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Chapter 2: Genome interactions

2.1 Fungal life styles: hyphal fusions are the key to advanced hyphal systems

Fungi differ from most other eukaryotes in that their vegetative body, the thing that grows as an individual and gives them body mass, shows indeterminate growth. Fungal mycelia will continue to grow and invade new substrates for as long as new substrates remain available and growth conditions remain satisfactory. Now, you could take that statement to mean that fungi would just keep on growing until they became enormous, and that does happen in some cases. The largest known organism on Earth is a tree root pathogen known as *Armillaria ostoyae*, a clone of which covers an area of 890 hectares in the Malheur National Forest in eastern Oregon, USA. It weighs in at around 150 metric tons and is at least 2,400 years old. However, it is the unusual life style of *Armillaria* that enables it to get so large; it forms rhizomorphs which scavenging widely through the soil for nutrients and hosts. It's probably true to say that the majority of fungi are 'microfungi' that live in very small habitats, like individual leaves, individual insects or even smaller grains of soil. The rate at which the fungus can grow will, like the extent of the habitat, also limit the extent of growth that can occur. For example, lichens can grow in some very extreme environments, from deserts to the Arctic wastes, but in the Arctic, lichen growth is around 5 cm in a thousand years, so colonies several thousand years old will be little more than the size of your hand. Nevertheless, unless other growth conditions impose a limitation, a fungus will continue to grow until it occupies all of the available substrate. Animals and plants cannot manage that.

The fungal kingdom is very large and diverse, and as a consequence of this the part played by sexual reproduction in the life cycle of fungi is also very diverse. At one extreme of the spectrum of behavior, there are fungi that are completely asexual organisms (most examples are usually classified among the group known as the deuteromycetes, or, more formally, the Deuteromycotina). These organisms are not static in an evolutionary sense because they can generate variation by modifying genetic expression or by adapting processes that occur during the mitotic division to produce asexual propagules in which chromosomes have segregated in new combinations or which contain recombinant chromosomes (see section 5.7). At the other extreme, fungi display many forms of sexuality that govern the bringing together of genetic information from different parents into some arrangement that eventually produces a (potentially heterozygous) diploid nucleus. This nucleus undergoes meiotic division during which chromosomal segregation and genetic recombination take place as in every other eukaryote.

Before we can take the discussion very much further, we have to define at least some of our vocabulary. A species is homothallic if an individual can complete the sexual cycle on its own, but as we will explain below, there are different ways in which homothallism can be achieved. We should also emphasize that a homothallic species is not limited to self-fertilization. Two homothallic strains may well interbreed, either in nature or with 'assistance' in the laboratory. The point is that a homothallic species does have the ability to self-fertilize.

This contrasts with a heterothallic species, which ***requires*** interaction of two different individuals to complete the sexual cycle. Individual isolates of heterothallic fungi are self-sterile or self-incompatible, but can be cross compatible. Heterokaryosis results from the fusion of hyphae of different isolates, followed by migration of nuclei from one hypha into the other, so that the hyphae come to have two kinds of nuclei. Such a hypha is a heterokaryon. If there is only one kind of nucleus in the hypha, we have a homokaryon. In the most highly adapted version of this behavior, in model basidiomycetes like *Coprinus cinereus* and *Schizophyllum commune*, a basidiospore germinates to produce homokaryotic mycelium with uninucleate cells, called a monokaryon. When two monokaryons meet, hyphal anastomoses occur, and, if they are vegetatively compatible, nuclei of one migrate into the mycelium of the other. If, in addition, the nuclei have compatible mating types, the new growth and the cells of much of the pre-existing mycelium now have binucleate cells, with one nucleus of each monokaryotic parent, and this mycelium is called a dikaryon.

If a sexual cycle is really important as a means of providing variation, the mechanisms that enable it to occur presumably evolved at a very early stage in evolution. To get an idea of how the mechanisms that organize sexual reproduction might have arisen, we can look at some members of the most ancient groups of fungi and fungal relatives. Analysis of molecular data indicates that all true fungi have a common origin, and there are some organisms that are relatives with structures and ecological roles similar to true fungi (see section 9.1). Phylum Oomycota (less formally known as the oomycetes) is among the latter and includes a number of important species with interesting sexual cycles. Members of phylum Zygomycota, a phylum of the true kingdom Fungi, reproduce sexually by fusion of two gametangia to form a thick-walled zygosporangium containing zygospores. The way they arrange this is also instructive.

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Vegetative hyphae of the oomycetes are diploid and lack cross walls except where reproductive organs or damaged parts of the mycelium are separated off. Though heterokaryons of *Phytophthora megasperma* have been forced by using complementing mutants *in vitro* and in plant tissue, a key feature is that hyphal anastomosis is rare except between specialized sexual organs. Sexual reproduction involves a female structure called the oogonium in which one or several uninucleate oospheres differentiate. When these are fertilized they give rise to thick-walled oospores. The fertilizing nuclei come from a male structure (antheridium) that develops alongside the oogonium. Meiosis occurs in the oogonia and antheridia; the post-meiotic haploid nuclei being packaged into gametes. Fusion of one male and one female nucleus occurs in the oospore.

The genus *Achlya* has been used to study Oomycete sexual reproduction. Self-sterile *Achlya* strains that reproduce only when paired with another strain are frequently found, and these can be described as being heterothallic. However, the population is not neatly differentiated into homothallic and heterothallic strains, but rather into strains with varied sexual potency. Some strains are predominantly males, acting as antheridial parents in crosses, or predominantly females, acting only as oogonial parents; still other strains are intermediate, acting as males in crosses with predominant females or as females when crossed with a predominant male. This is called relative sexuality.

The first step in the reproductive cycle is that the female produces a hormone (a steroid called antheridiol) that induces the male strain to produce antheridial branches. Then the induced male strain produces a second hormone (another steroid called oogoniol), which induces the female to form oogonial initials. There are specific receptor proteins in each sex for the hormones of the other, and activation of the receptors leads to acetylation of histone proteins, activation of specific mRNA synthesis, and consequential synthesis of specific proteins. The genus *Phytophthora* includes both homothallic and heterothallic species. For example, *P. cactorum*, *P. heveae*, and *P. erythroseptica* are homothallic, and *P. infestans*, *P. palmivora* and *P. cinnamomi* are heterothallic. In the heterothallic species there are two mating types, called Al and A2, both of which are capable of forming antheridia and oogonia, though neither of them can produce oospores when alone, only when paired. This involves two hormones; the one produced by the Al mating type that induces A2 is called al hormone, and that produced by A2 to induce Al, is called a2. However, it has been shown that *Phytophthora* and *Pythium* species require sterol supplements for sexual reproduction, as well as production of normal sporangia and motile zoospores, but not for vegetative growth. It may be that sterols provide precursors for hormones that control the development of antheridia and oogonia.

In the zygomycetes, typical members of which are the genera *Mucor*, *Rhizopus*, *Phycomyces* and *Pilobolus*, the characteristic sexual structure is the zygophore, and sexual reproduction results in the formation of zygospores. In heterothallic species, when strains of different mating type (called plus (+) and minus (-)) confront each other, the specialized branches called zygophores grow towards the opposing strain and make contact near their tips. They then swell at the point of contact, these swellings developing into progametangia. A septum forms in each progametangium to separate the multinucleate gametangium from the supporting cell (suspensor).

The wall between the opposed gametangia is degraded and the fusion cell becomes the zygospore, which eventually develops a thickened wall. Karyogamy occurs in the zygospore, which is the only diploid phase in the life cycle. A zygospore germinates by forming a sporangiophore hypha with a single germ sporangium that contains many haploid, uninucleate spores. Many sporangia contain the products of just a single meiosis (amplified by post-meiotic mitoses), but the multinucleate gametangia can form several fusion nuclei, so the products of several meioses can appear in the spores. On the other hand, it may also be that some of the products of meiosis fail to be represented in the germ sporangia.

As is the case with the oomycetes, fusions between vegetative hyphae do not occur in zygomycetes; only zygophores fuse. Consequently, zygophores must be highly differentiated hyphal branches. Development of zygophores is induced by trisporic acid in both mating types, and the hormone is active at concentrations as low as 10^{-8} M. Trisporic acid is formed by both plus and minus heterothallic strains in a confrontation, but its synthesis ceases if either partner is removed. The plus strain produces a precursor (methyl 4-dihydrotrisporate), which is converted to trisporic acid by the minus partner, and the minus strain produces a different precursor (trisporol), which the plus strain converts to trisporic acid. Both precursors are volatile and their diffusion in the atmosphere is responsible both for induction of zygophores and for directing their growth towards one another in a mutual positive chemotropism.

Modern fungi *are* modern fungi and we do not want to fall into the trap of suggesting that what we have just described represent ‘primitive’ or ‘early stages’ in the development of fungal mechanisms for sexual reproduction. But there are some lessons to be learned here, from modern representatives of groups that diverged early in the evolution of fungi. First, both oomycetes and zygomycetes lack hyphal anastomoses, and generally only form hyphal septa to partition-off reproductive or injured structures. The independent syncytial nature of their mycelium creates the need (a) for specialized hyphal structures (like oogonia, antheridia and

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zygophores) to be involved in sexual fusions, and (b) for mechanisms that induce and mutually attract these structures when potential mates encounter each other.

