best studied of these are the chromosomal Ty elements, present in about 30 copies in yeast cells. In addition to a role in altering gene expression, they could have significant effects on chromosomal rearrangements when the "delta sequences" on the ends of these elements combine with one another. These transposable elements seem to have no function, except for self-perpetuation.

The mating-type genes of *S. cerevisiae* are transposable casettes, causing mating-type switching. However, mating-type switching cannot occur in *Neurospora crassa*, because individual haploid strains consist of only one mating type.

Viruses and viral genes

Fungal viruses were first discovered in the 1960s, associated with "La France" disease of the cultivated mushroom *Agaricus bisporus*. (The name was coined by British mushroom growers, reflecting the entente cordiale (cordial understanding) that has long characterized Anglo-French relations). In this disease the fruit bodies are distorted and the fruit body yield is poor. Electron micrographs of both the hyphae and the fruit bodies showed the presence of many isometric virus-like particles (VLPs), assumed to be the cause of the problem. VLPs were then discovered in other fungi, and by the 1980s they were known in over 150 species, including representatives of all the major fungal groups. Most fungal viruses seem to be symptomless.

Studies on a range of fungi have shown that fungal viruses (or VLPs) have similar basic features:

- They are isometric particles, 25–50 nm diameter, with a genome of double-stranded RNA (dsRNA), a capsid composed of one major polypeptide, and they code for a dsRNA-dependent RNA polymerase for replication of the viral genome.
- The genome size is variable. Even within a single fungus it ranges from about 3.5 to 10 kb. In some cases this variation is due to internal deletions of a full-length molecule, but in other cases the genome is divided between different capsid particles.
- In most fungi the VLPs are found infrequently in hyphal tips, but they can occur as crystalline arrays in the cytoplasm of older hyphal regions, often closely associated with sheets of endoplasmic reticulum that enclose the aggregates.
- The natural means of transmission of VLPs is via the cytoplasm during hyphal anastomosis (hyphal fusions) or by passage into the asexual spores. VLPs can also enter the sexual spores of some Basidiomycota and in Saccharomyces, but this seems to be rare in the sexual spores of mycelial Ascomycota.

VLPs are resident genetic elements of fungi because they have no natural mechanism for crossing species barriers.

For several years this created a problem in determining their functions, because the association between VLPs and phenotypic characters was only correlative. However, two major developments have changed this and opened the field to critical investigation. First was the discovery that virus-like dsRNA can be present in fungi even when VLPs are absent. In these cases, it can be assumed that the virus has lost the ability – and the need – to produce a capsid. Second, protoplasting techniques and transformation systems have now been developed for several fungi, so that dsRNA can be extracted, purified and introduced into protoplasts, or complementary DNA (cDNA) can be derived from dsRNA in vitro and then transformed into protoplasts.

In the chestnut blight fungus *Cryphonectria parasitica*, the dsRNA causes a marked reduction in pathogenic virulence, creating hypovirulent strains that can potentially be used to control the serious chestnut blight disease.

In recognition of the unique properties of viral dsRNA and its role in reducing pathogenic virulence, a new name has been approved for this group of viruses – the hypovirus group (Hypoviridae).

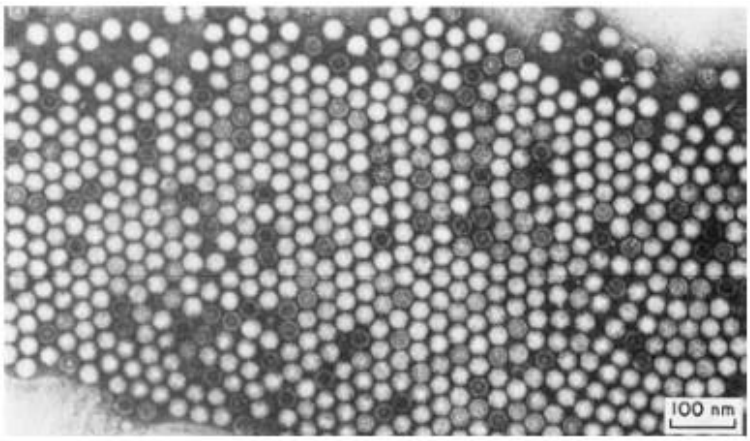

Fig. 9.4 Isometric virus-like particles extracted from hyphae of *Colletotrichum* sp. The particles aggregate in crystalline arrays *in vitro*. (Courtesy of Rawlinson *et al.* 1975.)

Genetics – Variation, Sexuality, and Evolution

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 recognizes 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, minimizing 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 geonomes. The ascomycetes *Aspergillus nidulans*, *Neurospora crassa*, and *Saccharomyces cerevisiae* have many of these features, and are among the genetically best understood organisms.

Individuals, populations, and species in fungi

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

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.

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.

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 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.

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 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).

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 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 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, epigeous 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 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 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.

Genetic variation in fungi

Nonsexual variation: the significance of haploidy

Mutation is the basis of all variation, but mutations are expressed and recombined in different ways depending on the biology of an organism. One of the most significant features of fungi is that they have a haploid genome, whereas all other major groups of eukaryotes are diploid.

Haploid organisms typically expose all their genes to selection pressure. Any mutation will either cause a loss of fitness, or an increase in fitness (e.g. antibiotic or fungicide resistance). This can be beneficial in the short term but the disadvantage is that haploid organisms cannot accumulate mutations that are not of immediate selective value. Diploid organisms have exactly the opposite features. Mutations often are recessive to the wild type, so they are not immediately expressed; instead they accumulate and can be recombined in various ways during sexual crossing.

However, mycelial fungi typically have several haploid nuclei in a common cytoplasm, and so recessive mutations can be shielded from selection pressure, being complemented by the wild-type nuclei. Mycelial fungi can also expose their genes to selection pressure periodically – whenever they produce uninucleate spores or when hyphal branches develop from only one

"founder" nucleus. In other words, mycelial fungi have many of the advantages of both haploidy and diploidy. This is not true for the Oomycota, which are diploid. The situation is different again for yeasts because these grow as uninucleate cells. Several yeasts (e.g. *Candida* spp.) are permanently diploid, and even *Saccharomyces* grows as a diploid yeast in nature, owing to mating-type switching.

Nonsexual variation: heterokaryosis

Heterokaryosis is defined as the presence of two or more genetically different nuclei in a common cytoplasm (hetero = different; karyos = kernel, or nucleus). Fungi that exhibit this are termed heterokaryons, in contrast to homokaryons which have only one nuclear type.

