8 Sexual development of higher fungi

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Introduction

Fungal mycelia will continue to grow and invade new substrates for as long as satisfactory conditions prevail (Chapter 9), typically producing numerous asexual spores (Chapter 7) and other mitotically derived invasive, reproductive and/or resistant structures (strands, rhizomorphs, sclerotia, stromata, etc.). Except for the Mycelia Sterilia (Deuteromycotina, Chapter 2), under particular conditions a fungus enters a sexual pathway, resulting in genetic segregation and production of recombinant progeny.

Since most fungi seem to be haploid for most of their life cycles, the first step in this process is to bring together two haploids so that nuclei can coexist in the same cytoplasm, undergo karyogamy followed by the meiotic division, and then generate and distribute progeny spores. These processes are considered in this chapter (see also Carlile and Watkinson, 1994; Elliott, 1994; Moore, 1998).

Sex: what and why?

Most fungi produce abundant asexual spores which are extremely effective in dispersing the organism. We have to ask why so many fungi invest more resources in a more complex sexual reproduction. There are, indeed, many fungi which only reproduce asexually but the majority still have a sexual cycle. Sex must have selective advantage if sexual stages are not to be replaced by asexual ones entirely (Maynard Smith, 1978).

The crucial point which provides the contrast with asexual reproduction is fusion of nuclei derived from different individuals. If the individuals differ in genotype, the fusion nucleus will be heterozygous and the products of the meiotic division can have recombinant genotypes. Thus, in one sexual cycle, new combinations of characters can be created.

This is the most usual 'explanation' for sex, namely that it promotes genetic variability through out-crossing and that variability is needed for the species to evolve to deal with competitors and environmental changes. Copious evidence exists to show that out-crossing certainly does promote variability, and that asexual lineages change little in time, in apparent support of the view that variability in the population enables the organism to survive ecological and environmental challenges.

However, this is a 'group selectionist' interpretation (variation generated in an *individual* meiosis is seen as benefiting the *group* or population to which the individual belongs) and current theory emphasizes, instead, that selection acts on individuals, so any feature which is argued to be advantageous in selection must be so because it benefits either the individual itself or its immediate progeny (Dawkins, 1976; Carlile, 1987). Bernstein *et al.* (1985) suggested that repair of damaged DNA is the crucial advantage of the meiotic sexual cycle, damaged DNA (caused by mutation or faulty replication) in one chromosome being repaired by recombination with the normal chromosome provided by the other parent.

Out-crossing might also give rise to heterozygous advantage, where the heterozygous phenotype is better than either of its homozygous parents, which has frequently been demonstrated in plants and animals and has also been demonstrated in the yeast *Saccharomyces cerevisiae.*

These alleged advantages of the sexual cycle (generation of variation and heterozygous advantage) are not mutually exclusive, nor of equal value; rather, they are themselves phenotypic characters which may or may not have selective value for the organism concerned. Different species have different life cycles and experience different evolutionary challenges and may therefore make use of, enhance or dispense with various aspects of sexual reproductive processes for any one or more than one of the interpretations outlined above. Generally, experimental evidence for the advantage of sex in the wild is lacking and fungi, which have both asexual and sexual cycles, are ideal organisms with which to perform such experiments.

Getting it together

Hyphal anastomosis (fusion between hyphae; or conjugation [cell fusion] in the unicellular fungi, yeasts) is the essential first step in the

sexual cycle of most fungi. The process involves breakdown of two hyphal (cell) walls and union between two separate plasma membranes to bring the cytoplasms of the fusing hyphae into continuity with each other. To maximize the advantage of sexual reproduction the parental nuclei should be genetically as different as possible, whereas safe operation of the cell requires that cytoplasms which are to mingle must be as similar as possible.

The problem at the cytoplasmic level is that hyphal anastomosis carries the risk of exposure to contamination with alien genetic information from defective or harmful cell organelles, viruses, transposons or plasmids. Protection against alien DNA is provided by expression of vegetative compatibility which is controlled by one to several nuclear genes which limit completion of hyphal anastomosis between colonies to those which belong to the same vegetative compatibility group (v-c group). Members of a v-c group possess the same vegetative compatibility genes (or alleles).

Cytoplasmic compatibility and the individualistic mycelium

Fungal isolates from nature usually show interactions which demonstrate self/non-self recognition. If the colonies involved are not compatible the cells immediately involved in anastomosis are killed. Vegetative compatibility (also called somatic or heterokaryon incompatibility) will prevent formation of heterokaryons except between strains which are sufficiently closely related to belong to the same v-c group.

Fusion incompatibility (where the compatibility system determines the ability to fuse) occurs in slime moulds, but the type of vegetative compatibility which is most usual in fungi is called post-fusion incompatibility. Hyphal anastomosis is consequently promiscuous, but compatibility of the cytoplasms determines whether the cytoplasmic exchange process will progress beyond the few hyphal compartments involved in the initial interaction. This strategy prevents transfer of nuclei and other organelles between incompatible strains, but if the incompatibility reaction is slow, a virus or cytoplasmic plasmid may be communicated to adjacent undamaged cells before the incompatibility reaction kills the hyphal compartments where anastomosis occurred.

It is these compatibility reactions (including vegetative and sexual) which define in real life what constitutes the fungal individual. In yeasts each cell is clearly an individual but a mycelial individual is not so obvious. Spores are individuals and colonies developed from single spores must also be individuals. Whether a heterokaryon is an individual, rather than a chimera or mosaic of two or more individuals, is more debatable. When individuals do exchange nuclei it is the mating systems which then regulate sexual exchange between the vegetatively compatible mycelia.

Sexual mating systems

Mating systems (or breeding systems) depend on nuclear genes that prevent mating between genetically identical mycelia. A mycelium which possesses a system to prevent mating between identical gametes will be self-sterile and, since *different* mycelia must come together for a successful mating to occur, the system is called *heterothallism*. The alternative, where there is nothing to prevent a single mycelium completing a successful mating, is called *homothallism*. A number of mating type systems have been recognized in fungi, they all regulate the sexual process so that meiosis occurs only if the two mycelia concerned carry different mating type factors.

In many fungi there are only two mating types. The common 'bread-mould' *Neurospora crassa*, the brewer's yeast *Saccharomyces cerevisiae*, the grass rust *Puccinia graminis* are examples. In these cases the 'mating type' of a culture depends on which allele it has at a *single* mating type locus (hence the name *unifactorial* incompatibility) and successful mating can only take place between (yeast) cells or mycelia that have different alleles at the mating type locus. The diploid nucleus which is eventually formed is, of course heterozygous at the mating type factor, and meiosis produces equal numbers of progeny of each of the two mating types (hence the alternative name bipolar heterothallism). A modification of this system is seen in *Coprinus comatus* ('Lawyer's wig' or 'Shaggy-cap' mushroom) which has a large number of mating type alleles at its single mating type locus. When there are only two alleles the likelihood that two unrelated individuals will be able to mate (which is the outbreeding potential) is 50 per cent. But if there are n mating types in a

population the outbreeding potential would be $[1/n \times (n-1)] \times 100$ per cent. The greater the number of mating type alleles, the greater the outbreeding potential.

Many of the Basidiomycotina have *two* unlinked mating type factors (designated A and B). This, of course, is called a *bifactorial* incompatibility system. In this case also, compatibility requires that two mycelia have different alleles, but this time *both* mating type factors must differ. The diploid nucleus will therefore be heterozygous at the two mating type loci and meiosis will generate progeny spores of four different mating types (so tetrapolar heterothallism is the alternative name of this system). The classic examples of this mating type system are the Hymenomycetes *Coprinus cinereus* and *Schizophyllum commune*. In both organisms the wild population contains many different A and B alleles, so the outbreeding potential is about 100 per cent. The advantage over the unifactorial system is that the inbreeding potential of bifactorial incompatibility (the likelihood of being able to mate with a sibling) is 25 per cent (because there are four different mating types among the progeny of a single meiosis) whereas it is 50 per cent in unifactorial incompatibility where there are only two mating types among the progeny. The percentage values quoted here are simplifications. Change in the mating type (by recombination or mating type switching) can increase inbreeding potential and is discussed below.

In self-fertile (homothallic) fungi the sexual process can occur between genetically identical cells or hyphae, but this does not mean that mating type factors are not involved. Primary homothallism does indeed occur in species completely lacking heterothallism, but secondary homothallism occurs in species which have an underlying heterothallism which is bypassed in some way. The best examples are found in *Neurospora tetrasperma* and *Agaricus bisporus*. In both cases, fewer spores are formed than there are post-meiotic nuclei with the result that spores are binucleate and heterozygous for mating type factors. When the spores germinate they form heterokaryotic vegetative mycelia which are able to complete the sexual cycle alone; i.e. they act like homothallics. A different example is provided by the yeast *Saccharomyces cerevisiae*, in which most strains are heterothallic with two mating types, a and α (see below, section: mating type factors in *S. cerevisiae*). However, in some strains mating

occurs between progeny of a single haploid ancestor; that is, the culture appears to be 'homothallic'. The apparent 'homothallism' results from a switch from one mating type to the other (see below) so that the clone comes to contain cells of both mating types.

Mating type factors in *Saccharomyces cerevisiae*

The yeast *Saccharomyces cerevisiae* has a life cycle which features an alternation of a haploid phase with a true diploid phase, and in this respect differs from other Ascomycotina in which the growth phase after anastomosis is a heterokaryon. Haploid yeast cells have one of two mating types which are symbolized a and α. Nuclear fusion (karyogamy) follows fusion of cells of opposite mating type and the first bud after these events contains a diploid nucleus.

Most natural cultures of yeast are diploid because the haploid meiotic products mate soon after meiosis while they are in close proximity. The diploid state is maintained by mitosis and budding until specific environmental conditions (deficiency in nitrogen and carbohydrate, but well aerated and with acetate or other carbon sources which favour the glyoxylate shunt) induce sporulation when the entire cell is converted into an ascus in which meiosis occurs and haploid ascospores are produced. Ascospore germination re-establishes the haploid phase, which is itself maintained by mitosis and budding (Figure 8.1).

The mating type factors in yeast are responsible for production of peptide hormones (pheromones called α- and a-factors; Figure 8.2) and pheromone-specific receptors. The pheromones organize the mating process by binding to pheromone receptors on the surface of cells of opposite mating type (Figure 8.1) acting through GTP binding proteins to alter metabolism and (i) causing recipient cells to produce an agglutinin, so that cells of opposite mating type adhere; (ii) stopping growth, so that cells are blocked in the G1 stage of the cell cycle; (iii) changing wall structure and consequently cell shape.

Both pheromones cause their target cells to elongate into projections but have no effect on cells of the same mating type or on diploids. Fusion occurs between the projections (Figure 8.1). The elongated cells are called 'shmoos'.