Both groups use chemical hormones. In the literature dealing with water moulds these tend to be called sex hormones or sex attractants, but the word pheromone is increasingly applied, and will be met in section 2.6 below, and in later sections, in relation to mating processes in yeasts and filamentous fungi. A pheromone is a chemical emitted into the environment by an organism as a specific signal to another organism, usually of the same species. Often effective at minute concentrations, pheromones play particularly important roles in the social behavior of animals, especially insects and mammals, being used to attract mates, to mark trails, and to promote social cohesion and coordination in colonies. This seems to be exactly what the sex hormones do in fungi, so the word has, not surprisingly, been adopted. It is now applied to a range of phenomena involved in the fungal mating process in which a molecule is released into the intra- or extracellular environment, is then recognized by a specific pheromone receptor, which is a transmembrane protein. Through a G-protein (which uses GTP), the complex of pheromone + pheromone receptor then triggers a protein kinase signal transduction pathway that eventually specifically regulates gene activity (see section 10.11).

The higher fungi (Ascomycotina and Basidiomycotina) have taken up and adapted the same strategy; for example, the trichogynes (specialized mating hyphae) of *N. crassa* protoperithecia grow towards hyphae of opposite mating type in response to a pheromone. However, there are some important differences in life style between higher fungi and other eukaryotes that have great genetic significance. First, many fungal mycelia can tolerate (in fact, more than tolerate, can benefit from) the presence of several genetically distinct nuclei within their hyphae. This probably arises from the second important difference, namely the fact that hyphal anastomoses occur very readily within the Ascomycotina and Basidiomycotina.

Hyphal anastomosis is the fusion between hyphae or hyphal branches, the process involving breakdown of two hyphal walls and union between two separate plasma membranes to bring the cytoplasms of the fusing hyphae into continuity with each other. Once they are in continuity, they can exchange nuclei and other organelles. The important point is that anastomosis is not limited to sexual reproduction; rather, hyphal fusions are essential to the efficient functioning of the mycelium of filamentous ascomycete and basidiomycete fungi because they convert the initially radiating system of hyphae into the fully interconnected (and three-dimensional) network. Hyphal fusions are common within the individual mycelium as it matures. The interconnections they establish enable transport of nutrient and signalling molecules anywhere in the colony.

In unicellular fungi, the yeasts, fusion between the individual cells is called conjugation, and is very similar to the fertilization process that is the first step in the sexual cycle of animals and plants. Yeast cell conjugation also requires many of the processes required for fusion of filamentous vegetative hyphae: signalling by diffusible substances, directed growth, attachment of the two cell types to one another, production and targeting of hydrolytic enzymes to the attachment site, fusion of the plasma membrane, and restructuring of the cell wall to form a connecting bridge between the two cells. Database searches of the *Neurospora* genome for sequences involved in these processes in yeast have revealed a number of potential *N. crassa* homologues. Some of the machinery involved in yeast cell fusion during mating may also be used for hyphal anastomosis in filamentous fungi.

Evidently, higher fungi are fully equipped with the machinery necessary for hyphal tips to target other hyphae and to produce and externalize the enzymes (for cell wall degradation, for example) needed for anastomosis. This is part of normal mycelial development; it is not a specialization of the sexual cycle. It is complex machinery, though, because during normal mycelial growth vegetative hyphae usually avoid each other (known as negative autotropism). This behavior pattern promotes exploration and exploitation of the available substrate. Anastomosis requires that hyphae grow towards each other (called positive autotropism). How and why the usual avoidance reactions between hyphae become reversed is unknown but, evidently, it is a change in the behavior of hyphal tips that occurs as the mycelium matures (and may depend on local population density).

Higher fungi, therefore, have a mechanism that promotes cell fusions to an extent that is never encountered in animals and plants, nor oomycetes and zygomycetes. Their problem is to regulate hyphal anastomosis so that its physiological and genetic advantages can be realized without hazard. And there are hazards. Hyphal anastomosis carries the risk of exposure to contamination with alien genetic information from defective or harmful cell organelles, viruses or plasmids.

If genetically different hyphae are to interact, to take advantage of the sexual cycle, for example, nuclear and cytoplasmic control requirements are very different. To maximize the advantage of sexual reproduction the controls must ensure that the nuclei are genetically as *different* as possible. In contrast, safe operation of the cell requires that cytoplasms that are to mingle must be as *similar* as possible. These features are under the control of genetic systems that regulate the ability of hyphae to fuse, generally called vegetative compatibility, and subsequently genes called mating type factors regulate the ability of nuclei that have been brought together to undergo karyogamy and meiosis. It is important to recognize that these different aspects are recognizable phenotypes.

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The phenotype of vegetative compatibility (also called vegetative, somatic, or heterokaryon incompatibility) is formation of a joint heterokaryotic mycelium, and the phenotype of compatible interaction between mating type factors is occurrence of sexual reproduction. Although we have to describe these systems separately because they are different phenotypes, and, further, that they must be described in terms of population biology, genetics and molecular biology, remember that they are interdependent functions. Most of what we know about compatibility genetics has been obtained from laboratory studies with model organisms. However, in nature, vegetative compatibility is different from, but has a controlling influence over, mating type function in terms of both population structure and genetic diversity.

2.2 Population biology aspects of compatibility systems

Vegetative compatibility is controlled by one to several nuclear genes that limit completion of hyphal anastomosis between colonies to those that belong to the same vegetative compatibility group (usually abbreviated to v-c group). Members of a v-c group possess the same vegetative compatibility alleles. The type of vegetative compatibility that is most usual in fungi is called post-fusion incompatibility. Hyphal anastomosis is promiscuous in fungi, but compatibility of the cytoplasms determines whether cytoplasmic exchange will progress beyond the first few hyphal compartments involved in the initial interaction. If the colonies involved are not compatible the cells immediately involved in anastomosis are killed (Fig. 2.1). 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.

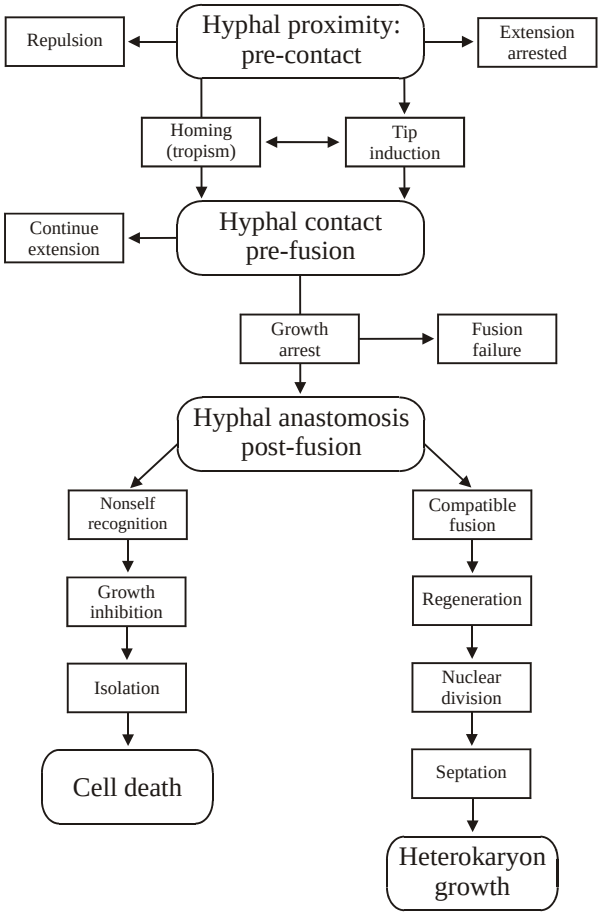

Fig. 2.1. Flow diagram illustrating the progress of hyphal interaction leading to operation of the vegetative compatibility systems. Recognition processes between hyphae take place at all three major steps: pre-contact hyphal proximity, pre-fusion hyphal contact and post-fusion self-nonself recognition. Fig. 2.1. adapted and redrawn from Figure 1 in Glass, Jacobson & Shiu (2000), *Annual Review of Genetics* **34**, p. 168.

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Vegetative compatibility prevents formation of heterokaryons except between strains that belong to the same v-c group. Hyphal fusions bring together nuclei of different origins into the same mycelium, and their ability to undergo karyogamy and meiosis to complete the sexual cycle is determined by the mating type factors. Evidently, the best life cycle strategy for the fungi is to produce some offspring sexually for generating novel genotypes to adapt to the unpredictable and fluctuating environment, *and* produce many offspring asexually to rapidly colonize the favorable environment (once it is found) and so establish territorial control in a competitive world.

Mating (also called breeding) systems rely on nuclear genes that control mating between mycelia. Basic analysis of such systems depends on making experimental confrontations between mycelia and scoring whether or not the sexual stage is completed. It is, therefore, important to emphasize that such experiments test for the *phenotype* of sexual reproduction, and the pattern of its occurrence and its inheritance allow deductions about the control of sexual reproduction. A mycelium that possesses genes that prevent mating between mycelia that are genetically identical will be self-sterile; since it ensures that different mycelia must come together for a successful mating to occur and this is why such a system is called heterothallism.