Heterokaryons can arise in two ways. First, when a mutation occurs in any of the nuclei of a hypha and the mutated nucleus proliferates along with the wild-type nuclei. This must happen very often, but a stable, functional heterokaryon will develop only if the genetically different nuclei proliferate in the apical cells so that all the newly formed hyphae contain both types. A second way in which heterokaryons arise is by tip-to-tip fusion (anastomosis) of the hyphae of two strains (see Fig. 3.6). Again, the nuclei would need to proliferate in the apical cells to form a stable heterokaryon.

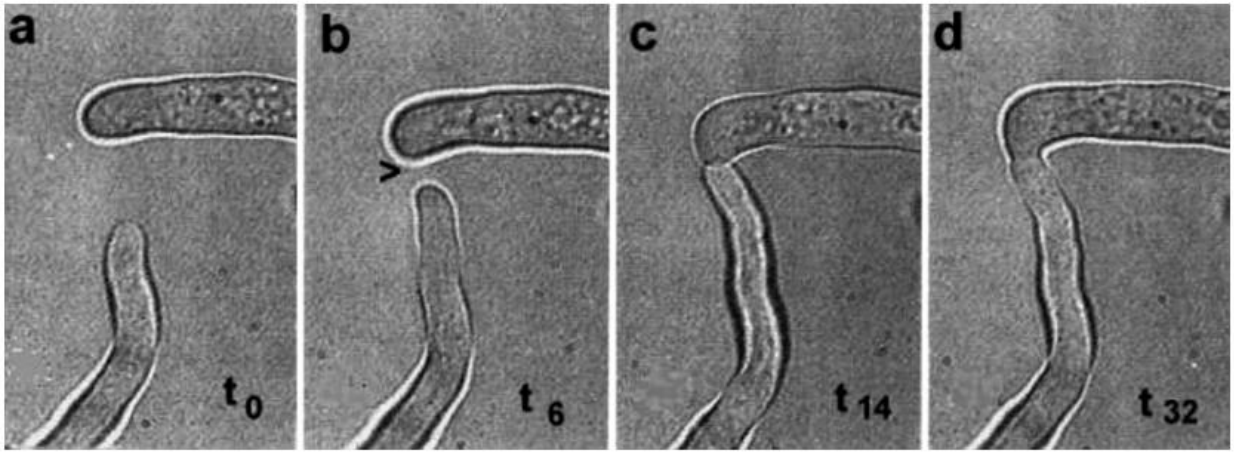

Fig. 3.6 Videotaped sequence of anastomosis of two hyphae of *Rhizoctonia solani*. The times shown are minutes after the start of video recording. The upper hypha had stopped growing at t_0 but began to produce a branch (arrowhead) at t_6 in response to the approaching hyphal tip. The hyphal tips met at t_{14}. Dissolution of the tip walls and complete hyphal fusion was achieved at t_{32}. (From McCabe *et al.* 1999.)

Most experimental studies on heterokaryosis have involved the pairing of strains with defined mutations, such as amino acid auxotrophs. The heterokaryon will then behave as a prototroph,

capable of growing on minimal medium. The most interesting feature in these cases is that the ratio of nuclear genotypes can vary within wide limits and is influenced by environmental conditions. So, at least in theory, a single heterokaryotic strain can change the frequency distribution of its nuclear types in response to selection pressure.

When the heterokaryon was grown on apple-pulp medium the proportion of B-type nuclei was very high, but as the amount of apple pulp was lowered so the proportion of A-type nuclei increased, and dramatically so when the heterokaryon was grown on minimal medium (table 9.3).

Table 9.3 Effects of composition of the growth medium on the ratio of nuclear types in a heterokaryon of *Penicillium cyclopium*. (Data from Jinks 1952.)

Composition of medium (%)		*% of nuclei in the heterokaryon*		*Relative growth rates of homokaryons A and B*
Minimal nutrients	*Apple pulp*	*Type A*	*Type B*	*A : B*
0	100	8.6	91.4	0.47 : 1
20	80	7.8	92.2	0.53 : 1
40	60	11.1	88.9	0.54 : 1
60	40	12.7	87.3	0.67 : 1
80	20	13.5	86.5	1 : 1
100	0	51.8	48.2	1.56 : 1

Breakdown of heterokaryons

Heterokaryons can break down in two ways (Fig. 9.5) – either during the production of uninucleate spores, or when branches arise that contain only one nuclear type. Many common fungi produce uninucleate spores, often by repeated mitotic division of a "mother" nucleus in a phialide – *Aspergillus*, *Penicillium*, *Trichoderma*, *Gliocladium*, etc. (Fig. 9.5a(i)). Multinucleate spores can also be produced from phialides, if a single nucleus enters the developing spore and then divides to produce several nuclei. For example, this is seen in many *Fusarium* species (Fig. 9.5b). But some other fungi (e.g. *Neurospora*, Fig. 9.5c) produce conidia directly from multinucleate hyphal tips or buds, and these spores will be either homokaryotic or heterokaryotic, depending on whether the cells that produced them were homokaryotic or heterokaryotic.

Heterokaryons also break down if a branch arises that, by chance, contains only one nuclear genotype. This branch can produce further branches and eventually give rise to a homokaryotic

sector of the colony (Fig. 9.5a(ii)). If the homokaryon is favored more than the heterokaryon in the prevailing environment then it will expand to occupy progressively more of the colony margin; if not favored it will be suppressed.