Mating phenotypes in *S. cerevisiae* are controlled by a complex

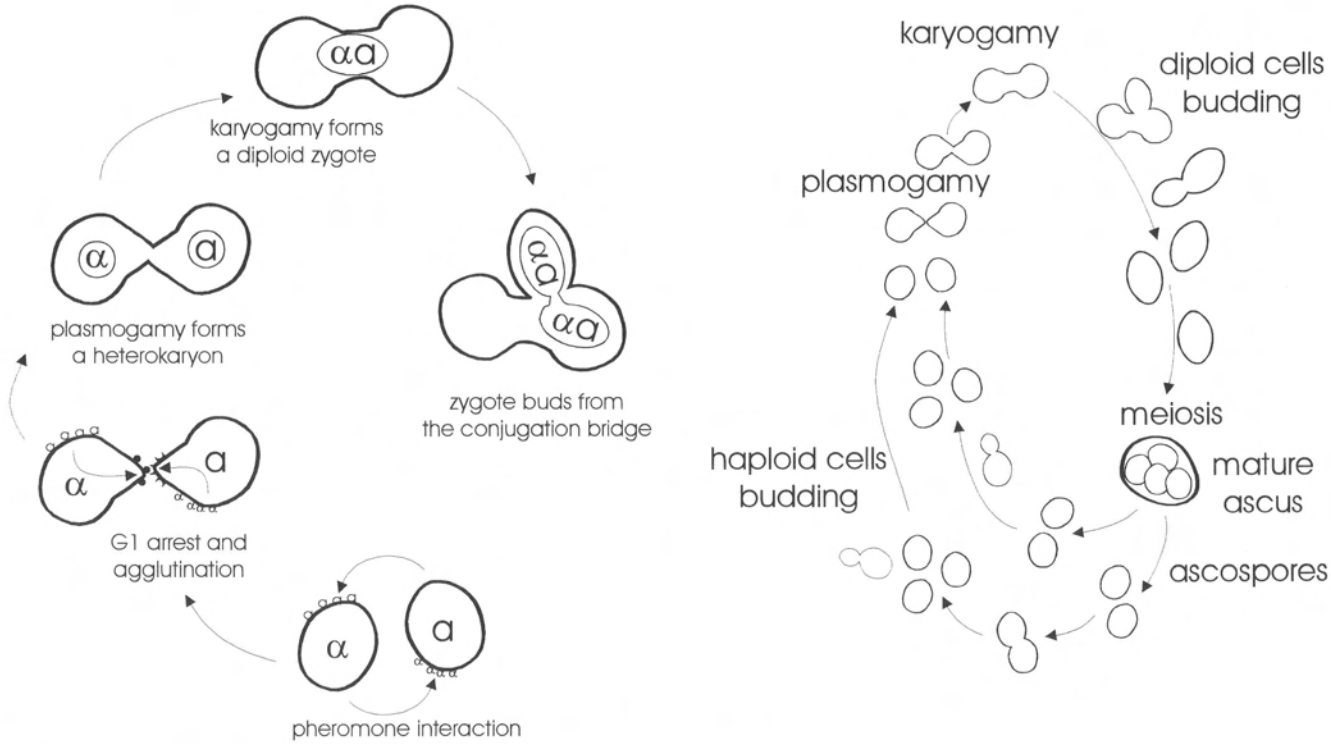
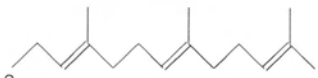


Figure 8.1 Mating process (left-hand panel) and life cycle of the yeast *Saccharomyces cerevisiae*. Yeast can reproduce asexually by budding. Haploid cells of different mating types fuse to form dumbell-shaped zygotes, which can themselves bud to establish a diploid clone. Well-nourished diploid cells which are exposed to starvation conditions enter meiosis, forming a four-spore ascus. Ascospores germinate by budding. In the laboratory, ascospores can be separated to form haploid clones but in nature ascospores usually mate immediately, so the haploid phase is greatly reduced.

α-factor

NH_2-Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr-COOH

a-factor



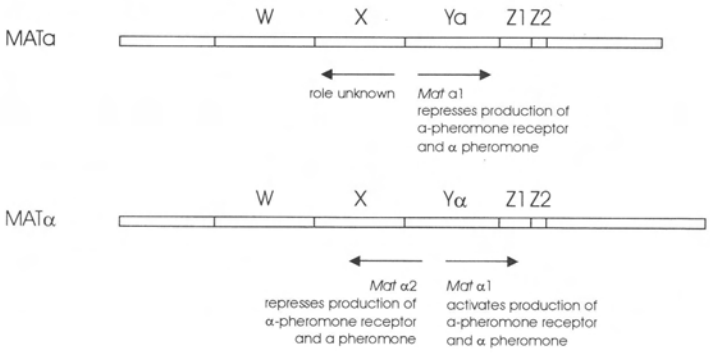
NH_2-Tyr-Ile-Ile-Lys-Gly-Val-Phe-Trp-Asp-Pro-Ala-Cys-$COOCH_3$

Figure 8.2 Simplified chemical structures of yeast pheromones. The a-factor contains a farnesyl group.

genetic locus called *MAT* where two linked genes are harboured (a1, a2 for mating type a and α1, α2 for mating type α). The *MAT*a locus encodes the divergently transcribed a1 and a2 polypeptides (Figure 8.3), and *MAT*α encodes polypeptides α1 and α2 (no function has been identified for the a2 gene product). Heterozygosity at *MAT* signals diploidy and eligibility to sporulate (i.e. even partial diploids carrying *MAT*a/*MAT*α will attempt to sporulate). The α2 polypeptide is a repressor of transcription of a-factor (in α-cells), whilst a1 represses αspecific genes in a-cells. The α1 protein activates transcription of genes coding for αpheromone and a-factor surface receptor. In a/α diploids, a1 and α2 polypeptides form a heterodimer which represses genes specific for the haploid phases, including a gene called *RME*1 (Repressor of Meiosis) which suppresses meiosis and sporulation (Figure 8.3).

S. cerevisiae is heterothallic but a clone of haploid cells of the same mating type frequently sporulates, producing equal numbers of a and αprogeny. Originally thought to result from mutation, this was eventually found to result from mating type switching controlled by the gene *HO* (HOmothallic) which exists in two allelic forms; rare occurrence of mating type switching (about once in 10^5 divisions) is the phenotype of allele *ho*, whereas in strains carrying *HO* the switch occurs at every cell division. *HO* is a gene encoding an endonuclease creating a double-strand break at locus *MAT* and its transcription is repressed by the a1/α2 heterodimer. The mating type of the yeast is determined by the expressed allele in the *MAT* locus, but on either side of this (and on the same

Saccharomyces cerevisiae



In a/α diploids, the MATa1/MATα2 heterodimer protein activates meiotic and sporulation functions, and represses haploid functions (turning off α-specific functions by repressing MATα1, a-specific functions being repressed by MATα2 alone).

Neurospora crassa

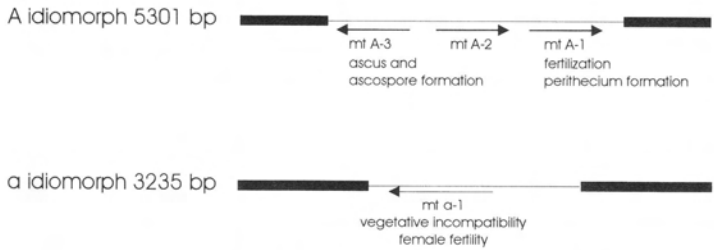


Figure 8.3 Functional regions of mating type factors in *Saccharomyces cerevisiae* (top) and *Neurospora crassa* (bottom). In both panels the arrows indicate direction of transcription and the legends beneath the arrows indicate functions of the gene products. In *S. cerevisiae* of mating type a, a general transcription activator is responsible for production of a-pheromone and the membrane-bound α-pheromone receptor. In the *N. crassa* illustration the black bars represent the conserved DNA sequences which flank the idiomorphs, the latter shown as lines. These diagrams are oriented so that the centromere is on the left, consequently the centromere-distal sequence is on the right.

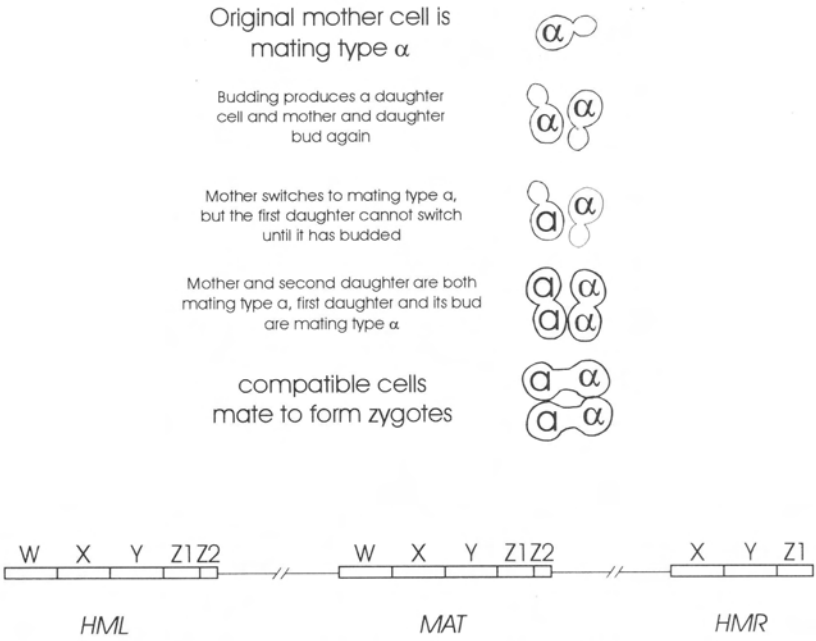


Figure 8.4 Top: pattern of mating type switching in *Saccharomyces cerevisiae* showing the consequences of a mating type switch in one mother cell. Bottom: the three loci involved in mating type switching, *HML*, *MAT* and *HMR*, are located on the same chromosome.

chromosome) there are storage but silent loci, one for each mating type, *HML* and *HMR*. Switching involves replacement of information at the *MAT* locus by that at either *HML* or *HMR* (Figure 8.4; see Schmidt and Gutz, 1994) by an intrachromosomal recombination event. *HO* is expressed in G1 phase of the cell cycle under the positive regulation of SWI5 protein which is synthesized in late S and G2 phases. As a result, the asymmetrical distribution of SWI5 protein in the mother cell, which has undergone one complete cell cycle, grants the cell the developmental potential of mating type switching (Figure 8.4).

Mating type switching also occurs in the distantly related fission yeast *Schizosaccharomyces pombe* but mating types in filamentous fungi tend to be far more stable although unidirectional switching of mating type has been reported in *Chromocrea spinulosa, Sclerotinia trifoliorum, Glomerella cingulata* and *Ophiostoma ulmi.*

Mating type factors in *Neurospora* species

The genus *Neurospora* has at least four markedly contrasting mating strategies: (i) bipolar heterothallism with mating types A and a (e.g. *N. crassa*, *N. sitophila*, *N. intermedia* and *N. discreta*), but unlike the yeast *Saccharomyces cerevisiae*, the mating type genes are present in a single copy per genome, and the two alleles do not share homology (hence the name idiomorph); (ii) secondary homothallism (e.g. *N. tetrasperma* which produces asci containing four ascospores each containing compatible nuclei); (iii) primary homothallism in which each haploid genome carries genetic information of both mating types (e.g. *N. terricola*, *N. pannonica*); and (iv) primary homothallism in which genetic information for only one mating type is detected (e.g. *N. africana* which possesses only the *A* idiomorph which shares 88 per cent similarity with that of *N. crassa*). In the primary homothallic species, meiosis produces a linear eight-spored ascus in which all progeny are self-fertile.

In bipolar heterothallic species, the mycelium of each mating type is hermaphroditic. Under nitrogen starvation, strains of either mating type develop female structures (protoperithecia and trichogynes). Mycelia and asexual spores (macroconidia or microconidia) of the strain of the opposite mating type can serve as the male in a sexual cross and secrete mating type-specific pheromones which orient trichogynes to cells of the opposite mating type. Mating is followed by the formation of perithecia which contain asci (see Figure 8.13). In *N. crassa*, the mating type locus is one of the 10 heterokaryon incompatibility (*het*) loci active during vegetative growth, preventing the formation of viable $a+A$ heterokaryons (see section: Cytoplasmic compatibility, above). Yet this mating type heterokaryon incompatibility is suppressible by an unlinked wild-type suppressor, tol^{+} (tolerant).