Many heterothallic fungi, indeed all known heterothallic ascomycetes, have only two mating types specified by a single locus with different 'alleles': *Neurospora crassa*, the brewer's yeast *Saccharomyces cerevisiae*, and the (basidiomycete) grass rust *Puccinia graminis* are examples. In such cases the mating type of a culture depends on which 'allele' it has at the single mating type locus (hence the alternative name of unifactorial incompatibility), successful mating only taking place between cells or mycelia that have different 'alleles' at the mating type locus. Of course, the diploid nucleus that results is heterozygous for the mating type factor, and meiosis produces equal numbers of progeny of each of the two mating types (hence the alternative name bipolar heterothallism). We put the word allele into quotes in the last few sentences because, although it is not evident from classical genetic analysis, one of the first things that molecular analysis revealed about the mating type factors is that the different forms of the mating type locus do not share the amount of DNA sequence homology you would expect of alleles. Their 'alleles' can be very different indeed, in some cases differing in length by thousands of base pairs. For this reason they have been called idiomorphs rather than alleles. Idiomorphic structure (not allelism) is common to all fungal mating type genes that are known.

With only two idiomorphs, the likelihood that two unrelated individuals will be able to mate (which is the outbreeding potential) is 50%. But if there were *n* mating type idiomorphs the outbreeding potential would be $[1/n \times (n\text{-}1)] \times 100\%$; so the greater the number of mating type idiomorphs, the greater the outbreeding potential. Many basidiomycetes have two unlinked mating type factors (designated *A* and *B*); this is called a bifactorial incompatibility system. In this case too, a compatible interaction is one between two mycelia with different idiomorphs, but this time both *A* and *B* mating type factors must differ. As a result, the diploid nucleus that is formed will 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).

Coprinus cinereus and *Schizophyllum commune* are the classic examples of this mating type system. In both of these the wild population contains many different *A* and *B* idiomorphs, and the outbreeding potential is about 100%. The inbreeding potential of bifactorial incompatibility (the likelihood of being able to mate with a sibling) is 25% (because there are four different mating types among the progeny of a single meiosis) whereas it is 50% in unifactorial incompatibility where there are only two mating types among the progeny. So a bifactorial system tends to favor outbreeding. About 90% of higher fungi are heterothallic, and 40% of these are bipolar and 60% tetrapolar.

In homothallic (self-fertile) fungi sexual reproduction can occur between genetically identical hyphae, but mating type factors may still be involved. Primary homothallism occurs in species completely lacking heterothallism, but secondary homothallism occurs in species that have an underlying heterothallism that is bypassed. *Neurospora tetrasperma*, *Coprinus bisporus* and *Agaricus bisporus* are good examples. In these cases, there are more post-meiotic nuclei than spores, so the spores become binucleate and heterozygous for mating type factors. Spore germination gives rise to heterokaryotic mycelia that are, consequently, able to complete the sexual cycle alone, that is they act like homothallics. The yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* exemplify a different process. Most strains are heterothallic with two mating types (see below), but 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, in a few cells in the population, from one mating type to the other (see section 2.6) so that the (still heterothallic) clone comes to contain cells of different mating type.

It is the compatibility reactions (including, for the moment, both vegetative and mating type compatibilities) that 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. But are ten spores from the same colony ten different individuals, or just ten bits separated from one individual? And then there are heterokaryons; mycelia that contain more than one

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nuclear type. Is a heterokaryon an individual, rather than a chimera or mosaic? These are important questions because in genetical terms a population consists of individuals that are able to interbreed. Individuals are important in evolution because selection operates on individuals. So to understand fungal populations we have to know where the individual begins and ends.

Populations are important because the fundamental unit of biological classification, the species, is conventionally defined in terms of mating success and production of viable offspring. This is the biological species (or genospecies); in fungi this is delimited by the experimenter carrying out mating tests between fungal isolates collected over a wide geographical area. This obviously applies to fungi that have a sexual process but in Deuteromycotina, which lack sexual reproduction, the concept of a genospecies is meaningless. In this latter case, individuals can be designated as a 'taxospecies' on the basis of their similarity in many phenotypic characters, and to achieve this molecular methods of analysis and computer aided numerical analysis have become increasingly important. In most fungi, both types of analysis can be done and biological species do not always correspond to taxospecies because different selection pressures in different geographical areas may cause local populations to adapt into distinct taxospecies, which, despite obvious phenotypic differences, can interbreed when combined artificially (that is, different taxospecies = one genospecies). At the other extreme, mating barriers may arise, separating populations that are still sufficiently similar to remain in one taxospecies into different genospecies.

Most fungi have life cycles consisting of various phases including haploid (n), dikaryotic (n + n) (or heterokaryotic with two or more types of nuclei in either a random or fixed ratio) and diploid (2n). Recessive alleles will not be expressed by heterozygous dikaryotic or heterokaryotic mycelia and such mycelia may show hybrid vigor. The nuclei of a heterokaryon are open to selection and can segregate into homokaryons (or heterokaryons with altered nuclear ratios) if adverse conditions impose selection pressure. That is, an individual with a new phenotype might segregate from a heterokaryon in response to selection, the mechanism being selective segregation of some of its constituent nuclei into hyphal branches. This is a developmental process that allows the heterokaryon to adapt to its environment using an evolutionary mechanism; hyphal branches containing a nuclear ratio best suited to the environment will be the ones to grow best. Consequently, a heterokaryon is a very tolerant and adaptable mycelium. Heterokaryon interactions and interrelationships are also governed by their vegetative compatibility and mating type systems. Most natural populations of fungi are composed of vegetatively incompatible, genetically and physiologically distinct individual heterokaryon mycelia that have been called genets.

Compatibility systems maintain the individuality of a mycelium and enable it to recognize unrelated mycelia of the same species with which it competes for territory and resources. In other words they provide an individual mycelium with a way of establishing whether hyphae it encounters belong to itself or not. When individuals do exchange nuclei it is the mating type systems that then regulate sexual exchange between the mycelia by ensuring that compatible nuclei undergo karyogamy and enter meiosis. Separate genes for cytoplasmic compatibility and mating type can be identified in ascomycetes, but in basidiomycetes the genetic factors that have been identified as mating type factors are complex multiple genes involved in determining self/nonself recognition, regulation of mycelial morphogenesis (the growth pattern of the dikaryon is different from that of the monokaryon) and regulation of karyogamy, meiosis and multicellular development.

2.3 Compatibility and the individualistic mycelium

Fungal isolates from nature that are confronted with each other on artificial media usually show interactions implying self/nonself recognition. Such confrontations are the classic test for compatibility, which have been used for many purposes from population studies to allele complementation tests (see section 4.1). They are set up by placing small pieces of the strains that are to be tested side by side on the surface of an agar medium. Tests should always be replicated; five replicates are best, but three will suffice. It is important that the confronted inocula are standardized as far as possible. They should be standardized in size and vigor by being cut to the same dimensions from a region about 2 mm behind the margin of a vigorous stock plate. The old medium should be removed from the inoculum before it is planted onto the fresh medium.

When the confrontations are incubated, leading hyphae may mingle and hyphal anastomoses occur between their branches. If the confronting strains are compatible the heterokaryon may proliferate so that the whole mycelium is heterokaryotic; this is what happens in *Neurospora crassa* and *Podospora anserina*. Alternatively, in species such as *Verticillium dahliae* and *Gibberella fujikuroi*, nuclei do not migrate between cells and heterokaryosis is limited to fusion cells. In this latter case, heterokaryons are continually formed by repeated fusion events within the mycelia. If the colonies involved are not compatible, the fusion cells are killed (Fig. 2.1). Cell death resulting from vegetative incompatibility involves plugging of the septal pores, to compartmentalize dying hyphal segments, vacuolization of the cytoplasm, DNA fragmentation, organelle degradation, and shrinkage of the plasma membrane from the cell wall. It is an internalized cell death, different

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Table 2.1 Genes involved in vegetative incompatibility that have been cloned and characterized

Neurospora crassa	
Mat A-1	Mating type transcription regulator, contains region similar to *Mat α1* of *Saccharomyces cerevisiae*
Mat a-1	Mating type transcription regulator with an HMG box (characteristic of *H*igh *M*obility *G*roup proteins, a class of proteins distinct from histones which are found in chromatin and represent a subclass of the non-histone proteins; the HMG proteins function in gene regulation and maintenance of chromosome structure)
het-c	Signal peptide (involved in endoplasmic reticulum targeting of secreted proteins) with glycine-rich repeats
het-6	Region of similarity to *tol* and *het-e* (of *P. anserina*)
un-24	Large subunit of type I ribonucleotide reductase
tol	Features a coiled-coil, leucine-rich repeat (a protein conformation found in extracellular matrix molecules), has regions similar to sequences in *het-e* (of *P. anserina*) and *het-6*
Podospora anserina	
het-c	Glycolipid transfer protein (glycolipids are involved in cell to cell interactions)
het-e	GTP-binding domain, region with similarity to *tol* and *het-6* of *N. crassa*
het-s	Prion-like protein (abnormally-folded variant can infectively communicate its abnormal conformation to normal proteins which then form aggregates)
idi-2	Signal peptide, induced by *het-R*/*V* incompatibility
idi-1, *idi-3*	Signal peptide, induced by nonallelic incompatibility
mod-A	SH3-binding domain (*s*rc *h*omology domain 3; a protein domain of about 50 amino acid residues present in proteins involved in signal transduction, and also in a number of cytoskeletal proteins, generally involved in protein-protein interactions)
mod-D	α-subunit of G-protein with GTP binding (such proteins are involved in signal transduction in eukaryotic cells), modifier of *het-C*/*E* incompatibility
mod-E	Heat-shock protein (belongs to the Hsp90 family of 90 kDa polypeptides with ATPase activity which are essential for the viability of yeast cells and found in association with many regulatory proteins in eukaryotes, like steroid receptors and protein kinases), modifier of *het-R*/*V* incompatibility
pspA	Vacuolar serine proteinase, induced by nonallelic incompatibility

from necrotic cell death, with many features in common with programmed cell death (PCD, or apoptosis) in other multicellular eukaryotes.