sectoring

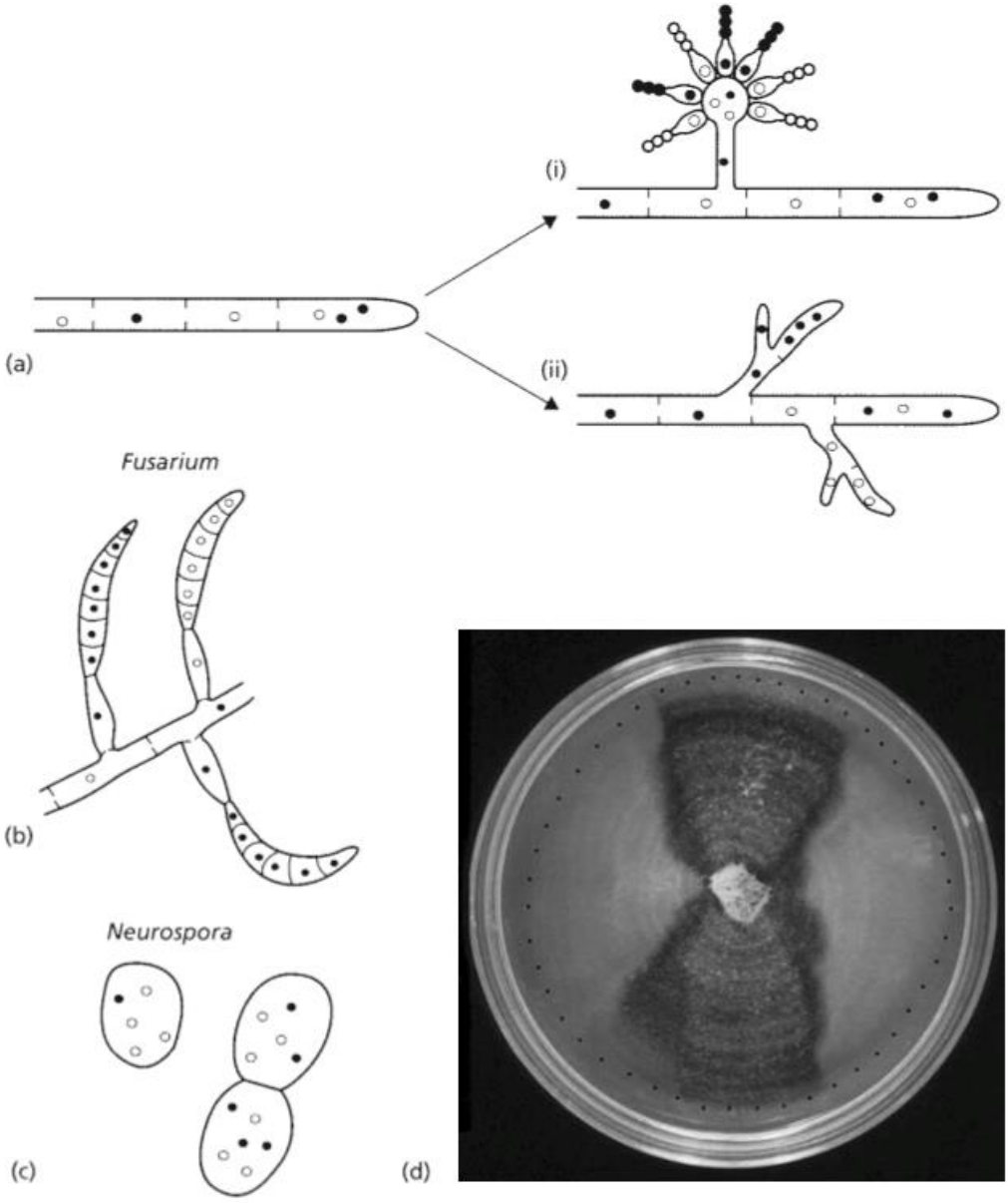

Fig. 9.5 (a–d) Heterokaryosis and the reversion to homokaryons. See text for details.

Figure 9.5d shows an example of this, where a fungal colony was initially darkly pigmented (with daily zones of white aerial hyphae). Two light-colored sectors soon developed and because of their faster growth they progressively expanded. This type of sectoring is quite often seen in

fungal colonies – either as differences in pigmentation or sporulation. But sectors can also differ in pathogenicity or biochemical properties, and these often go undetected.

The significance of heterokaryosis

Heterokaryosis is a potentially powerful phenomenon, but caution is required because the extent of heterokaryosis in natural environments is largely unknown. Many laboratory studies have involved paired auxotrophic mutants, and these can be regarded as "forced heterokaryons" – there is a strong selection pressure to maintain the heterokaryotic condition. Also, there are significant barriers to the creation of heterokaryons in nature, because many fungi have nuclear-encoded "heterokaryon incompatibility" (het) gene loci. For example, *Neurospora crassa* has at least 10 such loci, with two alleles at each locus, so there are potentially 210 (1024) different "vegetative" compatibility groups (VCGs). Pairings of strains of different VCGs lead to hyphal fusion at the points of contact, followed by different degrees of cytoplasmic incompatibility, depending at least partly on the number of het loci that any two strains have in common. Typically, the cytoplasm of the fused cells dies (Fig. 9.6) resulting in a clear demarcation zone between opposing colonies (Fig. 9.7).

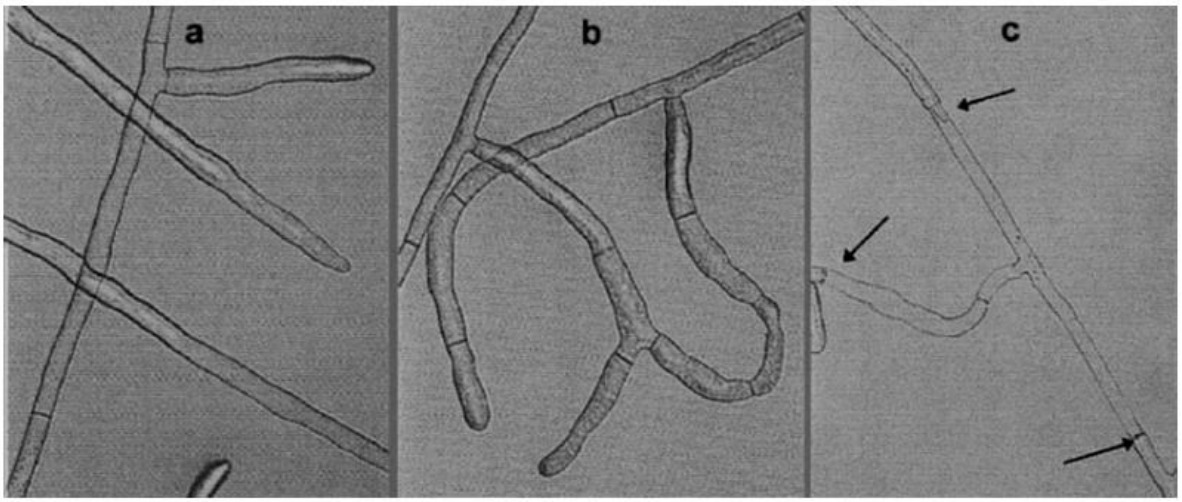

Fig. 9.6 Anastomosis reactions of *Rhizoctonia solani*. (a) No reaction, when strains of different anastomosis groups show no hyphal attraction. (b) Compatible reaction, when strains of the same anastomosis group orientate towards one another and fuse to form a continuous hyphal network. (c) Incompatible reaction, when strains of the same anastomosis group but with different vegetative compatibility genes undergo hyphal fusion, followed by cell death of the fused hyphal compartments. The fused hyphae between the three arrows are dead. (Courtesy of H.L. Robinson.)