The mating type genes *A* and *a* in *Neurospora crassa* have been cloned and sequenced (see Figure 8.3). In all the known *Neurospora A* and *a* idiomorphs and flanking regions, the centromere-proximal flank contains species-specific and/or mating type-specific DNA sequences. Immediately adjacent to the centromere-proximal are the variable regions, which are very different between species. Next to the variable regions are the idiomorphs themselves which are highly conserved between species but are completely dissimilar between the two mating

types within the species. These are then followed by a 'mating type common region' of 57–69 bp which separates an idiomorph from its nearby variable region and is highly similar between all species and between both mating types. These flanking regions may provide positional information for the proper expression of the idiomorph enclosed.

The *A* idiomorph of 5301 bp in length gives rise to at least three transcripts (A-1, A-2 and A-3), with the first two transcribed in the same direction (Figure 8.3). The first 85 amino acids at the N-terminal region of the mating type *A* product are minimally sufficient for female fertility. A region from position 1 to 111 confers vegetative incompatibility while amino acids from position 1 to 227 are required for male-mating activity. This mating type-specific mRNA is expressed constitutively in vegetative cultures, and during the sexual cycle both before and after fertilization. Transcript *A*-1 which shows a high degree similarity to *MAT*α1 of *Saccharomyces cerevisiae* is essential for fertilization and fruiting body formation and the other two transcripts are involved in post-fertilization functions including ascus and ascospore formation. The *A*-3 transcript is a HMG (high-mobility group) polypeptide with DNA-binding ability, indicating its potential function as a transcriptional factor and shares 50 per cent similarity to the mating type polypeptide (*Mc*) of yeast *Schizosaccharomyces pombe.*

In *N. crassa*, bases 2923–4596 of the *a* idiomorph give rise to a single mt *a*-1 transcript which encodes a polypeptide (288 amino acids) belonging to high-mobility group proteins (the HMG box) with DNA-binding activity. The HMG box is the domain for mating function whereas amino acids 216–220 of mt *a*-1 act in vegetative incompatibility. The separation of vegetative incompatibility from both mating and DNA binding indicates that vegetative incompatibility functions by a biochemically distinct mechanism. All *a* idiomorphic DNA sequences between 1409 and 2923 are non-essential. Unlike the case with *Saccharomyces cerevisiae* (see section: mating type factor in *S. cerevisiae*), the genes downstream to and regulated by the *A* and *a* transcripts, however, have not been characterized.

Mating type factors in *Ustilago maydis*

Ustilago maydis causes the smut disease of maize. It has a tetrapolar mating system. Haploid, yeast-like sporidia can be grown on synthetic

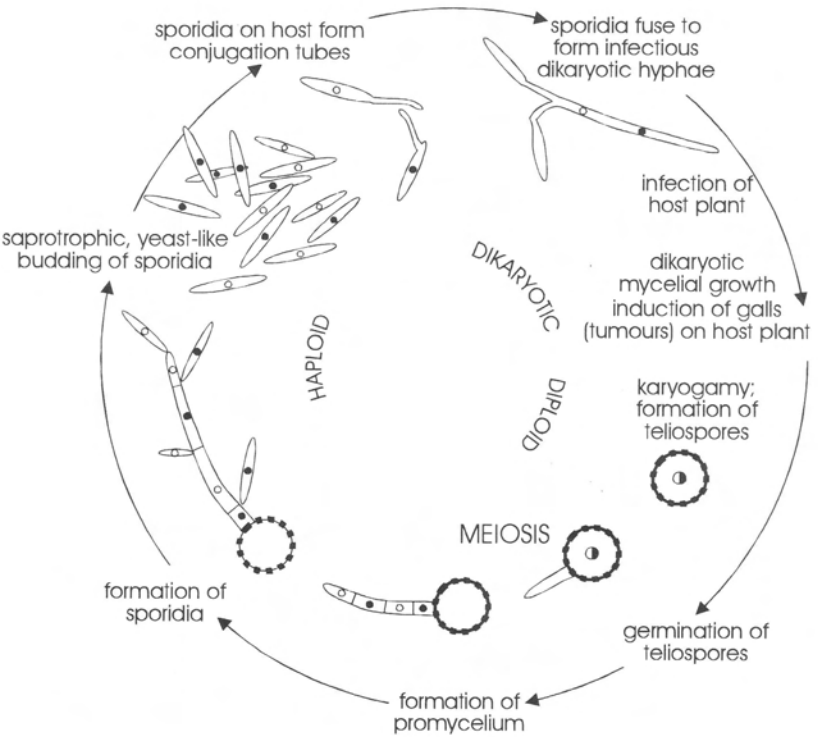


Figure 8.5 Diagram of the life cycle of *Ustilago maydis*. (Redrawn after Kämper *et al.*, 1994.)

media and are non pathogenic for the host plant (Figure 8.5). When sporidia of opposite mating types are mixed, conjugation tubes are formed and fusion of these produces the dikaryon which grows as a filamentous fungus. The dikaryon is the pathogenic stage, however, and its growth is strictly dependent on the host plant. The hyphal cells grow within the plant, causing tumour (or gall) formation. Within the galls, virtually all the hyphal segments differentiate into diploid teliospores which have thickened black walls. Eventually the host epidermis ruptures and galls break open, releasing teliospores. Teliospores germinate, undergo meiosis, and form a promycelium that usually consists of four haploid cells, each of which produces numerous sporidia by successive budding.

Fusion of sporidia is controlled by the biallelic '*a*' mating-type locus, a contrast with Homobasidiomycetes, where hyphal fusion is not

controlled by mating type factors, but by vegetative compatibility genes. The heterozygous *a*1/*a*2 genotype is required for the generation of structures similar to conjugation tubes for the proper transition between the yeast and filamentous growth form. The multiallelic '*b*' locus determines true filament (hyphal) growth form, pathogenicity and stops cells fusing after the formation of the diploid.

Formation of conjugation tubes is induced by the action of diffusible, mating type-specific pheromones released by haploid cells. Sporidia of each mating type secrete a pheromone specific to their mating type and sense the presence of pheromone from cells of opposite mating type with mating type-specific pheromone receptors. These pheromones are short hydrophobic lipopeptides. The peptide consists of 11 to 15 amino acids; they all have a C-terminal cysteine residue that is post-translationally modified. The cysteine's carboxyl group is methylated and a farnesyl group (a 15-C isoprenyl moiety) is attached to the sulfhydryl group. The latter makes the pheromone so hydrophobic that it is nearly insoluble in water and it may act as a membrane anchor.

The *a*1 allele comprises 4.5 kb of DNA, and the *a*2 allele 8 kb. Two mating type-specific genes have been identified in these regions: *mfa*1 (in *a*1) and *mfa*2 (in *a*2) code for precursors of the farnesylated pheromones and *pra*1/*pra*2 which encode pheromone receptors (Figure 8.6).

The *b* mating type factor in *U. maydis* contains a pair of genes (separated by a 260-bp spacer region) which are transcribed in opposite directions. The genes are called *bE* and *bW* (East and West) and their coding sequences are equivalent to polypeptides of 473 and 629 amino acids, respectively. There are a number of conserved sites with similarities to the homeodomain or DNA-binding regions known in regulatory proteins of many other eukaryotes. In different alleles the coding sequence shows a high degree of variation at the amino terminal end, whereas the carboxy-terminal ends are highly conserved.

The variable regions at the amino terminal end determine the allele specificity at this multiallelic locus; whilst the highly conserved region, and particularly the homeodomain, provide for its function in regulating sexual development. The current notion is that bW and bE proteins form a heterodimer which might act as an activator of genes required for the sexual cycle and/or repressor of haploid-specific genes. It must be

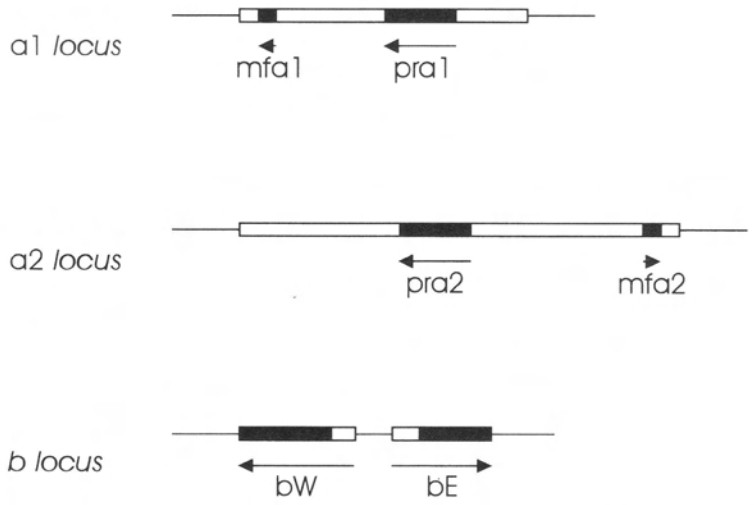


Figure 8.6 Schematic representations of the structures of the a and b mating type loci of *Ustilago maydis*. Alleles of the a locus consist of mating type-specific (i.e. variable) DNA sequences (4500 base pairs in a1, 8000 base pairs in a2), here shown as open boxes, within which are the genes for mating (*mfa* and *pra*). The b locus has two reading frames, bW and bE, which produce polypeptides containing domains of more than 90 per cent sequence identity (shown as black boxes) and variable domains (open boxes) which show 60 to 90 per cent identity. Arrows indicate the direction of transcription.

assumed, of course, that the complex comprised of bE & bW from the same allele is always inactive (perhaps the homeodomains are not properly exposed); only when the proteins come from different alleles will the heterodimer function properly.

Mating type factors in *Coprinus cinereus* and *Schizophyllum commune*

These two homobasidiomycetes have been the main subjects in studies of mating in saprotrophic basidiomycetes over many years. The attraction has been that in both organisms mating results in a major change in mycelial morphology (Figures 8.7 and 8.8) and growth pattern as it converts the sterile parental homokaryons (monokaryon) into a fertile heterokaryon (dikaryon). In *Coprinus cinereus* the monokaryon

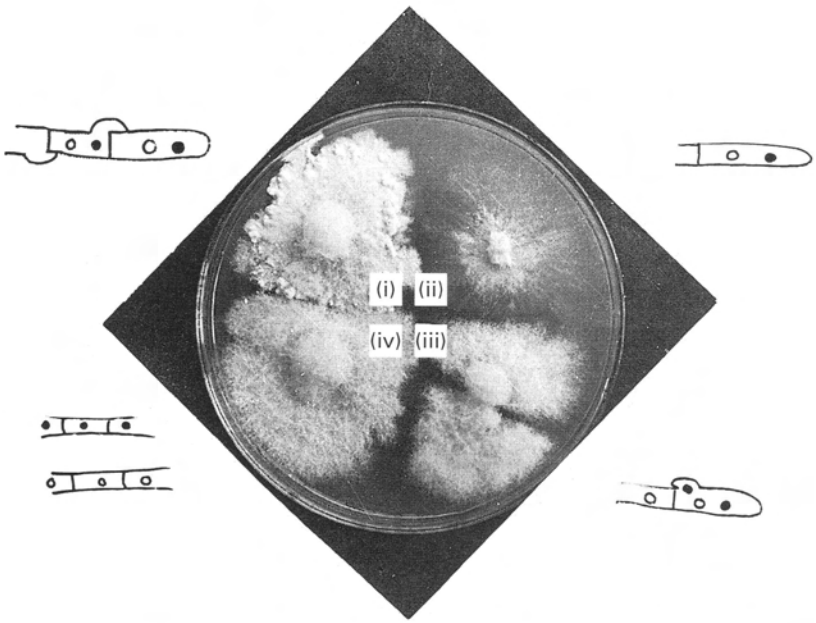


Figure 8.7 Mating reactions in *Schizophyllum commune*. (i) + reaction, compatible 'A different, B different' heterokaryon; (ii) flat reaction, common A heterokaryon; (iii) barrage reaction, common B heterokaryon; (iv) – reaction, common AB heterokaryon.

has uninucleate cells and produces abundant uninucleate arthrospores (oidia). The dikaryon has binucleate cells with characteristic clamp connections (Figure 8.8) at each septum. It does not produce oidia but under appropriate environmental conditions (nutrition, temperature and illumination) it does produce the mushroom fruit bodies. Hyphal fusions occur between any monokaryons (i.e. it is promiscuous, see section: Cytoplasmic compatibility, above), the mating type factors recognize compatibility intracellularly after hyphal fusion. Because of the clear phenotypic differences between homokaryons, heterokaryons and dikaryons the mating type factors have been subjects for classical genetic studies for many years and have recently been subjected to detailed molecular analysis.