Vegetative compatibility (= vegetative, somatic or heterokaryon incompatibility) will prevent formation of a heterokaryon unless the strains belong to the same v-c group. Incompatibility between strains in a confrontation is caused by genetic differences between the two individuals at particular gene loci, which are called *het* (for *het*erokaryon) or *vic* (for *v*egetative *inc*ompatibility) loci, although once the major genes were identified several others that affected or otherwise modified their expression were also identified and given other descriptive names (Table 2.1). The *het* genes might define allelic (the most frequent type, also called homogenic) or nonallelic systems (heterogenic). In allelic systems, incompatibility is triggered by the expression of two incompatible alleles at the same locus. With nonallelic systems, incompatibility results from interaction between two genes at different loci. Only allelic systems have been found in *N. crassa* and *Aspergillus nidulans*, although both allelic and nonallelic systems occur in *P. anserina*. There are usually about 10 *het* loci, but the number varies from one species to another: there are at least 11 *het* loci in *N. crassa*, 9 in *P. anserina*, 8 in *A. nidulans*, and 7 in *Cryphonectria parasitica*. Usually, two different alleles of a *het* gene are found in wild type isolates, although *het* loci with more than two alleles have been found.

In *Aspergillus nidulans*, conidial colour mutants have been induced in isolates from soil to study the extent of heterokaryon formation between pairs of strains. This approach works because the colour of the

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conidium depends on expression of genes in the single haploid nucleus the spore contains. So although the conidial head of *A. nidulans* is formed as spores bud off in chains from a multinucleate vesicle at the top of the conidiophore that could, in a heterokaryon, contain two kinds of nuclei, each chain of spores will be of one colour. If the conidiophore is heterokaryotic, then conidial heads of mixed colour are produced; homokaryotic conidiophores have only one kind of nucleus in the vesicle and all their chains of conidia will be the same colour. In one study, eighteen different isolates were collected from around Birmingham, UK, and from Durham, which is about 300 km away. When confronted with each other, incompatible strains formed either none or less than 0.2% mixed conidial heads, whilst those that formed heterokaryons readily produced up to 5% mixed heads. In the former case the strains were considered to belong to different compatibility groups (v-c groups); in the latter, to the same group. Five v-c groups were identified among the 18 isolates, the largest of which included seven isolates that were collected from both localities.

Subsequent genetic analysis revealed that eight genes (called *hetA*, *hetB*, *hetC*, etc.) determine a total of six different heterokaryon compatibility groups in the homothallic *A. nidulans*. Normally, a difference at any of the *het* gene loci prevents heterokaryon formation and effectively defines the individual of *A. nidulans*. An important function of vegetative compatibility might be the protection of a mycelium from invasion by 'diseases' following hyphal anastomosis. For example, an infectious cytoplasmically determined condition called 'vegetative death' has been shown to be readily transmitted within a v-c group in *Aspergillus amstelodami*, but is only rarely transmitted between v-c groups.

A similar circumstance has been identified in *Ophiostoma ulmi*, the fungus that causes Dutch elm disease. Isolates of *O. ulmi* fall into mutually incompatible v-c groups, which differentiate races, subspecies and even biological species. In compatible (called c-type) reactions between isolates there is no visible reaction or only a slight increase in density of the mycelium along the junction line between confronted cultures. A virus that causes a cytoplasmically transmitted disease of *O. ulmi* is transferable between strains that could establish c-type reactions, implying that hyphal anastomoses establish cytoplasmic continuity. Compatible reactions are rare (because most isolates tested are distinct individuals) and the most common reaction is an antagonistic one in which a broad mycelial barrage forms between the confronted cultures - this is the w or 'wide' reaction. The disease virus is not transmitted between strains in a w reaction, suggesting there is no cytoplasmic continuity. Narrow (n), 'line' (l) and 'line-gap' (lg) reactions are other antagonistic reactions that are sometimes observed. These reactions are different phenotypes of a vegetative compatibility system controlled by at least three genes that are functionally and genetically independent of the mating type locus. In the wide reaction, hyphae penetrate from one colony into the space occupied by the other and this can lead to sexual recombination between the individuals defined by vegetative compatibility. Genetic exchange is usually unilateral: a strain may be an efficient female parent in crosses but an inefficient male parent.

Neurospora crassa is similar to *Aspergillus* in that heterokaryon formation requires genetic identity at all *het* genes. One of these genes is the mating type locus of *N. cras*sa, and although this is unusual, association between mating-type and vegetative incompatibility is not restricted to *N. cras*sa, but has been reported in *Ascobolus stercorarius*, *A. heterothallicus*, and *Sordaria brevicollis*.

In *N. crassa*, heterokaryons made between strains of opposite mating type grow slowly and have an irregular colony outline as compared with the rapid, uniform growth of heterokaryons between strains with the same mating type. Evidently the mating type gene of *N. crassa* controls both sexual compatibility and heterokaryon compatibility, although the former requires that the mating types are different and the latter requires that the mating types are identical. It seems that nuclei of opposite mating type do not readily coexist in vegetative hyphae of *N. crassa*. Aggressive maintenance of individuality between mates is neither unusual nor difficult to understand in our own species; allegedly, men are from Mars, women from Venus. In *N. crassa*, the molecular basis of this mating aggression is that the MAT A-1 and MAT a-1 mating polypeptides (see section 2.7) encode transcription regulators that specify different cell types in the sexual phase, but they are lethal when expressed together in a vegetative cell. The mating function of MAT a-1 depends on its DNA-binding ability, but this is not needed for the vegetative incompatibility function. So different functional domains of the polypeptide serve these two different activities of the mating type idiomorphs.

A functional *tol* gene is required for expression of this mating type factor-mediated vegetative incompatibility in *N. crassa*. Incompatibility caused by the mating type factor is suppressed by mutations in *tol* (these make the strains *tol*erant of each other), but *tol* mutations do not disrupt the sexual cycle. An active (that is, non-mutant) *tol* allele is normally present in the heterothallic outbreeding species in which its mutant form was originally identified, but *N. tetrasperma*, which normally exists as a self-fertile (*mat A + mat a*) heterokaryon, has been shown to have an inactive *tol* allele.

Heterokaryons made between *N. crassa* strains of the same mating type (and the same *het* genotype) have nuclear ratios close to 1: 1, full cytoplasmic continuity, and they also produce up to 30% heterokaryotic conidia. In incompatible heterokaryon confrontations the pores in the septa of any cells that do fuse become blocked, and the cytoplasm becomes granular, then vacuolated, and finally dies. When such cytoplasm, or even

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a phosphate-buffer extract of it, was injected into a different strain, the same degenerative changes resulted. The activity of the extract was associated with its proteins, demonstrating that heterokaryon compatibility self/nonself recognition depends on the protein products of the *het* genes. The *un-24* gene of *N. crassa*, which is involved in *het-6* incompatibility, encodes the large subunit of ribonucleotide reductase, which is essential for DNA replication. Mutants in these *het* loci lose their nonself recognition ability and form vigorous heterokaryons with strains with which they were formerly incompatible.

Further insight into the mechanism of action of vegetative compatibility genes comes from work with *Podospora anserina*. When two incompatible colonies meet, hyphal fusion is followed by death of the fused cells and consequent absence of pigment, so a clear zone forms between the colonies called a barrage. The barrage is due to vegetative incompatibility, but the colonies might still be sexually compatible (controlled by one mating type locus with idiomorphs called plus and minus). If they are compatible, a line of perithecia can be formed on each side of the barrage because although fused vegetative cells are killed, lethality does not extend to fused trichogynes and spermatia. Originally, analysis of the interactions of different races of *P. anserina* revealed nine loci involved in barrage formation, several with multiple alleles. Incompatibility could result due to interaction between alleles of one gene, for example allele *t* with t_1, *u* with u_1 (called allelic or homogenic incompatibility), or to interaction between alleles of different genes, e.g. gene a_1 with gene *b*, gene c_1 with gene *v* (called heterogenic or nonallelic incompatibility).