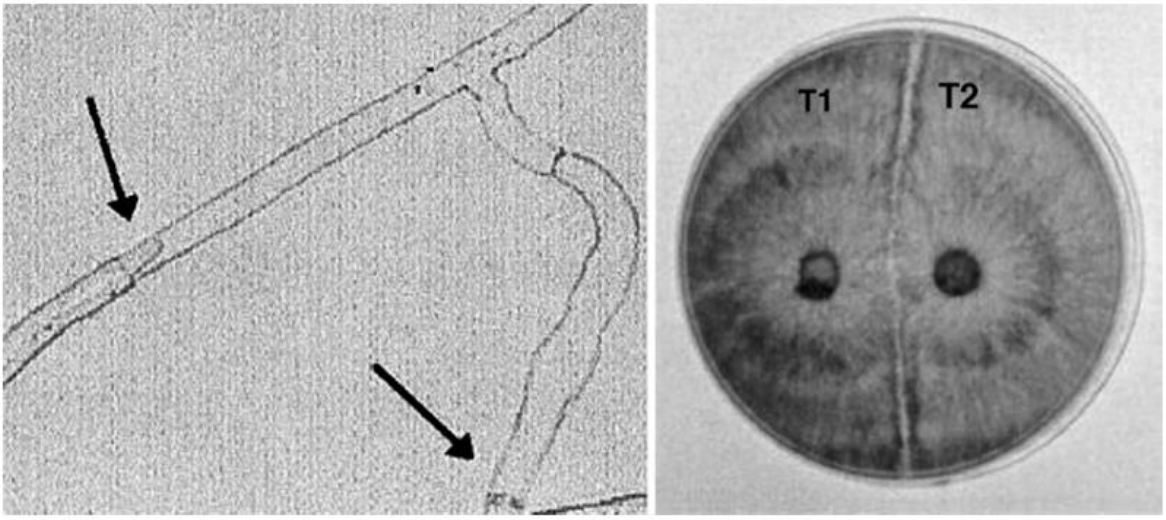

Fig. 9.7 (a) Part of Fig. 9.6c at higher magnification, showing regrowth of a hyphal tip into the dead hyphal compartment. (b) Vegetative incompatibility between two strains (T1 and T2) of *Rhizoctonia solani* (Basidiomycota) opposed on an agar plate. The clear demarcation zone between the colonies resulted from post-fusion death of the hyphae where the colony tips fused (Courtesy of P.M. McCabe.)

Nonsexual variation: parasexuality

Many common mitosporic fungi, such as Aspergillus, A. niger have never been found to produce a sexual stage. Penicillium, Fusarium, and Trichoderma, seem largely to have abandoned sexual reproduction, because their sexual stages are not seen in laboratory conditions and are found only infrequently, if at all, in nature. For example, the common fungi, *Aspergillus fumigatus* and *A. niger* have never been found to produce a sexual stage. This leads us to ask whether they have alternative mechanisms of genetic recombination, and the answer seems to be that they do have this potential through a mechanism termed parasexuality (or the parasexual cycle).

Parasexuality was discovered by Pontecorvo (Pontecorvo 1956), during studies on heterokaryosis in *Emericella* (*Aspergillus*) *nidulans*. He constructed a heterokaryon from two parental strains that had markers at two gene loci (we will call the strains **Ab** and **aB**) and was analyzing the homokaryotic spores produced by the heterokaryon. As expected, most of the spores had nuclei of the "parental" types, either **Ab** or **aB**, but a significant number were found to be recombinants (**AB** or **ab**) and their frequencies were too high to be explained by mutation. Evidently, the genes had recombined in the heterokaryon, although this cannot occur by heterokaryosis alone because the nuclei remain as distinct entities regardless of how they are mixed in the cytoplasm. Further investigation led Pontecorvo to propose a parasexual cycle, involving three stages:

1- **Diploidization.** Occasionally, two haploid nuclei fuse to form a diploid nucleus. The mechanism is largely unknown, and this seems to be a relatively rare event, but once a diploid nucleus has been formed it can be very stable and divide to form further diploid nuclei, along with the normal haploid nuclei. Thus the heterokaryon consists of a mixture of the two original haploid nuclear types as well as diploid fusion nuclei.

2- **Mitotic chiasma formation.** Chiasma formation is common in meiosis, where two homologous chromosomes break and rejoin, leading to chromosomes that are hybrids of the parental types. It can also occur during mitosis but at a much lower frequency because the chromosomes do not pair in a regular arrangement. Nevertheless, the result will be the same when it does occur – the recombination of genes.

3- **Haploidization**. Occasionally, nondisjunction of chromosomes occurs during division of a diploid nucleus, such that one of the daughter nuclei has 2n + 1 chromosomes and the other has 2n – 1 chromosomes. Such nuclei with incomplete multiples of the haploid number are termed aneuploid (as opposed to euploid nuclei, with **n** or complete multiples of n). They tend to be unstable and to lose further chromosomes during subsequent divisions. So the 2n + 1 nucleus would revert to 2n, whereas the 2n − 1 nucleus would progressively revert to **n**. Consistent with this, in *E. nidulans* (n = 8) nuclei have been found with 17 (2n + 1), 16 (2n), 15 (2n − 1), 12, 11, 10, and 9 chromosomes.

It must be emphasized that each of these events is relatively rare, and they do not constitute a regular cycle like the sexual cycle. But the outcome would be similar. Once a diploid nucleus has formed by fusion of two haploid nuclei from different parents, the parental genes can potentially recombine. And, the chromosomes that are lost from an aneuploid nucleus during its reversion to a euploid could be a mixture of those in the parental strains.

Significance of parasexuality

Parasexuality has become a valuable tool for industrial mycologists to produce strains with desired combinations of properties. However its significance in nature is largely unknown and will depend on the frequency of heterokaryosis, determined by cytoplasmic incompatibility barriers. Assuming that heterokaryosis does occur, we can ask why several (obviously successful) fungi have abandoned an efficient sexual mechanism of genetic recombination in favor of a more

random and seemingly less efficient process. The answer might be that the parasexual events can occur at any time during normal, somatic growth and with no preconditions like those for sexual reproduction. Although each stage of the parasexual process is relatively rare, there are many millions of nuclei in a single colony, so the chances of the parasexual cycle occurring within the colony as a whole may be quite high.