C. cinereus and *S. commune* exhibit tetrapolar heterothallism which is determined by two mating type factors, called *A* and *B*. The natural

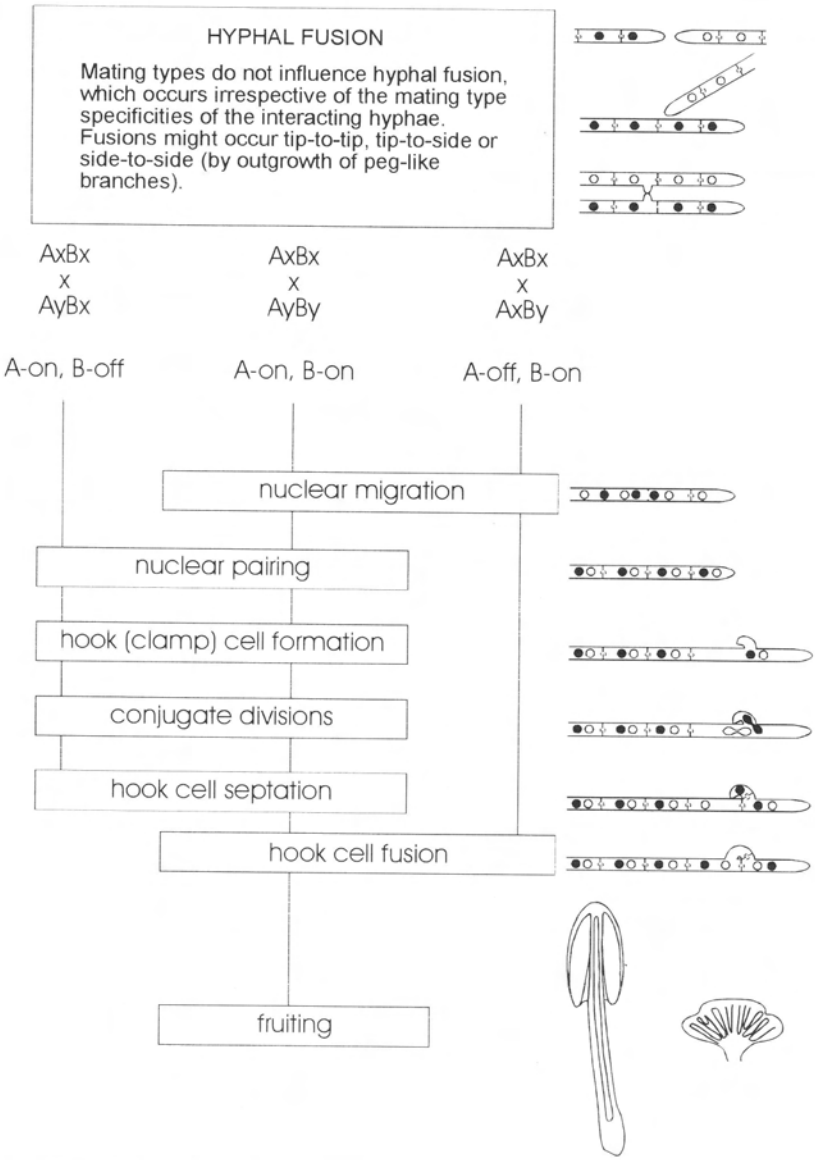


Figure 8.8 Flow chart diagram of A and B mating type factor activity in the homobasidiomycetes *Coprinus cinereus* and *Schizophyllum commune*. See text for details. (Revised and modified after Wendland *et al.*, 1995.)

Coprinus cinereus

Schizophyllum commune

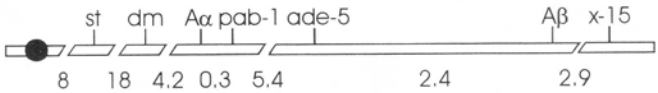


Figure 8.9 Linkage maps of the chromosomes which carry the A mating type factor in *Coprinus cinereus* and *Schizophyllum commune*.

population contains many different mating types which behave in crosses as though they are multiple alleles at the two mating type loci. In fact, each mating type factor is a complex genetic region (which is why we refer to mating type *factors* rather than genes). The mating type factors are located on different chromosomes, and even conventional genetic analysis was able to demonstrate Aα, Aβ, Bα and Bβ subloci, the subloci being relatively far apart in *S. commune* but much closer together in *C. cinereus* (Figure 8.9). The subloci also exhibit multiple allelism and recombination between subloci can generate new mating type specificities.

In both fungi, a compatible mating with the clamp connections (hook) and conjugate nuclei in the mated hyphae requires heterozygosity at both *A* and *B* (A-on, B-on). In terms of cytological observations, mating type factor *A* controls nuclear pairing, clamp cell formation and synchronized (conjugate) mitosis whereas mating type locus *B* controls nuclear migration and clamp cell fusion (Figure 8.8). Nuclei migrate through the existing mycelium of the recipient and this requires breakdown of the septa between adjacent cells of that mycelium. Heterokaryons can also be formed in matings in which one of the mating type factors is common (i.e. homozygous). When the *A* factors are the same (A-off, B-on), nuclear migration occurs but no clamp connections form. A mating between strains carrying the same *B* factor (A-on, B-off) forms a heterokaryon only where the mated monokaryons meet because

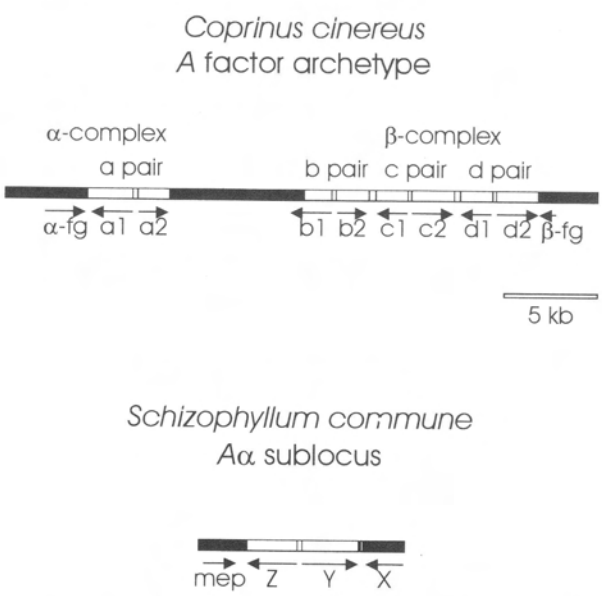


Figure 8.10 Schematic representations of the structures of parts of the A mating type factors in *Coprinus cinereus* and *Schizophyllum commune*. Arrows show the direction of transcription. The (predicted) archetypal A factor from *Coprinus cinereus* has four pairs of functionally redundant genes (a, b, c and d) which feature the homeodomain 1 (HD1 in a1, b1, c1 and d1) and homeodomain 2 (HD2 in a2, b2, c2 and d2) sequences. Interaction between HD1 and HD2 proteins is the basis of the compatible reaction (see Figure 8.11). A factors examined in different strains of *C. cinereus* isolated from nature contain different combinations, and different numbers, of these genes. In *Schizophyllum commune* the mating type genes are called X and Y and carry HD1 and HD2 respectively. Again, different alleles are found in different natural mating types; indeed, the Z gene is absent in the Aα1 mating type. The sequences shown as mep and α-fg are homologous, encoding a metalloendopeptidase; X is a flanking gene of unknown function.

nuclear migration is blocked. Terminal cells of heterokaryotic hyphae initiate clamp connections and nuclei divide but the hook cell fails to fuse with the subterminal cell and its nucleus remains trapped.

Molecular analysis of the *A* mating type factors showed that they are composed of many more mating type genes/gene pairs than the classical genetic analysis revealed (Figure 8.10). These gene pairs encode two

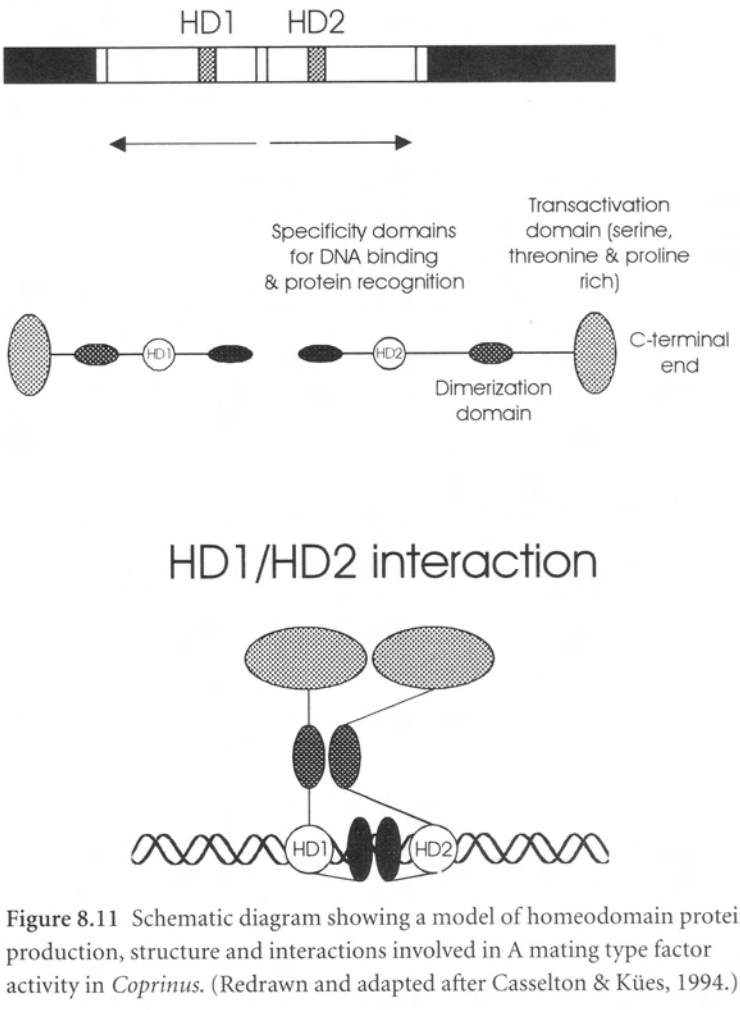


Figure 8.11 Schematic diagram showing a model of homeodomain protein production, structure and interactions involved in A mating type factor activity in *Coprinus*. (Redrawn and adapted after Casselton & Kües, 1994.)

families of proteins (HD1 and HD2) with homeodomain regions which may encode transcriptional factors. The compatibility reaction required for sexual development is triggered by heterodimerization between HD1 and HD2 proteins from the different *A* mating type factors of compatible individuals (Figure 8.11). The N-terminal regions of these proteins are essential for choosing a compatible partner but not for regulating gene transcription.

Although most work has been aimed at determining the structure

of the *A* mating type factors, recently the B sequences have been cloned and sequence analysis has shown that the multiallelic B mating type factor encodes several pheromone and receptor genes which might be involved in controlling the growth of the clamp connection.