Heterogenic incompatibility reactions are often associated with fertility differences that, in some cases, are due to cytoplasmic factors transmitted through the hyphae. For example, interactions between the *R/r* and *V/v* genes indicate that the *V* gene product diffuses through the cytoplasm, while the *R* gene product is probably fixed to the plasma membrane. Observations like this give rise to the belief that vegetative incompatibility results from interaction of protein products of the incompatibility genes that trigger activity of proteins and enzymes during, and possibly causing, the senescence and death of the incompatible fusions (Table 2.1). So, the incompatibility genes are probably regulators of such enzymes, and it has been argued that they may have arisen as mutations in genes normally concerned with housekeeping functions that ensure proper progress of the female (protoperithecial) part of the sexual cycle.

Several *het* loci of *P. anserina* have been characterized, including the allelic incompatibility locus, *het-s*, and the nonallelic *het* loci, *het-c* and *het-e* (Table 2.1). Note that *het-c* in *P. anserin*a has no relationship to the *het-c* of *N. crassa*. Just as in *N. crassa*, the *P. anserina het* loci evidently encode varied gene products. The *P. anserina het-c* locus encodes a glycolipid transfer protein; and, interestingly, disruption of *het-c* impairs ascospore maturation. Although mutants of *het-s* exhibit no other phenotype than promiscuous vegetative compatibility, the *het-s* gene product behaves like a prion protein. A prion is a 'proteinaceous infectious particle', a cellular protein that can assume an abnormal conformation that is infectious in the sense that it can convert the normal form of the protein into the abnormal (see section 5.11). Hyphal anastomosis between *het-s* and the neutral *het-s** strain results in the cytoplasmic transmission and infectious propagation of the *het-s* phenotype.

Although the *het* loci encode very different gene products, three regions of similarity can be detected between predicted products of the *het-6* locus and the *tol* locus of *N. crassa*, and the predicted product of the *het-e* locus of *P. anserina*. These regions might represent domains necessary for some aspect of vegetative incompatibility in which all three of these *het* loci are involved. Alleles of *het-c* that are found in *N. crassa* are present in other *Neurospora* species and related genera, indicating there was a common ancestor and conservation during evolution of this sequence. However, despite this indication that there may be some underlying similarity in function, a *het* locus from one species does not necessarily confer vegetative incompatibility in a different species.

2.4 Nuclear migration

The incompatible reaction evidently sets in train a large number of changes that result in the fusion cells being sealed off from the rest of the two interacting mycelia and killed. An equally dramatic change occurs following the compatible reaction in which hyphal fusion is followed, in many species, by migration of nuclei to give rise to a heterokaryon. Nuclei can migrate through pre-existing mycelium, either bi-directionally or unilaterally. The direction of migration depends on the genes and alleles involved in the compatibility reaction.

When confrontations are made between cultures of the two mating types of *Gelasinospora tetrasperma*, hyphae anastomose and nuclei migrate from one strain into the other. Perithecia develop along the junction line in one of the cultures because migration is regularly unilateral. Nuclear migration is fast: a typical migration rate of 4 mm h^{-1} compares with a typical hyphal growth rate of only 0.7 mm h^{-1}.

Nuclear migration has also been studied in some basidiomycetes. In the species that have been examined in most detail, *Coprinus cinereus* and *Schizophyllum commune*, basidiospores germinate to form homokaryotic mycelia with uninucleate cells, which is usually called a monokaryon or homokaryon. Two

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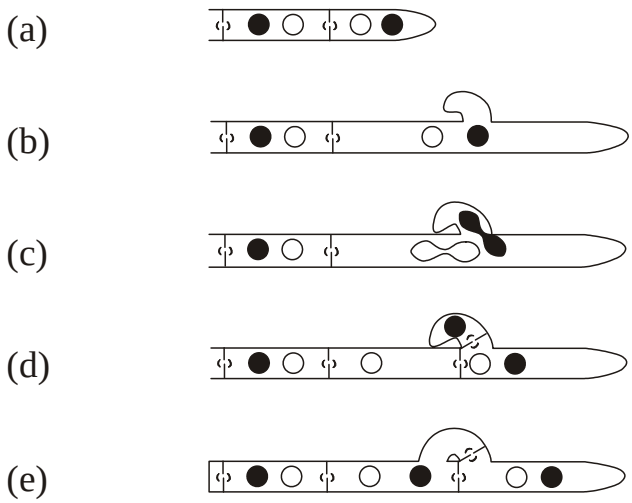

Fig. 2.2. Formation of a clamp connection. In dikaryotic hyphal cells (Fig. 2.2a) nuclei are paired, with one haploid nucleus of each mating type in each cell (here indicated by the closed and open circles). The apical cell extends, mitosis is initiated, and a clamp connection, or hook cell, emerges as a branch (Fig. 2.2b) that grows backwards (away from the hyphal apex). Conjugate mitoses then occur, that is the two nuclei enter mitosis in synchrony, one daughter nucleus enters the clamp connection (Fig. 2.2c), and a daughter nucleus of opposite mating type migrates away from the hyphal apex. Two septa are laid down (Fig. 2.2d), one traps a daughter nucleus in the clamp connection and the other divides the tip cell into two compartments at a location just behind the point of emergence of the clamp connection. Finally, the apex of the clamp connection anastomoses with its parent hypha (Fig. 2.2e) releasing its trapped nucleus into what is now the sub-terminal cell of the hypha.

monokaryons will form hyphal anastomoses, and if they are compatible, nuclei will migrate from one mycelium into the other. This establishes a new mycelium, called a dikaryon, which has regularly binucleate cells containing one nucleus of each parental type. The growth of dikaryotic hyphal tips requires that the two nuclei complete mitosis together (conjugate division) and a mechanism of nuclear migration and sorting that depends on a small backward-growing branch (called a clamp connection or hook cell) at each hyphal septum (Fig. 2.2). Other characteristic differences between the monokaryotic and dikaryotic mycelia of *C. cinereus* are that branches emerge from monokaryotic hyphae at a wide angle (40 to 90°), but at an acute angle (10 to 45°) from dikaryotic hyphae; monokaryons, but not the dikaryons, produce asexual arthrospores, called oidia, in droplets of fluid; and the aerial mycelium of monokaryons is generally less dense and fluffy than that of dikaryons.

Two multiallelic mating type factors, called *A* and *B* control sexual compatibility in *C. cinereus* and *S. commune*. To be compatible, the parental monokaryons must have different *A* and different *B* mating type factors. Clearly, the mating type factors determine the initial self/nonself recognition that follows the first anastomoses of the encounter; they also regulate mycelial morphogenesis (presumably upregulating features that characterise the dikaryon and downregulating some monokaryotic features, like oidiation) but as it is only dikaryons that fruit in normal circumstances, the *A* and *B* mating type factors also regulate fertility. This is why they are called mating type factors. Dikaryon formation requires that both the *A* and *B* factors are different (which is taken to mean that both *A* and *B* are turned on (*A*-on, *B*-on, see Fig. 2.3). In a common-*A* heterokaryon the *A* factors are the same but *B* factors are different (*A*-off, *B*-on) and nuclear migration can occur, but without conjugate divisions or clamp connection formation. When only A functions are turned on (A-on, B-off), in a common-*B* heterokaryon, conjugate divisions and clamp connection formation occur, but the clamp connections remain incomplete and without clamp cell fusion nuclear migration cannot take place (Fig. 2.3).

This ‘division of labor’ between *A* and *B* factors is not universal; nuclear migration is regulated by the *B* factor only in *C. cinereus* and *S. commune*, but in *C. patouillardii* migration is regulated by both factors but only *A* affects fertility. In *Ustilago* and *Tremella*, cell fusion is controlled by a locus with two idiomorphs, but the ability of fused cells to grow as dikaryons depends on a second locus with multiple idiomorphs (*Ustilago* mating types are described in section 2.8 below).