Sexual variation

Sex is the major mechanism for producing genetic recombinants, through crossing-over (chiasma formation) and independent assortment of homologous chromosomes during meiosis. A fungus such as *Emericella* (*Aspergillus*) *nidulans*, with eight chromosomes, could generate 2^8 (i.e. 256) different chromosome combinations by independent assortment alone. This would depend on an efficient outcrossing mechanism. Many fungi are heterothallic (outcrossing), requiring the fusion of cells of two different mating types. But some are homothallic (e.g. most *Pythium* spp., about 10% of Ascomycota, and a few Basidiomycota) and some exhibit secondary homothallism – the sexual spores are binucleate with one nucleus of each mating type (e.g. *Neurospora tetrasperma* and *Agaricus bisporus*). Another variation is seen in *Saccharomyces cerevisiae* and the distantly related fission yeast, *Schizosaccharomyces pombe*. Both of these undergo mating-type switching. These variations on the normal mechanisms of outcrossing have probably evolved because the sexual spores of fungi function as dormant spores to survive adverse conditions. At least in the short term, survival is more important than sex!

The place of fungi in genetical research (fungi as model organisms)

For more than 60 years, fungi have been major tools for classical genetical research because they have a combination of features unmatched by other eukaryotes:

1- They are easy to grow in laboratory conditions and they complete the life cycle in a short time.
2- Most fungi are haploid so they are easy to mutate and to select for mutants.
3- They have a sexual stage for analysis of the segregation and recombination of genes, and all the products of meiosis can be retrieved in the haploid sexual spores.

4- They produce asexual spores so that genetically uniform populations can be bulked up and maintained.
5- Because they are eukaryotic cells they exhibit many of the properties and functions characteristic of human cells, and thus served as a better model for many cellular processes than the bacterial systems that had been investigated in depth previously.

In addition to these points, fungi are eminently suitable for biochemical studies because of their simple nutrient requirements, and because "classical genetics" has provided excellent physical maps of the chromosomal genes. Studies on one fungus in particular – *Neurospora crassa* – led to the classical concept of "one gene, one enzyme", for which Beadle & Tatum received the Nobel Prize in 1945. However, it is more accurate to say that "one gene can encode one enzyme" – the situation is complicated because gene splicing occurs to remove noncoding introns in the pre-messenger RNA.

At the time of writing, the genomes of nearly 200 organisms have been sequenced - mainly bacteria and archaea, but also the genomes of "mouse and man." The "high-quality draft" genome sequences of ten fungi have been published, including *Saccharomyces cerevisiae*, *Neurospora crassa*, *Emericella nidulans*, *Schizosaccharomyces pombe*, the rice blast pathogen *Magnaporthe grisea*, and a wood-rotting fungus, *Phanerochaete chrysosporium*. The first four of these are Ascomycota with well-mapped chromosomes, providing a basis for combining classical and molecular genetics.

Fungal Life Cycles

Ascomycete Yeast (*Saccharomyces cerevisiae*)

S. cerevisiae is an extremely well-studied organism, with a clearly defined and experimentally manipulable life cycle. The life cycle of yeast involves mitotically propagating haploid forms of two distinct mating types, and a diploid form that can either grow vegetatively or can be induced into a meiotic developmental pathway through manipulation of the nutrient conditions of the growth media. The cellular pathways regulating processes such as mitotic proliferation, cell recognition and mating, meiosis and sporulation have been extensively studied on a molecular level, and are generally well understood.

Mitotic growth of yeast cells involves budding (Figure 2.1). During this process, growth of the cell is directed to a specific location on the surface of the mother cell, and a new cell is formed somewhat like blowing up a balloon through a hole in the mother cell. This involves highly polarized growth of the developing daughter cell, implicating both the actin and microtubule-based cytoskeletal networks, and is tightly coordinated with the cell cycle. This coordination ensures that the daughter cell receives a complete copy of the genetic material. Both haploid and diploid cells divide by the budding process, although there are subtle differences in the choice of the sites of bud emergence between haploids and diploids. In addition, some diploid cells can also modify the coordination of the cell cycle and polarized growth to switch to a **pseudohyphal** growth mode. In this growth pattern, individual cells are more elongate, and the budding pattern leads to the formation of chains of cells rather than compact colonies characteristic of the true budding mode.

Genetic analysis is highly developed in *S. cerevisiae*. When vegetatively growing haploid cells of opposite mating types are brought into proximity, they communicate with each other by diffusible pheromones, synchronize their cell cycles, conjugate and then fuse their nuclei to create non-mating, meiosis-proficient diploids. These diploids can be identified visually in their initial zygote form, and separated from the haploids by micromanipulation, or identified selectively because they contain a pattern of genetic traits not possessed by either haploid parent.

Under rich growth conditions, such diploid cells themselves propagate vegetatively; but under conditions of nitrogen and fermentable carbon limitation, the diploid cells are induced to initiate meiosis and sporulation. The ability to propagate the diploid allows the amplification of the initial mating product, and provides an essentially unlimited source of potential meiotic events from a single mating.