Overview of mating type factors

Not all fungi possess mating type genes. In those that do, mating type genes may encode or positively regulate the transcription of pheromone and pheromone receptors involved in some aspect of the mating process (ranging from recognition between sexually competent cells in yeast to governing growth of clamp connections in homobasidiomycetes). Also, heterodimerization of homeodomains from different alleles is employed to trigger further aspects of sexual development and may at the same time repress haploid-specific events. In terms of sequence and functionality, the mating type alleles are usually conserved within the same family (e.g. mating type gene of *Neurospora crassa* in other members of the Sordariaceae). The sequences of the mating type alleles are highly dissimilar and have been interpreted as equivalent to the highly variable region in major histocompatibility loci in mammals as part of a self/non-self recognition system. The N-terminal region of the proteins is essential for such self/non-self recognition while the other region codes for DNA-binding. As some of the mating type loci show a series of linked genes, some of the genes are transcribed in opposite directions to prevent intragenic recombination.

Superior to the simple biallelic system, the functional redundancy of the multiallelic system is clearly to promote outbreeding. For instance, an estimated 28 000 mating types for *Schizophyllum commune* result from the combination of 9, 32, 9 and 9 different specificities found in the world-wide population for Aα, Aβ, Bα and Bβ, respectively. In *Saccharomyces cerevisiae*, the mating type genes are known to act as master genes (producing transcription factors) controlling the expression of multiple genes (downstream regulation) which then impact on the sexual development pathway such as fertilization and sporulation. However, in *Podospora*, the meiocyte pathway (meiosis and sporulation) does not require heterozygous mating type alleles. In addition the formation of apparently normal fruiting bodies by haploid cultures is not

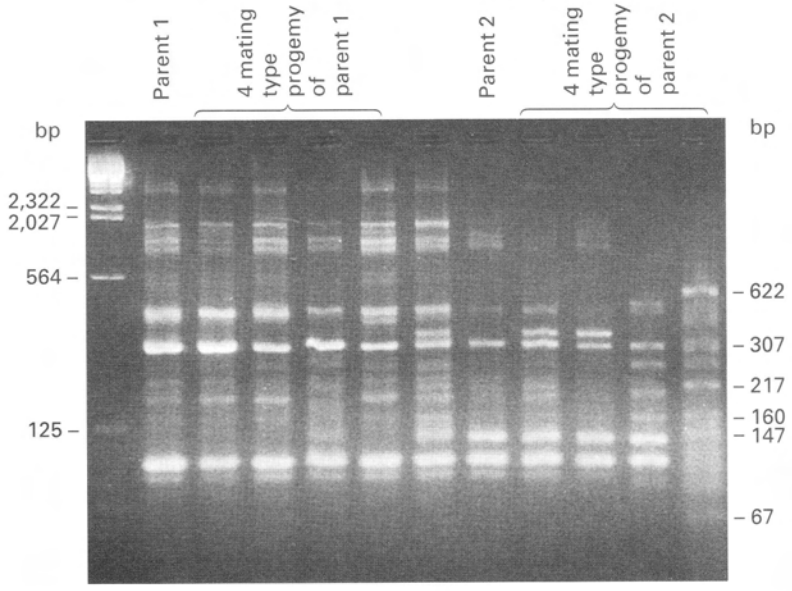


Figure 8.12 Segregation of DNA markers generated by the polymerase chain reaction in the sexual progenies of the basidiomycete, *Lentinula edodes* which is a tetrapolar heterothallic species. Some of the DNA bands are found in both parent and progeny while some bands are polymorphic (present/absent in/from some of the progeny).

uncommon in fungi and fruit body formation can usually be separated from other parts of the sexual pathway by mutation (discussion in Moore, 1994, 1998). Therefore, the significance of mating type factors in regulating events beyond the initial mating reaction is uncertain. Even the target genes within that mating reaction are unknown and it is also uncertain how the products of the multiple genes which make up the complex mating type factors avoid producing active multimers in unmated mycelia. Much remains to be learned about these mating type factors.

The sexual cycle

The outcome of mating is the recombination and segregation of the genetic elements which were brought together (Figure 8.12). The progeny have new genotypes but to achieve the expression of these the nuclei must

be 'packaged' into progeny spores which can be distributed into the environment.

Sporulation in higher fungi

Unlike most animals and plants, after mating a persistent and independent heterokaryotic or diploid phase exists in fungi. Only under particular environmental triggers is the sexual cycle initiated. Taking a filamentous ascomycete as an example: hyphal fusion or similar mating between male and female structures results in nuclei moving from the male into the female to form an ascogonium in which male and female nuclei may pair but do not fuse (dikaryon). Ascogenous hyphae grow from the ascogonium. Most cells in these hyphae are dikaryotic, containing one maternal and one paternal nucleus, the pairs of nuclei undergoing conjugate divisions as the hypha extends. In typical development, the ascogenous hypha bends over to form a crozier. The two nuclei in the hooked cell undergo conjugate mitosis and then two septa are formed, creating three cells (Figure 8.13). The cell at the bend of the crozier is binucleate but the other two cells are uninucleate. The binucleate cell becomes the ascus mother cell, in which karyogamy takes place. In the young ascus meiosis results in four haploid daughter nuclei, each of which divides by mitosis to form the eight ascospore nuclei (Figure 8.13). Formation of ascospores results from the infolding of membranes around the daughter nuclei so that eight ascospores are delimited. A spore wall then forms around each ascospore. Ascus cytoplasm left outside the spores, called the epiplasm, may provide nutrients to the maturing spores, contribute to the outer layers of the spore wall, or contribute to the osmotic potential of the ascus to aid subsequent spore discharge. Ascus and ascospore morphogenesis has been reviewed by Read and Beckett (1996).

Typically, eight uninucleate and haploid ascospores are formed, though there are variations on this theme. In some species a further mitotic division forms binucleate spores, in others the immature spores become multinucleate prior to being divided up by septa. Some ascomycetes form only four ascospores as a result of the spore membranes enclosing a pair of nuclei rather than just one nucleus, while others may produce fewer spores in each ascus as a result of nuclear disintegration or spore abortion.

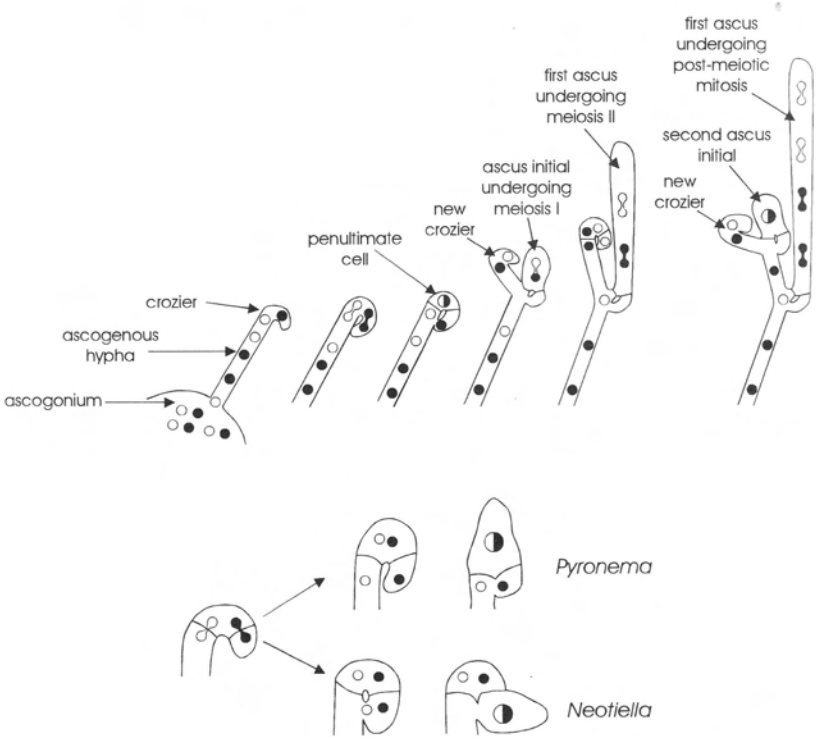


Figure 8.13 Diagram of ascus formation, as described in the text (adapted and redrawn from Webster, 1980). Karyogamy may occur in the penultimate cell of the crozier (top diagram and *Pyronema* in the lower diagram) or the terminal or stalk cell (*Neotiella* in the lower diagram, which is adapted and redrawn from Read and Beckett, 1996).

In basidiomycetes, karyogamy and meiosis take place in the basidium and basidiospores (usually four) are produced externally on outgrowths of the basidial wall which are called sterigmata (Figure 8.14). The walls of the sterigma and of the early spore initial are continuous and homologous. Starting with the spherical growth of the spore initial and nuclear migration into the maturing spore, a basidiospore grows further to attain the species-specific form and dimension, and the spore protection is increased by further wall layers formed with/without pigmentation, ornamentation and germ pore.

All of these spore formation processes include steps which require precise nuclear positioning and/or active nuclear movement. Various

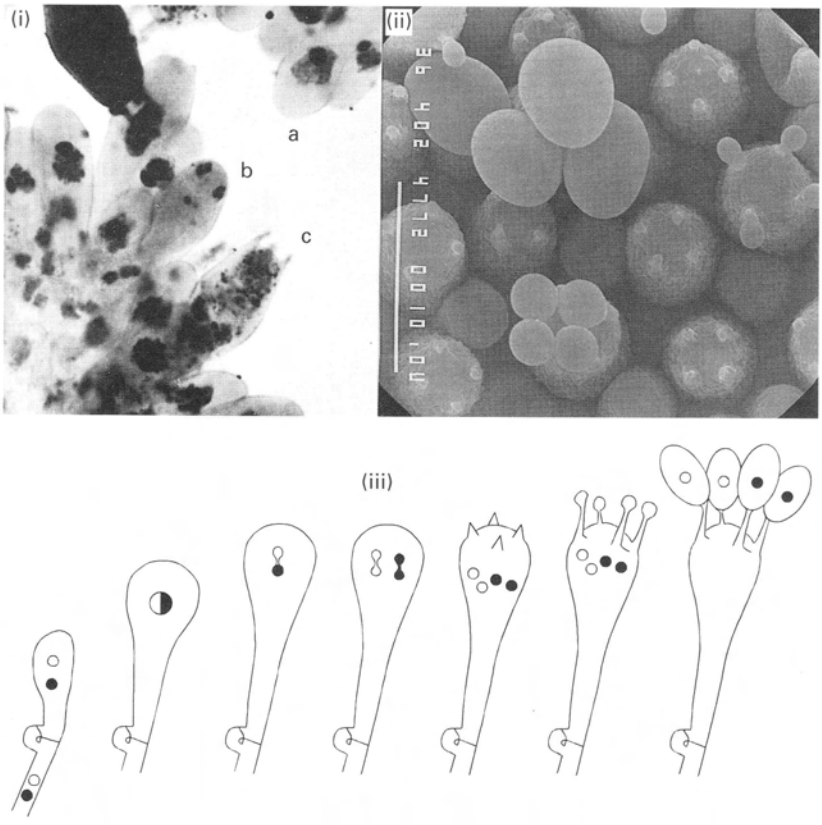


Figure 8.14 Meiosis and sporulation in the homobasidiomycete, *Volvariella bombycina*. (i) Light micrographs showing meiosis. a, prophase I; b, meiosis II; c, interphase II and basidium with sterigmata; (ii) scanning electron micrograph showing the initiation of sterigmata in the final spore maturation process; (iii) diagram of basidium formation: in a 'classic' homobasidiomycete the basidium arises as the terminal cell of a dikaryotic hyphal branch (see also Figure 8.16) which inflates and undergoes karyogamy and meiosis. At the conclusion of the meiotic division four outgrowths (sterigmata) emerge from the basidial apex and inflation of each sterigma tip produces the basidiospore (which is an exospore, produced outside the meiocyte in contrast to the endospores of ascomycetes). Nuclei then migrate from the basidium into the newly formed basidiospores. Mitosis may take place within the basidiospores before they are discharged. Comparison of this diagram with Figure 8.13 will indicate readily how tempting it is to suggest some evolutionary relationship between crozier formation and the early stages of basidium and clamp connection formation.

cytoskeletal structures have been associated with nuclear division, nuclear migration and cytokinesis. Benomyl-resistant (*ben*) mutants of *Coprinus cinereus* have defects in structural genes for α- or β-tubulin. The *ben* mutants were blocked in migration of nuclei during formation of dikaryotic hyphae with clamp cells, but migration of nuclei into developing spores was unaffected. This may indicate that there are at least two nuclear movement systems, only one being dependent on tubulin. A key functional difference may be that microtubules are involved in the pairing of the two conjugate nuclei in the dikaryon.