Nuclei migrate from a compatible mycelium and convert a monokaryon into a dikaryon. In experiments similar to those described above with *Gelasinospora tetrasperma*, it was found that nuclei of *Coprinus radiatus* (a close relative of *C. cinereus*) invade mycelia in such circumstances at a rate of 1.5 mm h^{-1}, which is at least

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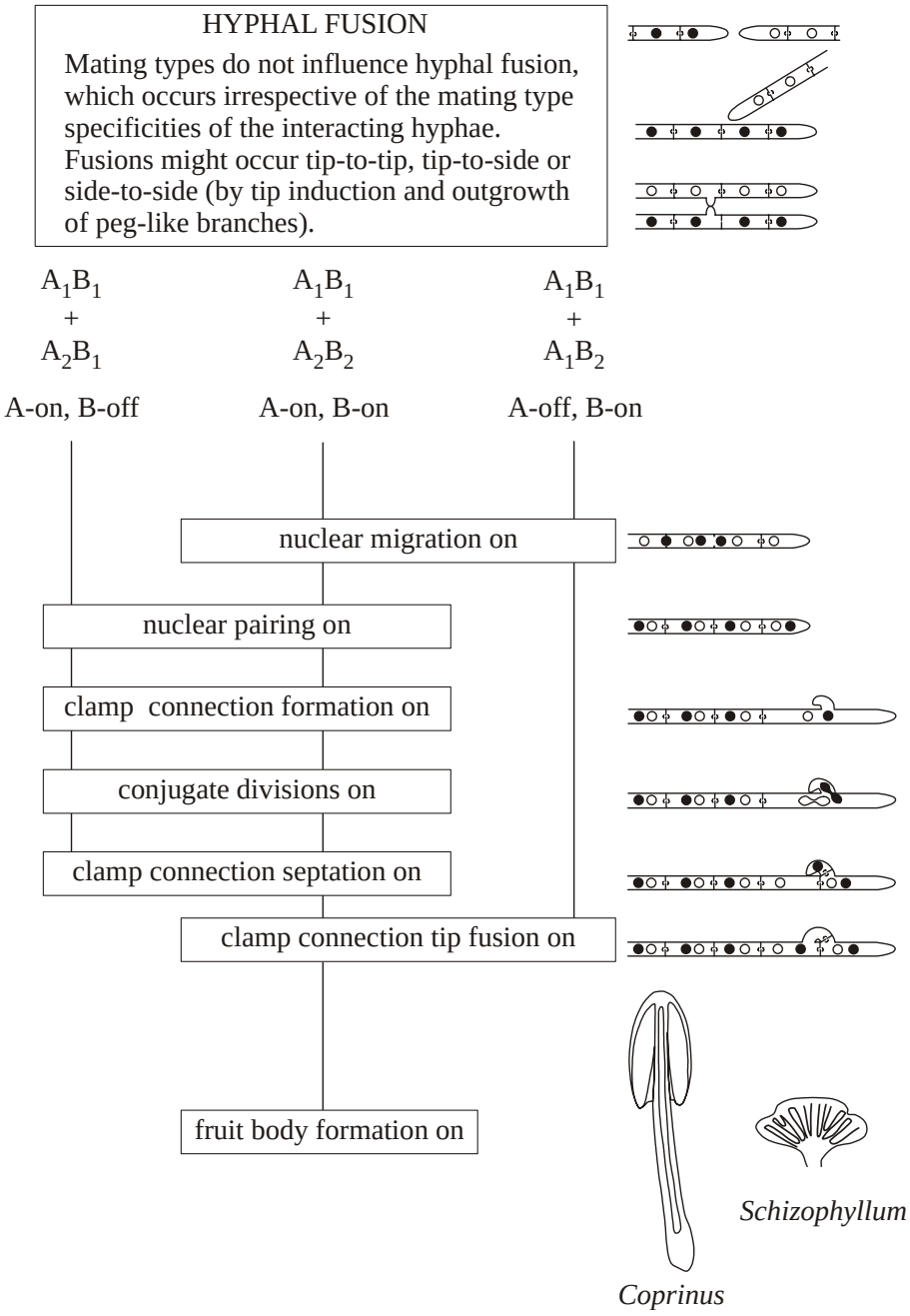

Fig. 2.3. Flow chart diagram of *A* and *B* mating type factor activity in the basidiomycetes *Coprinus cinereus* and *Schizophyllum commune*. From top to bottom the flow chart depicts the events that take place when two haploid mycelia confront each other. Dikaryon formation requires that both *A* and *B* factors are different (which is taken to mean that both *A* and *B* functions are turned on (*A*-on, *B*-on)). This is depicted in the central vertical line for a confrontation of $A_1B_1 + A_2B_2$, the vertical line connecting 'function boxes', the phenotypes of which are indicated in the cartoons on the right hand side. When only A functions are turned on (A-on, B-off), in a common-*B* heterokaryon ($A_1B_1 + A_2B_1$) conjugate divisions and clamp formation occur, but the clamp connections remain incomplete and without clamp cell fusion nuclear migration cannot take place. In a common-*A* heterokaryon ($A_1B_1 + A_1B_2$) the *A* factors are the same but *B* factors are different (*A*-off, *B*-on) and nuclear migration can occur, but without conjugate division or clamp connection formation. Figs 2.3 to 2.7 adapted and redrawn from Chiu & Moore (1999). Sexual development in higher fungi. Chapter 8 in *Molecular Fungal Biology* (ed. R. P. Oliver & M. Schweizer), pp. 231-271. Cambridge University Press: Cambridge, U.K.

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four times higher than the hyphal growth rate. In *C. congregatus*, a nuclear migration rate of 4 cm h^{-1} (yes, *centimeters*!) has been reported, and migration rates in *Schizophyllum commune* range up to 2.7 mm h^{-1} with a hyphal growth rate of only 0.22 mm h^{-1}.

During nuclear migration the invading nucleus undergoes regular division and one of the daughter nuclei moves into the next cell, the intervening dolipore septa between adjacent cells of the monokaryon being broken down into simple pores through which the nuclei can be squeezed. Although some corresponding cytoplasmic movement has been observed in *Schizophyllum commune*, nuclear migration occurs more commonly without visible cytoplasmic flow. Nuclear migration is a highly active transport process involving microtubules in a manner analogous to the involvement of spindle fibers in the movement of chromosomes during division, and this presumably accounts for its specificity. Clearly, in most cases a specific nuclear type is being transported in a specific direction and in all this discussion of nuclear migration it is important to emphasize that *only* nuclei migrate; mitochondria are not exchanged between compatible mycelia. During migratory dikaryotization, anucleate cells and multinucleate cells are observed, so the dikaryotic state is not set up as soon as compatible cells fuse. Rather, ordered dikaryotic growth emerges after an interval of disorganized and irregular growth.

2.5 Other incompatibility reactions

There are obviously numerous breeding systems and many genera contain species exhibiting a range of them. Although the tetrapolar system of *Coprinus cinereus* is one of the most extensively studied mating systems, other species of *Coprinus* are heterothallic bipolar, and some are homothallic. In the homothallic *Coprinus sterquilinus*, basidiospores germinate to form monokaryons but after some growth they spontaneously convert to dikaryons with clamp connections.

Sistotrema brinkmannii exemplifies a different, but not uncommon, situation among the fungi. It is an aggregate species, isolates of which are difficult, even impossible, to distinguish on morphological grounds. The aggregate includes homothallic and heterothallic isolates, and the latter can be bipolar or tetrapolar. Homothallics fall into a single interfertile population, while bipolar and tetrapolar isolates fall into a number of intersterile groups. Hybrid progeny can be obtained from crosses between some homothallic and bipolar isolates, and gene segregation is quite regular. This pattern of behavior suggests that homothallism was derived from a bipolar heterothallic system by loss or inactivation of the mating type factor. Evidently, therefore, the incompatibility systems can change as populations diverge in evolution.

The tone of the descriptions so far might imply that mycelial interactions take place only between homokaryons, but this is far from the truth. Study of the interactions *in vitro* is certainly easier with homokaryons, but when fungal mycelia are isolated from nature, the overwhelming majority proves to be heterokaryotic, so in the real world the most normal interactions are those between heterokaryons. For example a monokaryon of *Coprinus* can be dikaryotized by a dikaryon as well as by another monokaryon. In these 'di-mon' (dikaryon × monokaryon) matings, if both dikaryon nuclei are compatible with the monokaryon (e.g. $[A_1B_1 + A_2B_2] \times A_3B_3$) either dikaryon nucleus (but generally only one of them) migrates into the monokaryon; if only one dikaryon nucleus is compatible with the monokaryon ($[A_1B_1 + A_2B_2] \times A_1B_1$), then the compatible nucleus will migrate into the monokaryon; and if neither dikaryon nucleus is compatible with the monokaryon ($[A_1B_1 + A_2B_2] \times A_1B_2$) there is often no migration. However, in about half the cases where this confrontation was done in *Schizophyllum* it was found that the monokaryon became dikaryotized. In about half of those, both dikaryon nuclei migrated into the monokaryon, and in the others a mitotic recombination event in the dikaryon had produced a new nucleus that was compatible with the monokaryon. Common-*A* and common-*B* heterokaryons can also donate nuclei to monokaryons, providing the monokaryon's nucleus has a different *B* factor (because *B*-functions are required for nucleus migration). Dikaryons and common-*B* heterokaryons of *Coprinus cinereus* can accept nuclei, but in *Schizophyllum commune* they only donate nuclei.

Diploid strains of *Coprinus cinereus* arise from common-*A* heterokaryons when rare nuclear fusions form a diploid nucleus that is packaged into an oidium. So, the common-*A* heterokaryon, $[A_1B_1 + A_1B_2]$ would give rise to a diploid $A_1A_1B_1B_2$. This diploid behaves like a monokaryon and could form dikaryons with monokaryons of mating type A_2B_1 and A_2B_2. Dikaryosis requires that different factors are present in the two nuclei; that is, it is nonself idiomorphs that are compatible rather than self-idiomorphs being incompatible. Such diploids of *Coprinus* can only act as donors, not recipients, of migrating nuclei. Migration is sometimes unilateral in conventional monokaryon + monokaryon dikaryosis. The inability to accept nuclei is not related to the mating type factor, but is regulated by other genes.