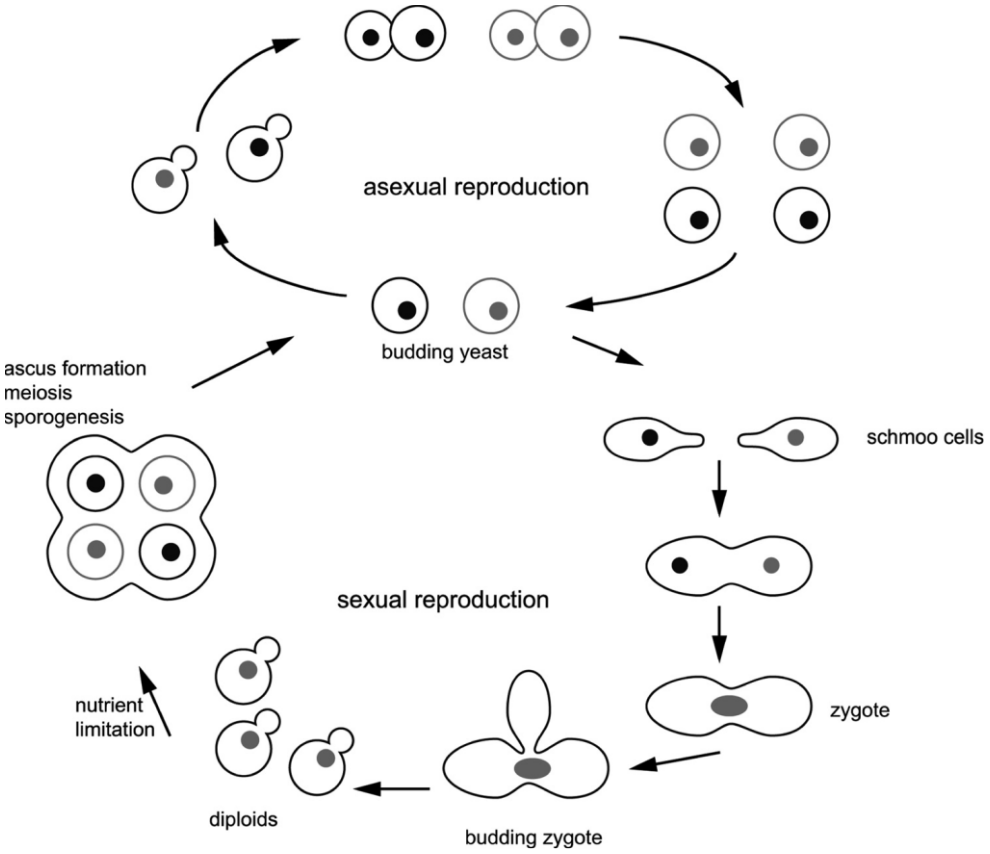

Figure 2.1 Life cycles of *Saccharomyces cerevisiae*.

Ascomycete Filamentous Fungi (*Neurospora crassa* and *Aspergillus nidulans*)

The filamentous fungi differ from the yeasts in that they grow vegetatively as hyphae, which are highly polarized filaments that extend indefinitely at their tips. The hypha initiates new tips in the form of branches from sub-apical regions, and together the growing mass constitutes the mycelium. Hyphae are predominantly multinucleated, with cross-walls called septa dividing the hypha into compartments. The compartments are connected through pores in the septa and, therefore, display cytoplasmic continuity. The hypha functions primarily in acquisition of nutrients and exploration of the environment. Enzyme secretion at the tip assists both processes. In pathogenic fungi, the hyphal growth form can also be important for virulence. In filamentous fungi, vegetative hyphal growth initiates from a spore. Spores are products of either sexual (ascospores, basidiospores) or asexual (conidia) reproduction. Conidia are typically produced from a differentiated structure called a conidiophore, whereas ascospores and basidiospores are produced within an ascus or basidium respectively contained within the fruiting body called an ascocarp or basidiocarp.

During asexual reproduction in the ascomycetes, such as *A. nidulans* (Figure 2.2), a spore containing a single nucleus (monokaryotic) germinates into a multinucleate, homokaryotic hypha. The hypha grows and develops branches for a period of time and then initiates a specialized branch called the conidiophore. Development of the conidiophore involves numerous different cell types, and is investigated as a model developmental process. The nucleus divides mitotically within the conidiophore, allowing the ultimate production of asexual, haploid conidia. Upon release, conidia germinate into vegetatively growing hyphae, and the cycle continues. The factors that trigger initial conidiophore development in *Aspergillus* are not clear, but involve the supply of carbon and nitrogen. The process can normally only occur in cultures grown on solid media with an air interphase; conidiation does not occur in liquid.

Sexual reproduction in *A. nidulans* begins when vegetatively growing hyphae fuse to create a heterokaryon, or dikaryotic hypha (Figure 2.2). The dikaryotic hyphae differentiate into a developing fruiting body called a cleistothecia. The fruiting body is a complex structure composed of many cell types, including both sterile and fertile hyphae. The dikaryotic fertile hyphae within the cleistothecium develop into hooked structures called croziers, which then differentiate into developing asci. Karyogamy or nuclear fusion occurs within the crozier, creating a diploid. The diploid undergoes meiosis and the four meiotic products then undergo mitosis, creating eight haploid ascospores. The ascospores undergo another round of mitosis and are thus binucleate. Thousands of asci are contained within a cleistothecium and are fragile, hampering their individual isolation. Upon release, the ascospores germinate into hyphae as described. *Aspergillus* is homothallic, or self-fertile, and sexual reproduction can be initiated within one colony containing genetically identical nuclei. In the absence of heterokaryon formation with another strain, the individual strain differentiates a cleistothecium as described, into which the hypha develops into a crozier and an ascogenous hypha. Unlike *S. cerevisiae*, *A. nidulans* does not undergo any mating-type switching. *A. nidulans* hyphae can also grow as heterokaryons and diploids as part of a parasexual cycle.