The spindle pole body (the centrosome in other organisms; SPB in abbreviation) shows a duplication cycle in meiosis and mitosis. Using mutant analysis, it has been deduced that structural modification of SPB in meiosis II is required to nucleate the endospore-wall formation in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Esposito and Klapholz, 1981). In homobasidiomycetes, the SPB always leads the daughter nucleus migrating into the maturing basidiospore.

Meiosis

Although there are some inevitable modifications, karyogamy and meiosis in heterothallic fungi go through stages fairly typical for eukaryotic haploids. In particular the major round of DNA replication is premeiotic, occurring before karyogamy. Indeed, it was research with the ascomycete *Neottiella* which first demonstrated this aspect of meiosis (Westergaard and von Wettstein, 1970). Meiosis takes place in the meiocytes (basidia or asci) and chromosome behaviour in meiosis follows the Mendelian laws of segregation, independent assortment, linkage and crossing over.

Until recently meiosis has been described primarily as comprising the sequence synapsis, recombination and segregation. It is now known that chromosome pairing and synapsis are distinct processes in terms of both mechanism and timing. Premeiotic DNA replication and formation of the synaptonemal complex are independent events. Synapsis does not require DNA homology. At prophase I, homologous chromosomes are aligned prior to appearance of tripartite synaptonemal complex, and at this time double-strand breaks (providing sites for meiotic recombination) usually occur. The fission yeast, *Schizosaccharomyces pombe*, has

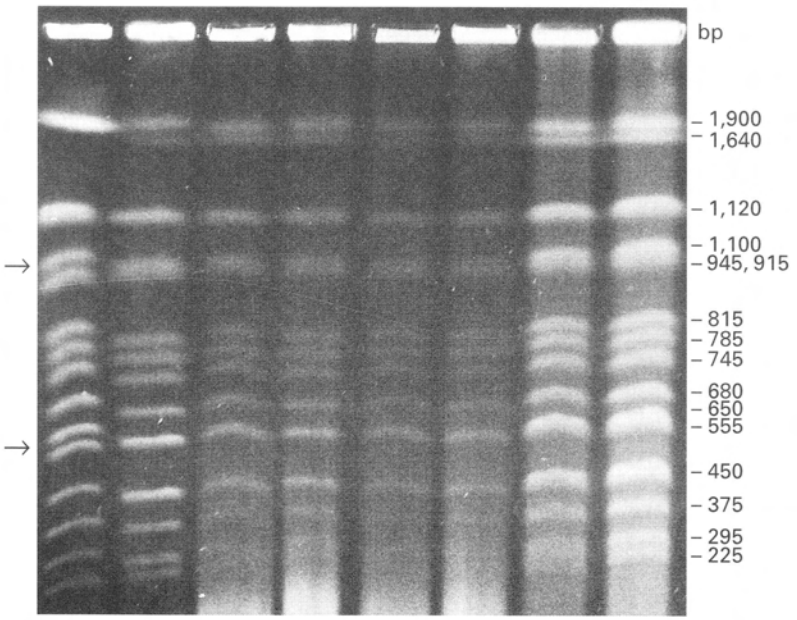


Figure 8.15 Electrophoretic karyotypes of two strains of *Saccharomyces cerevisiae* revealed by pulsed field gel electrophoresis showing chromosomal length polymorphisms. Arrows point to the polymorphic chromosomal DNA bands.

normal levels of meiotic recombination but does not make any synaptonemal complex, though structures resembling the axial core of the synaptonemal complex do occur. Mutants of *Saccharomyces cerevisiae* defective in synaptonemal complex structure showed defects in chromosome condensation but still underwent some meiotically induced homology pairing, revealing the independence of homology pairing and meiotic recombination. In meiotic recombination, heteroduplex DNA (hDNA), which refers to the hybrid DNA formed following strand exchange and with one or more mismatched base pairs, is an essential intermediate. The frequency of homologous recombination is 100- to 1000-fold higher during meiosis than during mitosis.

The synaptonemal complex, if formed, converts several sites of alignment and exchange into a functionally intact bivalent, consisting of kinetochores, which are sites for attachment to fibres of the division

spindle, and the four strands connected by chiasmata. Chiasmata are the cytologically visible connections between homologous chromosomes which have long been regarded as being equivalent to genetic cross-overs formed in the recombination process. Yet the number of chiasmata observed in meiosis of some organisms is much less than that of cross-over events deduced from progeny analysis, so recombination events and chiasmata may not be numerically equivalent. Rather, chiasmata may be only those cross-overs which are required to balance mechanical forces exerted by the division spindle on the kinetochores to ensure that homologues move away from each other in the first meiotic division (Hawley and Arbel, 1993; Moens, 1994).

Mutants defective in DNA repair have been demonstrated to have defects in meiotic chromosome condensation, synapsis and recombination in meiosis, perhaps culminating in defects such as formation of non-viable spores or abortion in sporulation. Using mutant analysis, it has been found that recombination events are not sufficient in themselves to ensure disjunction (Hawley and Arbel, 1993; Pukkila, 1994). On the other hand, experiments with artificially constructed chromosomes in *Saccharomyces cerevisiae* have shown that neither chromosome size nor homologous DNA sequence had much effect on chromosome disjunction. The crucial factor was that when a cross-over occurred the chromosomes nearly always disjoined at meiosis I. Clearly, much more work needs to be done to establish the precise relationships between, on the one hand, synapsis, sequence homology and synaptonemal complex formation, and on the other hand between molecular recombination events, genetic cross-overs and chiasmata.

Duplicated DNA sequences in *Neurospora crassa* are extensively point mutated during the sexual cycle by a process known as repeat-induced point mutation (RIP)(Davis, 1995). The effect occurs at high frequency during pre-meiosis; 50 to 100 per cent of linked, direct duplications can be RIPed and many deleted as well. RIP and recombination are correlated but not interdependent, indicating that the two processes are distinct but perhaps one provokes the other. Although the likely function of RIP is to protect against the potential instability in chromosomal interactions which direct duplications threaten, the effect can extend for one to four kilobases from the duplication boundary. Single-copy

sequences adjacent to a duplicated sequence are consequently more prone to mutation. Although other fungi detect and modify (by methylation) duplicated sequences, RIP is only known to occur in *Neurospora.*

Unlike most plants and animals, fungi carry out meiosis with the nuclear membrane remaining intact in prophase I. Meiosis I is a reductional division and meiosis II, an equational division. The meiotic II division is mitosis-like, sharing the same machinery with mitosis. At least in *Saccharomyces cerevisiae* following proper environmental triggers, the a1/α2 heterodimer activates IME1 (Inducer of Meiosis) to synthesize a transcriptional factor which, in turn, switches on various meiotic genes (Figure 8.3). Yeast is the only fungus for which the physiological, biochemical and molecular controls of meiosis are sufficiently well known for some understanding to emerge; it may or may not be representative.

In fungi, the leptotene of prophase I may be very brief or even absent. Also, in contrast to plant and animal systems, fungal karyotype analysis is usually done at the pachytene stage when chromosomes are paired and appear as long and thick threads rather than at metaphase I because fungal chromosomes are usually so small at the latter stage. Lu (1993) developed a method of spreading and staining chromosomes with silver nitrate which greatly improves conventional examination of synaptonemal complex and chromosomal rearrangements by both light and electron microscopy. Fluorescence *in situ* hybridization (FISH technique or 'chromosome painting') has been applied to chromosome spreads of *Saccharomyces cerevisiae.* With a probe derived from the homologous genomic library, the technique allows the study of specific individual chromosomes during meiosis. The karyotypes of most fungi can be resolved electrophoretically by pulsed field gel electrophoresis (PFGE) and Southern hybridization. Southern hybridization with homologous probes can establish the ploidy levels of different isolates and reveal gene amplification during differentiation. This technique can also reveal the loss of supernumerary chromosomes, which are usually less than one million base pairs in size and are dispensable, and generation of novel-sized chromosomes (chromosome-length polymorphisms; Zolan, 1995). Chromosome length polymorphisms are widespread in both sexual and asexual species, revealing a general genome plasticity (Figure 8.15). Tandem repeats, e.g. repeats of rRNA genes, frequently vary in length and

dispensable chromosomes and dispensable chromosome regions also occur. Many karyotype changes are genetically neutral, others may be advantageous in allowing adaptation to new environments (Zolan, 1995).

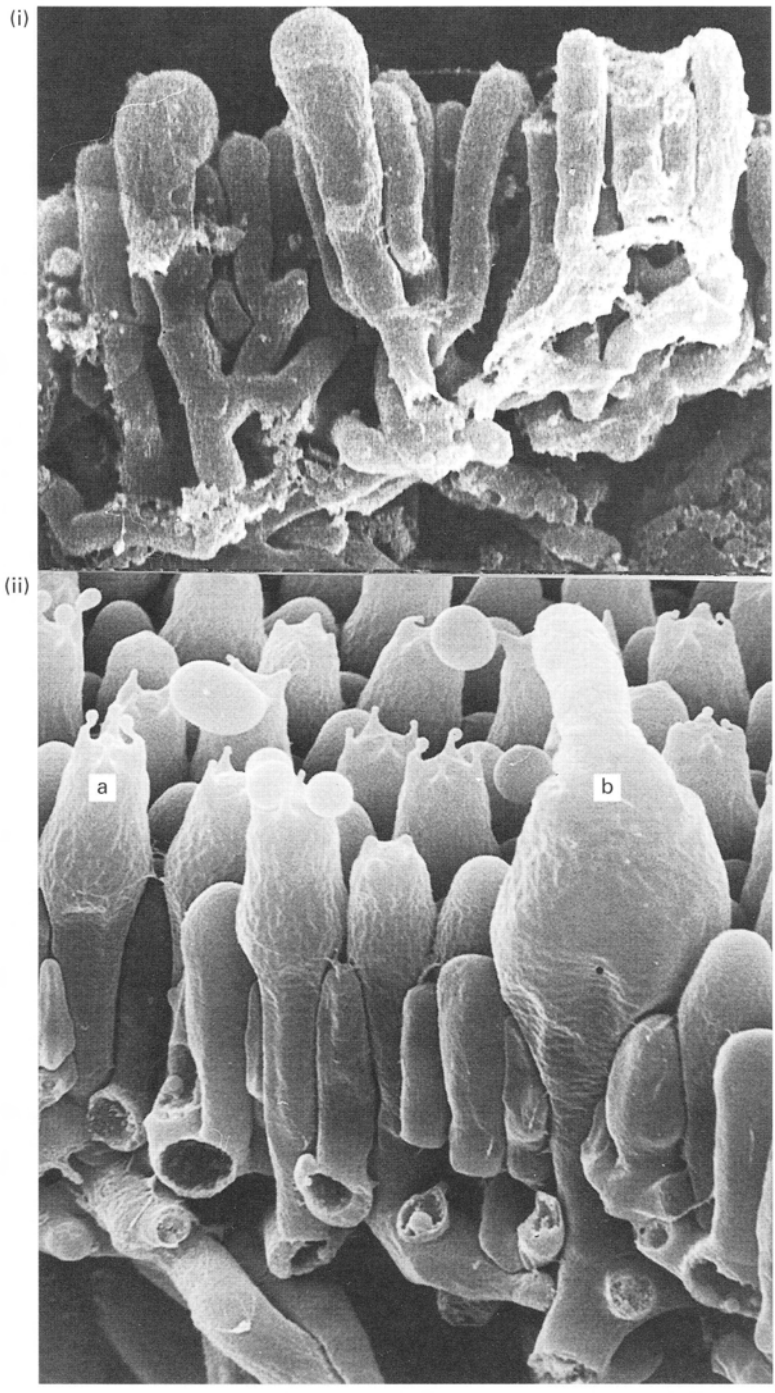
Spreading it around

In yeasts producing naked asci the mother cell becomes the ascus. There is no specialized dispersal apparatus and the ascospores formed endogenously are simply released by rupture of the ascus (mother cell) wall. Yeasts are not alone in producing 'naked' asci, but the majority of ascomycetes produce their asci in multicellular fruiting bodies called ascomata (see next section). Basidiomycetous yeasts also lack a fruiting body and after meiosis produce basidiospores by budding (sporidia; see section: Mating type factors in *U. maydis* as an example) or show pleomorphism, forming pseudohyphae or true hyphae and bearing naked basidia, e.g. *Cryptococcus neoformans.*