Armillaria mellea has a tetrapolar mating system but is unusual in normally having one diploid nucleus in each cell of its rhizomorphs and in most cells of its fruit body stem and cap. However, in the gill tissues there are multinucleate cells from which arise dikaryotic hyphae with paired nuclei and clamp connections. Young basidia are also binucleate, and seem to undergo a perfectly normal karyogamy and meiosis. Nuclei in the binucleate cells seem to be haploid but how the haploids are derived from diploid nuclei in fruit body tissue is

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unknown. On the other hand, compatible matings between haploid spores give rise to the uninucleate diploid as the only stable mycelial growth, the diploid condition arising as initial conjugate divisions form unusual patterns that result in uninucleate, diploid, cells. Interestingly, there is a recessive mutation of *Schizophyllum commune*, called *dik*, which converts dikaryotic hyphae into diploids when homozygous.

All of the emphasis in studies of incompatibility in *Coprinus cinereus* and *Schizophyllum commune* has centered on the mating type factors. Indeed, deliberately inbred strains of *S. commune* have often been used to provide an isogenic background in which the gene of interest can be the only one that differs between test strains. Similarly, a few closely related strains of *C. cinereus* have been used for the bulk of the research in the hope of minimizing complications arising from the genetic background. Both of these strategies minimize or even abolish vegetative incompatibility, so it is not surprising that incompatibilities due to genes other than mating type factors are rarely, if ever, encountered with these 'model organisms'. This does not mean that vegetative incompatibility does not occur in basidiomycetes.

Coprinus bisporus is bipolar in terms of mating type but shows additional incompatibilities due to interactions between other loci; this is similar to the heterogenic incompatibility of *Podospora* in which two monokaryons are incompatible if one has an allele C2 and the other allele D2. The combinations C1 + D1, C1 + D2, and C2 + D1 are compatible (providing the mating type factors are also a compatible combination). This heterogenic incompatibility was found to be common in isolates of the species collected from nature. *C. bisporus* is secondarily homothallic and its dikaryons do not normally donate nuclei to each other. These features tend to preserve heterozygosis in the population and the heterogenic incompatibility reinforces isolation between strains. *Heterobasidion annosus*, which is bipolar, has incompatibility due to five genes, each with two alleles called plus (+) and minus (-), superimposed on the A factor compatibility reaction. Compatibility requires that the confronting strains both have the plus allele of any one of these five genes (homozygosity for the minus allele does not confer compatibility). The system results in the natural population being comprised of several intersterile groups, genetically isolated from each other and with the potential to show further evolutionary divergence.

In *Stereum hirsutum* the primary mycelium emerging from a single basidiospore, and secondary mycelium, formed by hyphal fusions between compatible primary mycelia, both have multinucleate cells with multiple clamp connections at the septa. In this species (and other species of *Stereum*, *Phanerochaete* and *Coniophora*) two different breeding strategies occur, called outcrossing and non-outcrossing. Some non-outcrossing reactions between non-sibling primary mycelia result in a macroscopic rejection response where the mycelia confront each other, and this can be used to assign isolates to non-outcrossing interaction groups. However, some non-outcrossing reactions result in non-sibling mycelia intermingling without rejection. Rejection is taken to mean genetic difference between the isolates, but intermingling does not correspond to genetic identity. For example, in interactions between three interaction groups of *S. sanguinolentum*, two isolates that rejected each other both intermingled with the third. Outcrossing interactions between non-sibling primary mycelial isolates produce a range of recognition responses encompassing compatibility, resulting in general or localized formation of a stable secondary mycelium. Rejection is signified by the formation of narrow demarcation zones of sparse, pigmented mycelium (with numerous vacuolated cells) between the colonies, and the inhibition of extension of the colony margin of one or both of the mycelia, or the formation of flattened (called appressed) mycelium containing abnormal hyphae beyond the interaction interface. The pattern of formation of secondary mycelium indicates a multiallelic unifactorial (that is, bipolar) mating type system on which the other phenotypes (controlled by several other genes) are superimposed.

We have described a wide range of incompatibility reactions in the last few sections. You will have noticed that what is true for one organism may not apply to another, even closely related, species. Don't get confused. Remember two things. First, fungi are very flexible organisms and those we find in nature today have each evolved a strategy to manage their interactions which best suits them in the environment in which they are competing. Second, in studying that strategy we need to use a standard approach that involves confronting one mycelium with another, and then studying what happens. We do this in the hope of recognizing general categories that describe the manner in which the fungi interact, but in many cases our understanding is only partial, and may even be biased by the techniques chosen to make the observations. The crucially important point is that individuality ***is*** of prime importance to fungi and they have many ways of expressing their individuality.

2.6 Structure and function of mating type factors: mating type factors in *Saccharomyces cerevisiae*

The life cycle of the yeast *Saccharomyces cerevisiae* features an alternation of a haploid phase with a true diploid phase, and in this respect differs from filamentous Ascomycotina in which the growth phase after anastomosis is a heterokaryon. There are two mating types: haploid yeast cells may be of mating type a, or α. Karyogamy (nuclear fusion) follows the fusion of cells of opposite mating type and then the next daughter cell that is budded off contains a diploid nucleus. Most natural yeast populations are diploid because the haploid

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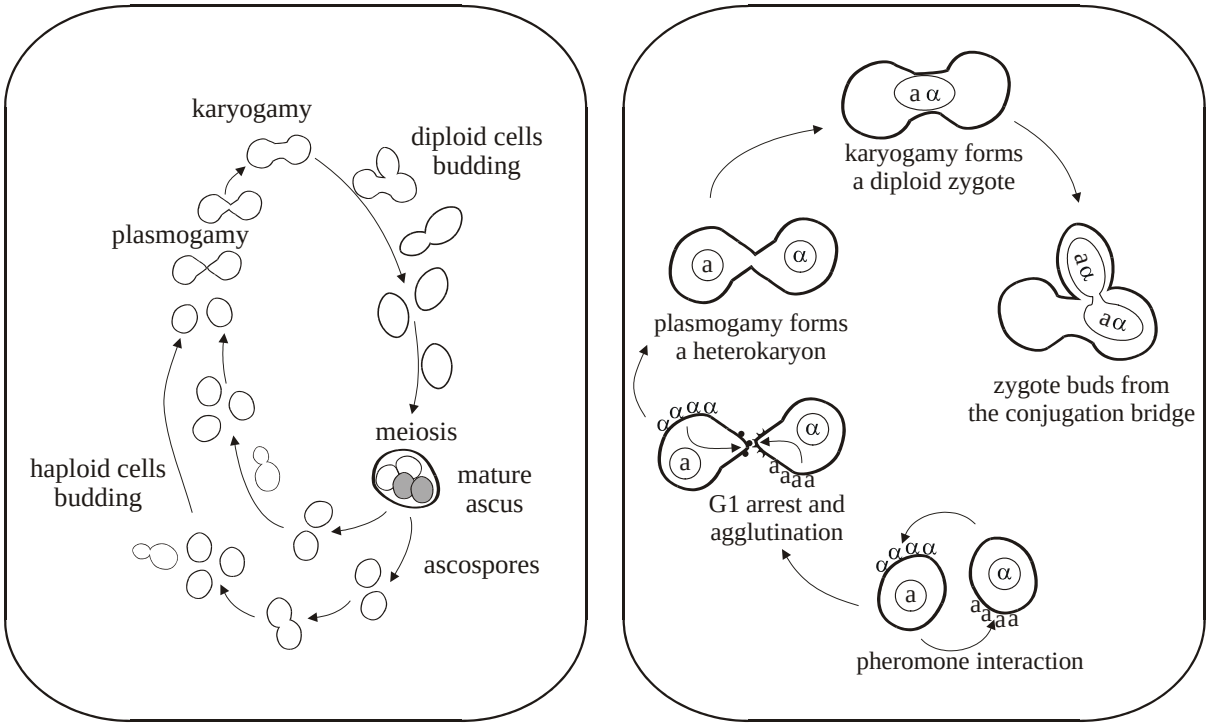

Fig. 2.4. Life cycle (left hand panel) and mating process of the yeast *Saccharomyces cerevisiae*. Yeast can reproduce asexually by budding. Haploid cells of different mating types fuse to form dumbbell-shaped zygotes, which can themselves bud to establish a diploid clone. Well-nourished diploid cells, which are exposed to starvation conditions, enter meiosis, forming a 4-spores 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. The right hand panel depicts pheromone interaction, agglutination and the mating process in a little more detail.

meiotic products mate while they are still close together immediately after meiosis. Diploid cells reproduce vegetatively by mitosis and budding until particular environmental conditions (deficiency in nitrogen and carbohydrate, but well aerated and with acetate or other carbon sources which favor the glyoxylate shunt) induce sporulation. When that happens, the entire cell becomes an ascus mother cell; meiosis occurs and haploid ascospores are produced. Ascospore germination re-establishes the haploid phase, which is itself maintained by mitosis and budding if the spores are separated from one another (by an experimenter in a laboratory, or by some disturbance in nature) to prevent immediate mating (Fig. 2.4).