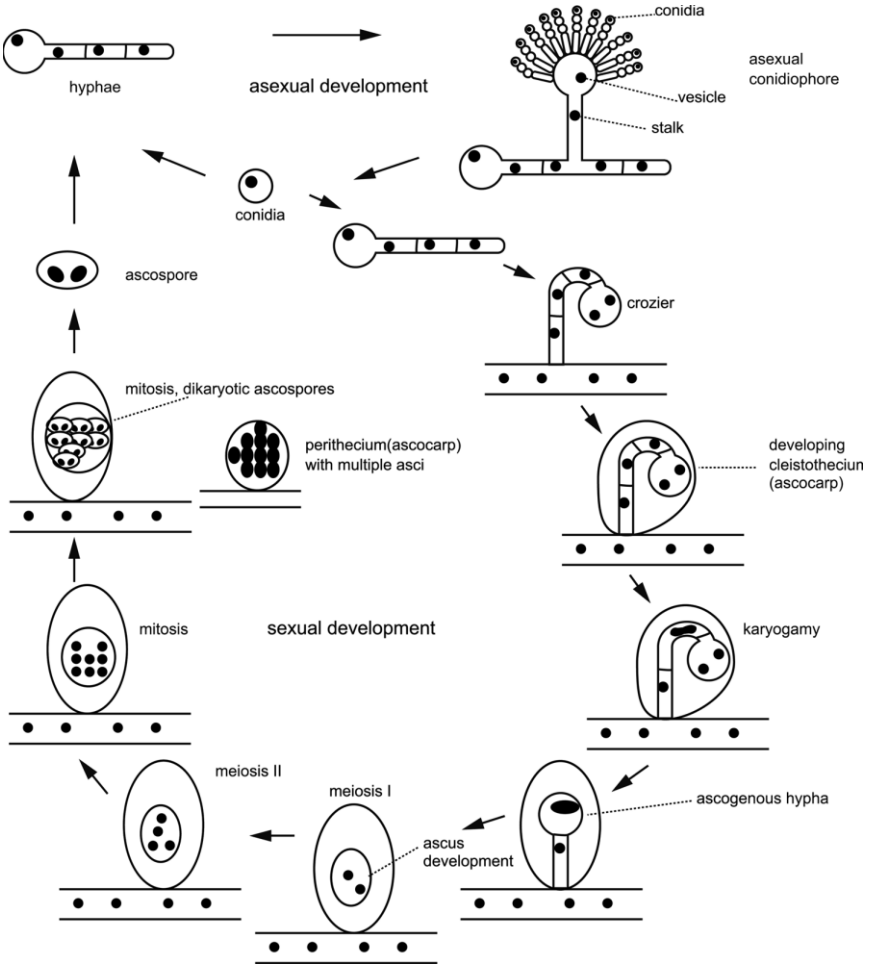

Figure 2.2 Life cycles of *Aspergillus nidulans*.

Life cycle of *Neurospora crassa*

In *N. crassa* (Figure 2.3), asexual reproduction is triggered by circadian (diurnal) rhythms, or an internal clock mechanism, and produces both macro and microconidia. Macroconidia are produced first from aerial hyphae and are used for subculturing strains, whereas microconidia are produced later in the growth process and have poor viability. Macroconidia germinate into vegetatively growing hyphae, but also serve a function during sexual reproduction.

The sexual cycle is initiated in response to nitrogen starvation, or changes in temperature or light. *N. crassa* is heterothallic and, therefore, requires genetically different mating partners. Macroconidia or microconidia produced from hyphae serve as the 'male' and produce a pheromone, which is a hydrophobic peptide. The opposite strain serving as the female develops

a fruiting body intermediate called a protoperithecia. A polarized structure called a trichogyne grows from the protoperithecium of one mating-type female and fuses with the male conidia of the opposite mating type. The nucleus from the latter moves through the trichogyne into the ascogonium within the protoperithecium, which is then referred to as the perithecium. Nuclei from both mating partners divide within a developing dikaryotic ascogenous hyphal structure. The ascogenous hypha develops a crozier, where nuclear fusion or karyogamy takes place, followed quickly by meiosis within the developing ascus. Mitosis and subsequent ascosporogenesis results in eight spores within an ascus within the perithecium. Acsi are long and slender in *Neurospora*, allowing for individual dissection and separation of ordered ascospores.

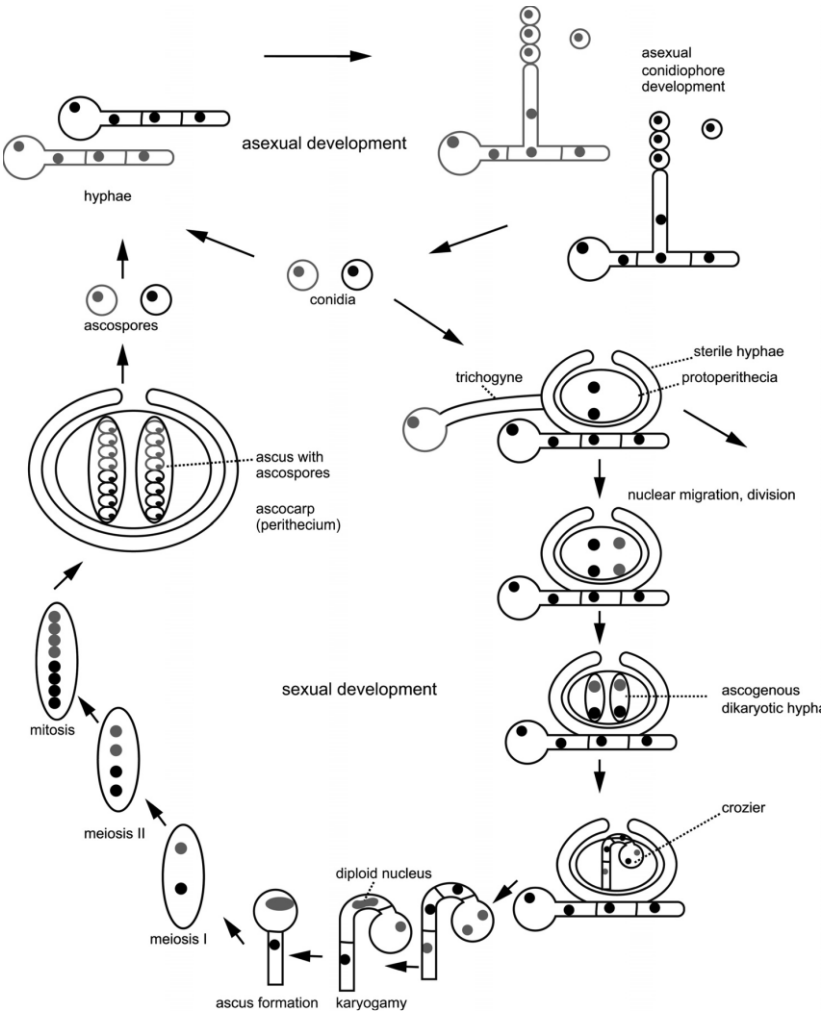

Figure 2.3 Life cycles of *Neurospora crassa*.

Neurospora and classical (mendelian) genetics

Neurospora crassa is one of the most intensively studied fungi for genetical research. It is one of four (possibly five) *Neurospora* species that are heterothallic, requiring haploid strains of two different mating types, termed A and a, for sexual reproduction. Essentially, it begins when a female receptive hypha termed a trichogyne is fertilized by a "male" spore, the spermatium, of opposite mating type. The cells that will eventually become the asci are separated by septa. These ascus mother cells contain two haploid nuclei, one of each mating type. The nuclei fuse to form a diploid, and the ascus elongates. Meiosis within each ascus results in the production of four haploid nuclei, and each of these undergoes one round of mitosis to produce eight nuclei, which are packaged into eight ascospores, linearly arranged within each ascus (Fig. 9.1).