Fungal multicellular structures – ascomata and basidiomata

Macroscopic fungal structures are formed by hyphal aggregation. When seen in microscope sections fungal tissues appear to be comprised of tightly packed cells and often resemble plant tissue (Figure 8.16). It is crucial to remember, though, that fungal tissue is made up of a community of hyphae. Primary septa in fungal hyphae usually have a pore which may be elaborated with the parenthesome apparatus in most basidiomycetes or be associated with Woronin bodies in ascomycetes; in either case the movement or migration of cytoplasmic components between neighbouring compartments is under effective control. So the hypha is separated into compartments which can exhibit contrasting patterns of differentiation. So fungi can quite reasonably be considered to be cellular organisms able to produce differentiated tissues comprised of cells which derive from an initial cell community which is induced to start

Figure 8.16 Scanning electron micrographs of hyphal tissues in the fruit body of *Volvariella bombycina.* (i) Highly branched hyphae in the gill at a young stage; (ii) the closely packed hymenium with hyphal tips differentiated into different cell types (a, basidium; b, cystidium).


(i)
(ii)
a
b

multiplication and differentiation. Nevertheless, there are fundamental differences between the fungal cell concept and one that might be applied to plants because the hypha grows only at its tip and cross walls form only at right angles to the long axis of the hypha. Consequently, fungal morphogenesis depends on hyphal branching. Proliferation in communities of fungal hyphae requires branching, and formation of a particular structure demands that the position of branch emergence and direction of growth are controlled (Moore, 1994, 1996, 1998; Watling and Moore, 1994; Chiu and Moore, 1996). Unfortunately, the cellular machinery involved in creating a new hyphal tip in a new lateral branch and determining its position, orientation and direction of outgrowth from the parent hypha is completely unknown at present.

Making ascomata

Ascogenous hyphae may branch repeatedly to form clusters of asci. Often the crook cell elongates into a new crozier rather than becoming an ascus mother cell immediately, and the tip and basal cells of the crozier fuse, producing another dikaryotic crozier beside the first (Figure 8.13). As a result, asci may arise singly, scattered within the fruiting body or may be in 'bunches' (called a fascicle). When asci form a distinct layer it is called a hymenium. Sterile hyphae interspersed between the asci are called paraphyses (singular paraphysis) and aid ascospore discharge.

Most ascomycetes form asci in a fruiting body called an ascoma (plural ascomata). Ascomata might be completely closed (a cleistothecium); more or less closed, but with an opening (ostiole) through which the ascospores escape when mature (a perithecium); completely open (an apothecium); or a cavity (locule) within a larger mass of tissue called a stroma (the whole structure is called an ascostroma or pseudothecium) (Figure 8.17). In some ascomycetes, an 'embryonic' ascoma develops before mating and the sexual organs are formed from hyphae within the developing ascoma (e.g. the protoperithecium of *Neurospora crassa*). In others, mating stimulates ascoma development by prompting mycelial hyphae around the ascogonium to grow and branch to generate the tissues of the ascoma.

In both cases, the ascoma develops from hyphae (most often maternal hyphae) that have not been involved in the mating process. Thus

Figure 8.17 Line drawings showing construction patterns of some ascomata in the form of simplified diagrammatic sectional drawings (redrawn after Burnett, 1968); in each case the hymenial tissue is represented by the black line.

formation of the ascoma and coordination of its formation with sexual reproduction must involve important signalling processes between the different hyphal populations involved; nothing is known about the nature of these signals (Novak Frazer, 1996).

Making basidiomata

The fruiting bodies of Basidiomycotina, the mushrooms, toadstools, bracket fungi, puff-balls, stinkhorns, bird's nest fungi, etc., are all examples of basidiomata (singular basidioma) which bear the sexually produced basidiospores on basidia. Simplified diagrammatic drawings of some of the different types of basidiomata are shown in Figure 8.18. The majority of these fruit bodies, which vary in size from a few milligrams to tens of kilograms fresh weight, are modifications of the basic theme of an umbrella-shape, made up of a cap on top of a stem, so that the spore-release

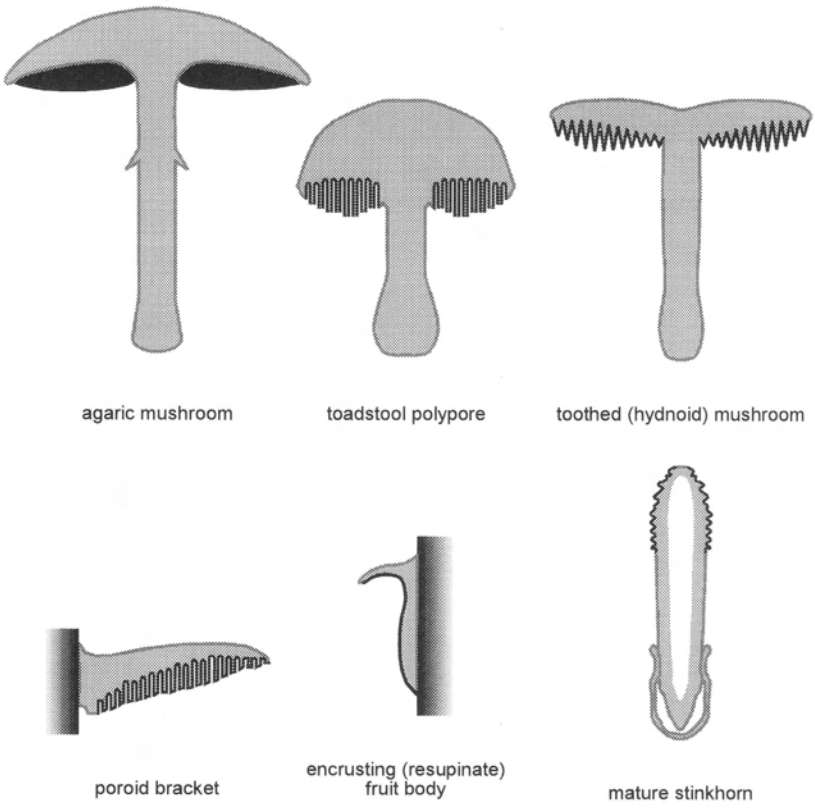


Figure 8.18 Line drawings showing construction patterns of basidiomata in the form of simplified diagrammatic sectional drawings with the hymenial tissue represented by a black line (redrawn after Burnett, 1968).

mechanism is protected from rain (Watling and Moore, 1994; Watling, 1996).

The tissue patterns in these structures are established very early in development. In *Coprinus cinereus*, fruit body initials only 800 μm tall, are clearly differentiated into cap and stem though this is only 1 per cent of the size of a mature fruit body (Moore, 1994, 1996). Examples like this bring to mind how early the basic body plan is established during development of an animal embryo and the concept of mushroom 'embryology' can be used to show that processes known to occur during animal embryo development have their analogues during basidioma development. These

include formation of inhomogeneous cell (hyphal) populations from homogeneous ones; regional specification of tissues (pattern formation) directed by organizers producing morphogens; specification and commitment of particular cells to particular fates; cell differentiation; and regulation of gene activity in ways specifically geared to morphogenesis. All of these processes are so well researched in animals (and, increasingly, in plants too); that other great Kingdom, Fungi, is a fertile area for research. So while the occurrence of these processes during fungal morphogenesis may be implied by, or can be inferred from, indirect observations, there are very few specific examples of research aimed directly at understanding how fungal multicellular structures develop (Chiu and Moore, 1996). Taxonomically, families are characterized by specific morphological features of fruit bodies, implying strict temporal and spatial control of morphogenesis. However, basidiome plasticity is also well known (FIGURE 8.19) showing that the developmental programme has great flexibility to adapt. The key function of the multicellular structure, regardless of its form, is to ensure spore dispersal even under stress.

Conclusions

In the sections above we have given some indication of the diversity of sexual behaviour in fungi. At one extreme of the spectrum of behaviour are fungi which are completely asexual organisms (e.g. the deuteromycetes) but which nevertheless generate variation by modifying genetic expression or adapting mitotic processes to produce recombinants or segregants as asexual propagules. At the other extreme are the diverse forms of sexuality, ranging from bipolar (unifactorial) incompatibility systems to (bifactorial) incompatibility systems, with mating type switching possible in some organisms. The uniqueness of fungi is the possession of complex multiallelic system in the mating type gene and the redundancy of linked functional mating type genes in an incompatibility locus. The highest expression of this is in the basidiomycetes with mating type systems which can generate thousands of compatibility genotypes, producing multicellular fruiting structures capable of releasing hundreds of millions of basidiospores.

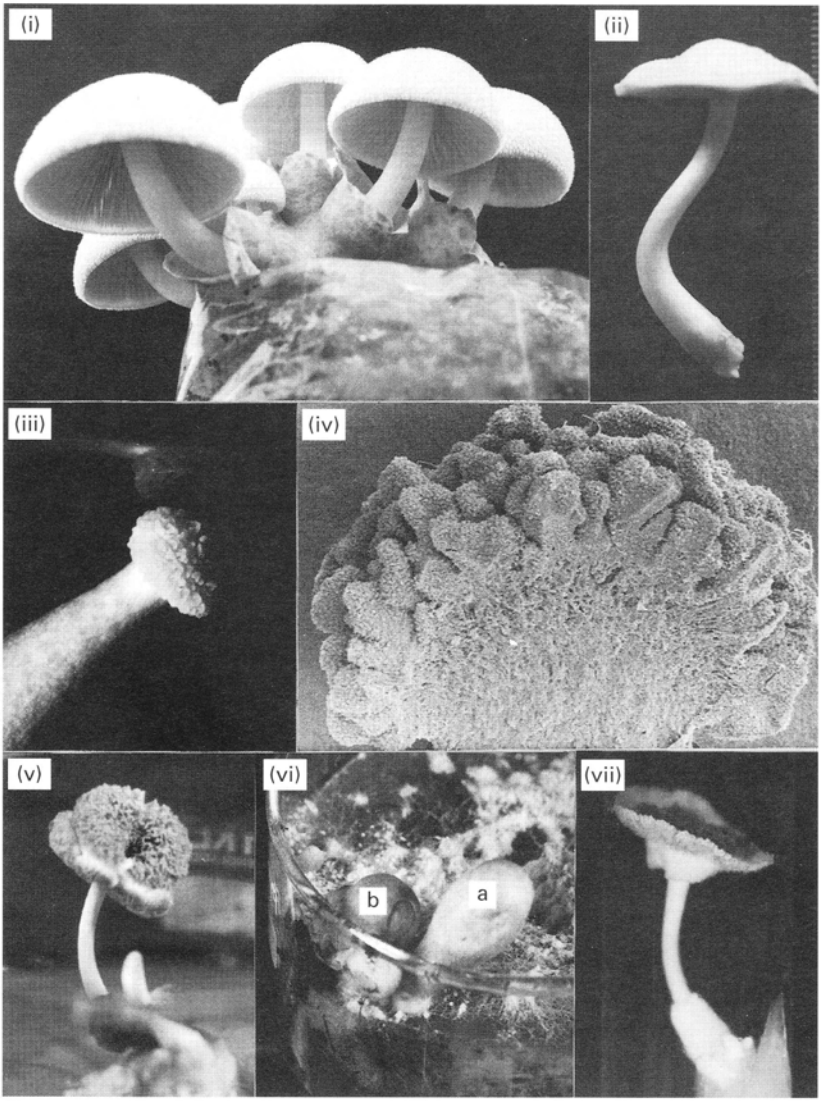


Figure 8.19 Spontaneous basidiome polymorphism in the basidomycete *Volvariella bombycina.* (i) Normal mushroom showing the genus-specific basal volva; (ii) spontaneous fruit body variant without volva; (iii) morchelloid fruit body variant; (iv) scanning electron micrograph of the longitudinal section of the morchelloid fruit body showing the convoluted hymenium without the formation of cap; (v) a fruit body variant with additional hymenium; (vi) a gasteroid fruit body variant (a) with gill maturation completed without rupture of the universal veil, next to a fruit body variant (b) which developed a hole in the universal veil early in development; (vii) a fruit body variant with inverted cap.

Most fungi have polyphasic life cycles, consisting of various phases such as haploid (N), dikaryotic (N + N) (or heterokaryotic with the two nuclear types in random ratio) and diploid (2N). In most species, reproduction is both sexual and asexual. Under a stable environment, haploidy and diploidy show equal selective advantage (Jenkins, 1993). The prolonged dikaryotic or heterokaryotic stage, however, may show hybrid vigour and can segregate into homokaryons if adverse selection pressure is imposed. Meanwhile, the cost (in metabolism and cellular resource) of producing a multicellular fruiting structure is in most cases greater than that of the simple conidiophore (for example) used for asexual reproduction. A sexual or asexual propagule, once separated from the parent, restarts the life developmental programme again. Thus, it seems that the best strategy to be adopted by fungi is to produce some offspring sexually for generating novel genotypes to adapt the unpredictable and fluctuating environment, and the rest asexually to rapidly colonize the favourable environment (once it is found) as well as to establish territorial control in a competitive world.

The mating type genes are involved in outcrossing and sexual development. The advantages of sexual reproduction include, obviously, the generation of genetic variation, but less obviously the process offers an escape from DNA parasites and a means to repair DNA damage. Sex may, therefore, be an important means to enhance the overall rate of adaptation (Hurst and Peck, 1996).

The beauty of a multicellular fruiting structure is not only the amplification in production of meiotic progeny widely differed in genetic make-up, but also the protection of the spore production against the fluctuating environment as well as the adaptation of the fungus towards its habitat. Regardless of its form, then, the multicellular fruiting structure, serves the same functions: to protect, to produce and to maximize the dispersal of the meiotic progeny for species propagation. In some cases, such as gasteromycetes bearing enclosed fruit bodies and hemiascomycetes producing naked asci, the spores are liberated by the 'mother structure' breaking open. In other cases the fruit body may be a complex mechanism which actively contributes to spore dispersal.

Rain splash is employed for spore dispersal in the bird's nest fungi in which the fruit body is adapted to redirect incoming raindrops so that

they pick up the propagules and splash out, well away from the parent fruit body. Aquatic fungi, such as *Saprolegnia* produce flagellated zoospores which are able to swim through the water to find new territory. Insects are important vectors for some fungi. For instance, the stinkhorn autolyses after spore maturation to produce a sugary, sticky, stinking solution together with the spore mass to attract flies, which then take the sticky fungal spores with them when they leave.

Other fungal spores are ornamented making them rough or hooked which may be used for surface attachment or the outer layers of their walls may be wet and sticky to ensure adhesion to the surface. Active discharge of spores is widespread in fungi. Ascospores of many ascomycetes are forcibly discharged from the ascus by a pressurised extrusion process. In many basidiomycetes the basidiospores are actively discharged as ballistospores. The mechanism of this discharge has been a topic of hot debate for most of this century. It now appears that as a ballistospore matures, hygroscopic substances (including mannitol) are secreted at the base of the spore, causing condensation of water from the atmosphere to collect into a drop ('Buller's drop'). When this drop grows to a critical size it finally attempts to spread over the spore but the hydrophobicity of the wall amplifies surface tension and causes the spore to recoil away from the advancing liquid and lift off into the air, each successfully discharged spore representing a triumph of surface tension over gravity.

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References and further reading

Bernstein, H., Byerly, H. C., Hopf, F. A. and Michod, R. E. (1985). Genetic damage, mutation and the evolution of sex. *Science* 229:1277–1281.

Birdsell, J. and Wills C. (1996). Significant competitive advantage conferred by meiosis and syngamy in the yeast *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA* 93:908–912.

Bourne, A. N., Chiu, S.-W. and Moore, D. (1996). Experimental approaches to the study of pattern formation in *Coprinus cinereus.* Pp. 126–155 in S.-W. Chiu and D. Moore, eds. *Patterns in Fungal Development.* Cambridge University Press, Cambridge, UK.

Burnett, J. H. (1968). *Fundamentals of Mycology.* Edward Arnold, London.

Carlile, M. J. (1987). Genetic exchange and gene flow: their promotion and prevention. Pp. 203–213 in A. D. M. Rayner *et al.*, eds. *Evolutionary Biology of the Fungi.* Cambridge University Press, Cambridge, UK.

Carlile, M. J. and Watkinson, S. C. (1994). *The Fungi.* Academic Press, London.

Casselton, L. A. and Kües, U. (1994). Mating-type genes in homobasidiomycetes. Pp. 307–321 in J. G. H. Wessels and F. Meinhardt, eds. *The Mycota, vol. I, Growth, Differentiation and Sexuality.* Springer-Verlag, Berlin.

Chiu, S.-W. (1996). Nuclear changes during fungal development. Pp. 105–125 in S.-W. Chiu & D. Moore, eds. *Patterns in Fungal Development.* Cambridge University Press: Cambridge, UK.

Chiu, S.-W. and Moore, D. (eds.) (1996). *Patterns in Fungal Development.* Cambridge University Press, Cambridge, UK.

Davis, R. H. (1995). Genetics of *Neurospora.* Pp. 3–18 in U. Kück, ed. *The Mycota,* vol II, *Genetics and Biotechnology.* Springer-Verlag, Berlin.

Dawkins, R. (1976). *The Selfish Gene.* Oxford University Press, Oxford.

Elliott, C. G. (1994). *Reproduction in Fungi. Genetical and Physiological Aspects.* Chapman & Hall, London.

Esposito, R. E. and Klapholz, S. (1981). Meiosis and ascospore development. Pp. 211–287 in J. N. Strathern *et al.*, eds. *The Molecular Biology of the Yeast* Saccharomyces cerevisiae, vol. 1, *Life Cycle and Inheritance.* Cold Spring Harbor Laboratory Press, New York.

Hawley, R. S. and Arbel, T. (1993). Yeast genetics and the fall of the classical view of meiosis. *Cell* 72:301–303.

Hurst, L. D. and Peck, J. R. (1996). Recent advances in understanding of the evolution and maintenance of sex. *Trends Ecol. Evol.* 11:79–82.

Jenkins, C. D. (1993). Selection and the evolution of genetic life cycles. *Genetics* 133:401–410.

Kämper, J., Bölker, M. and Kahmann, R. (1994). Mating-type genes in Heterobasidiomycetes. Pp. 323–332 in J. G. Wessels and F. Meindhardt, eds. *The Mycota,* vol. I, *Growth, Differentiation and Sexuality.* Springer-Verlag, Berlin.

Lu, B. C. (1993). Spreading the synaptonemal complex of *Neurospora crassa*. *Chromosoma* 102:464–472.

Maynard Smith, J. (1978). *The Evolution of Sex*. Cambridge University Press, Cambridge, UK.

Metzenberg, R. L. (1990). The role of similarity and difference in fungal mating. *Genetics* 125:457–462.

Metzenberg, R. L. and Glass, N. L. (1990). Mating type and mating strategies in *Neurospora*. *BioEssays* 12:53–60.

Metzenberg, R. L. and Randall, T. A. (1995). Mating type in *Neurospora* and closely related ascomycetes: some current problems. *Can. J. Bot.* 73(Suppl. 1):S251–S257.

Moens, P. B. (1994). Molecular perspectives of chromosome pairing at meiosis. *BioEssays* 16:101–106.

Moore, D. (1994). Tissue formation. Pp. 423–465 in N. A. R. Gow & G. M. Gadd eds. *The Growing Fungus*. Chapman & Hall, London.

Moore, D. (1996). Inside the developing mushroom: cells, tissues and tissue patterns. Pp. 1–36 in S.-W. Chiu and D. Moore, eds. *Patterns in Fungal Development*. Cambridge University Press, Cambridge, UK.

Moore, D. (1998). *Fungal Morphogenesis*. Cambridge University Press, Cambridge, UK.

Novak Frazer, L. (1996). Control of growth and patterning in the fungal fruiting structure. A case for the involvement of hormones. Pp. 156–181 in S.-W. Chiu and D. Moore, eds. *Patterns in Fungal Development*. Cambridge University Press, Cambridge, UK.

Pukkila, P. J. (1994). Meiosis in mycelial fungi. Pp. 267–281 in J. G. H. Wessels and F. Meinhardt, eds. *The Mycota, vol. I, Growth, Differentiation and Sexuality*. Springer-Verlag, Berlin.

Read, N. D. and Beckett, A. (1996). Centenary review. Ascus and ascospore morphogenesis. *Mycol. Res.* 100:1281–1314.

Schmidt, H. and Gutz, H. (1994). The mating type switch in yeasts. Pp. 283–294 in J. G. H. Wessels and F. Meinhardt, eds. *The Mycota, vol. I, Growth, Differentiation and Sexuality*. Springer-Verlag, Berlin.

Watling, R. (1996). Patterns in fungal development – fruiting patterns in nature. Pp. 182–222 in S.-W. Chiu and D. Moore, eds. *Patterns in Fungal Development*. Cambridge University Press, Cambridge, UK.

Watling, R. and Moore, D. (1994). Moulding moulds into mushrooms: shape and form in the higher fungi. Pp. 270–290 in D. S. Ingram and A. Hudson, eds. *Shape and Form in Plants and Fungi*. Academic Press, London.

Webster, J. (1980). *Introduction to Fungi*, Second edition. Cambridge University Press, Cambridge, UK.

Wendland, J., Vaillancourt, L., Hegener, J. *et al.* (1995). The mating-type locus Bα1 of *Schizopyllum commune* contains a pheromone receptor gene and putative pheromone genes. *EMBO J.* 14:5271–5278.

Westergaard, M. and von Wettstein, D. (1970). Studies on the mechanism of crossing over. IV. The molecular organization of the synaptinemal complex in *Neottiella* (Cooke) Saccardo (Ascomycetes). *Comtes Rendus des Travaux du Laboratoire Carlsberg* 37:239–268.

Zolan, M. E. (1995). Chromosome-length polymorphism in fungi. *Microbiol. Rev.* 59:686–698.