The pattern of gene segregation in an ascus can be followed by making crosses between strains that differ in biochemical features or spore coat color. For example, Fig. 9.1a shows the pattern of meiosis in a fungus heterozygous at a locus that determines spore coat color. The allele B codes for dark spores and the allele b codes for pale spores. (Note that each chromosome consists of two chromatids, attached to a centromere, but only one arm of each chromosome is shown.) During the first meiotic division the chromosomes separate. The chromatids then separate in the second meiotic division, and this is followed by mitosis, leading to an ascus containing a linear arrangement of four black ascospores and four pale ascospores.

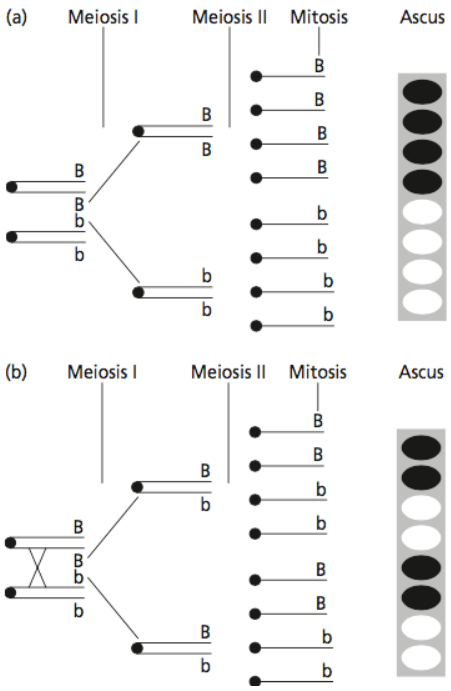

Fig. 9.1 Illustration of the segregation of spore color genes during the first division (a) or second division (b) of meiosis in Ascomycota. See text for details.

Figure 9.1b shows a different pattern of segregation of spore color, resulting from crossing over, in which two homologous chromatids break and rejoin, with reciprocal exchange of DNA. The subsequent pattern of spore coat color is different in the final ascus.

Normally, there would be several crossover events (chiasmata, singular chiasma) on any one arm of a chromosome, but this would best be detected by crossing strains that differ at three different loci – for example, loci X, Y, and Z – on one arm of the chromosome. Broadly, the chance of a crossover event occurring between any two gene loci on a chromosome depends on the physical distance between these loci. Similarly, the chance of crossing-over between a gene locus and a centromere depends on the distance between these. So it is possible to construct physical maps of the relative positions of different gene loci on any one arm of a chromosome (chromosome mapping) by making repeated crosses involving different gene loci. (This is not exactly true because the frequency of crossing-over tends to be lower near the centromere and higher near the ends of the chromosomes – the telomeres – but it does allow the order of genes to be determined.).

To provide a "real" example of the patterns of gene segregation and recombination in an ascus, Fig. 9.2 shows the results of a cross between two parental strains of *Sordaria brevicollis*, a fungus closely related to *Neurospora crassa*. One strain has the wild-type alleles for buff-colored (**b**) and yellow-colored (**y**) ascospores on one arm of the chromosome. The other strain has mutations at both of these gene loci, indicated as $\mathbf{b_m}$ and $\mathbf{y_m}$, where the subscript m denotes a mutation (see label 1 in Fig. 9.2).

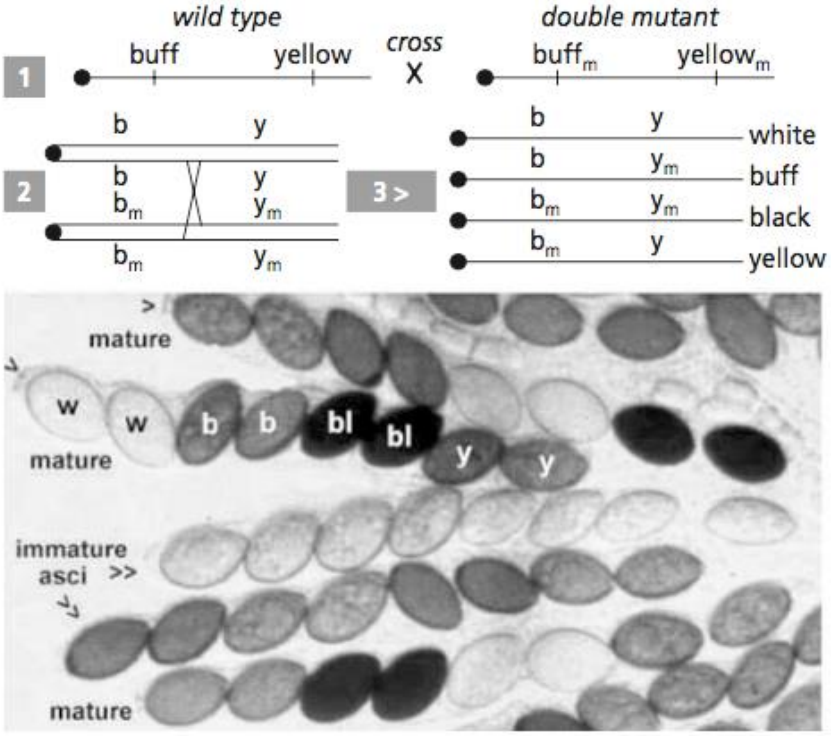

Fig. 9.2 Part of a crushed perithecium of *Sordaria*, showing several asci containing eight ascospores. In the normal, intact perithecium the ascospores would be released through small pores (arrowheads) at the ascus tips.

In this particular example, the outcome of such a cross would normally produce either buff spores (with the alleles **b** and $\mathbf{y_m}$) or yellow spores (with the alleles $\mathbf{b_m}$ and **y**). But the **b** and **y** loci are sufficiently far apart that there is about 20% probability of a crossover occurring between these loci (see label 2) so that after meiosis and a subsequent round of mitosis, the eight mature ascospores will display four different colors (see label 3). Spores with the **b** and **y** alleles are white, those with **b** and $\mathbf{y_m}$ are buff-colored; those with $\mathbf{b_m}$ and $\mathbf{y_m}$ are black, and those with $\mathbf{b_m}$ and **y** are yellow-colored. Three of the asci in Fig. 9.2 are labeled "mature" – the spores are labeled **w**

(white), **b** (buff), **bl** (black), and **y** (yellow). Each of these asci shows a different pattern of spore segregation, depending on which chromatids were involved in chiasma formation.

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