Mating type factors of yeast specify peptide hormones; these are called pheromones (the term originally applied to mate-attracting hormones of insects and mammals) and there are both pheromone α- and a-factors (Fig. 2.5), and corresponding receptors specific for each pheromone. Pheromones organize the mating process; they have no effect on cells of the same mating type or on diploids but their binding to pheromone receptors on the surface of cells of opposite mating type (Fig. 2.4) act through GTP binding proteins to alter metabolism and: (i) cause recipient cells to produce an agglutinin that enables cells of opposite mating type to adhere; (ii) stop growth in the G1 stage of the cell cycle; (iii) and change wall structure to alter the shape of the cell into elongated projections. Fusion eventually occurs between the projections.

The mating process of *S. cerevisiae* is controlled by a complex genetic locus called *MAT* at which two linked genes are located (a1, a2 for mating type a and α1, α 2 for mating type α). The *MAT*a locus encodes a1 and a2 polypeptides, the messengers for which are transcribed in opposite directions (Fig. 2.6), and *MAT* α encodes polypeptides α 1 and α 2. Heterozygosity at *MAT* is a sign of diploidy and eligibility to sporulate; even partial diploids carrying *MAT*a/*MAT* α will attempt to sporulate. In haploid cells, the α 2 polypeptide represses transcription of a-factor in α -cells, whilst a1 represses α -specific genes in a-cells. The α 1 protein activates transcription of genes coding for α -pheromone and the surface receptor for the a-factor. In a/ α diploids, interaction occurs between a1 and α 2 polypeptides to form a heterodimer, which represses genes specific for the haploid phases, including a gene called *RME*1, which itself suppresses meiosis and sporulation (Fig. 2.6).

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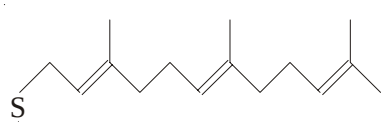
α-factor

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-$COOCH_3$

Fig. 2.5. Simplified chemical structures of yeast pheromones.

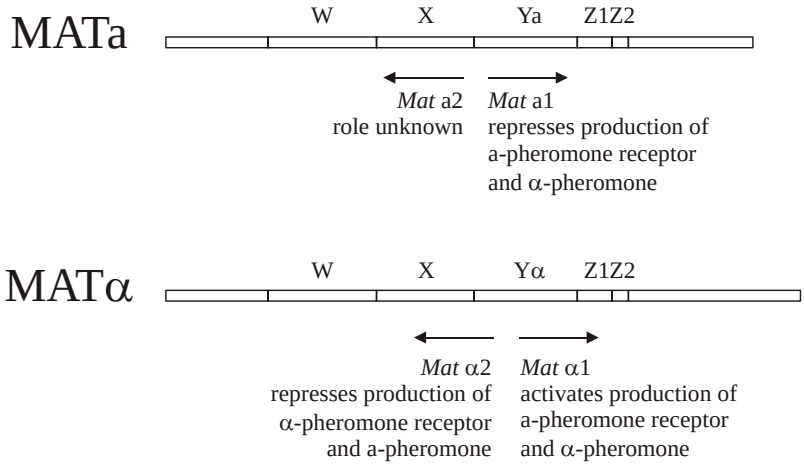


Fig. 2.6. Functional domains in mating type factors of *Saccharomyces cerevisiae*. Region Y is the location of the mating type idiomorphs, which have very little homology with each other. Ya is 642 bp long, Yα is 747 bp long. Regions W, X, and Z1 and Z2 are homologous terminal regions. The arrows indicate 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 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).

Saccharomyces cerevisiae is heterothallic but a clone of haploid cells of the same mating type frequently sporulates, and there will be equal numbers of a and α cells amongst the progeny. This results from mating type switching controlled by the gene *HO* (HOmothallic) that exists in two allelic forms (dominant *HO* and recessive *ho*), and encodes an endonuclease. On either side of the *MAT* locus, and on the same chromosome, there are silent storage loci for each mating type, called *HML* and *HMR*. The *HO*/*ho* endonuclease creates a double-strand break at the *MAT* locus that initiates switching of information, by an homologous recombination event between the two parts of the same chromosome, at the *MAT* locus with that at either *HML* or *HMR* (Fig. 2.7).

Since yeasts can live in very small habitats, like flower nectaries and surfaces of individual fruits, rare mating type switching will give isolated populations the opportunity to undergo sexual reproduction; this is presumably its selective advantage. Mating type switching occurs about once in 10^5 divisions in cultures carrying allele *ho*, whereas in strains carrying *HO* the switch occurs at every cell division. However, there is an asymmetry in the cell division in that a new daughter bud is not able to switch mating types until it has itself budded. In *S. cerevisiae*, this is achieved by actively transporting into the budding daughter cell the mRNA of a gene called *Ash1*, which encodes an inhibitor of the HO-endonuclease. Consequently, immediately after each division only the mother cell is switchable, which means that even if there is only one cell to start with, a single division cycle will produce two cells of opposite mating type. If you think that's a clever arrangement, the switch to *opposite* mating type is assured because a 250 bp recombination enhancer controls recombination in the arm of chromosome III on which all these genes are located. This control region ensures that in *MATa* cells

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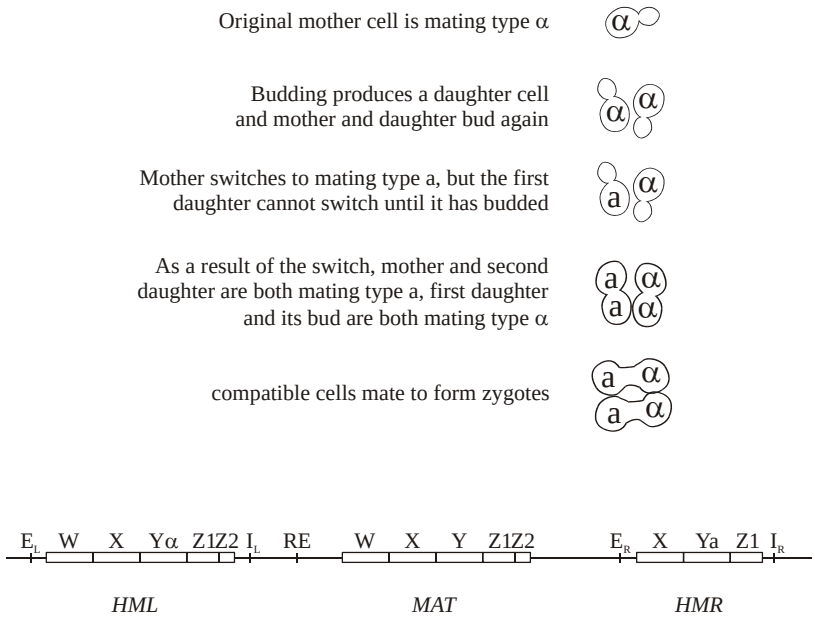

Fig. 2.7. Top: pattern of mating type switching in *Saccharomyces cerevisiae* showing the consequences of a mating type switch in one mother cell. Bottom: the three loci involved in mating type switching, *HML*, *MAT* and *HMR*, are located on the same chromosome (***not*** drawn to scale). *HML* is about 180 kb from *MAT*, and *HMR* about 120 kb from *MAT*; the centromere is located between RE and the *MAT* locus. A double strand break at the *MAT* locus, caused by the HO endonuclease, initiates a recombination event that replaces the Y region of the *MAT* locus with Y sequences from one of the storage loci. *HML* and *HMR* contain complete copies of the mating type genes but are not expressed because they have a repressed chromatin structure imposed by the E and I silencer sequences. *HML* shares more of the *MAT* sequences (W, X, Z1 and Z2) than does *HMR*. RE is a recombination enhancer that controls preferential recombination between *MAT*a and *HML*, or between *MAT*α and *HMR*.

the resident *MATa* locus recombines with *HML*, which contains a silent *MAT*α locus, whereas in *MAT*α cells the resident locus recombines with *HMR*, which contains a silent *MATa* locus. Now, *that's* clever!

Mating type switching also occurs in the distantly related fission yeast *Schizosaccharomyces pombe* but this organism uses the asymmetry of DNA replication to establish an asymmetrical mating-type switching pattern. When *S. pombe* divides, the two daughter cells exhibit different developmental programmes: one is mating type switchable, the other is unswitchable. Genetic experiments show that in switchable cells the expressed (*mat1*) mating type locus has an imprint that marks it as a candidate for the intrachromosomal recombination event that makes the mating type switch. The imprint is a modification in one strand of the DNA, possibly a 'nick' (a broken phosphodiester bond) or an RNA primer left from the DNA synthesis during the previous mitotic division. During DNA replication the strand-specific imprint is made at the *mat1* locus only during lagging-strand synthesis, so only one of the sister chromatids will carry the imprint. The cell that inherits the imprinted chromosome becomes a switchable cell, while its sister remains unswitchable. When the imprinted chromosome is replicated, the DNA replication complex runs into the imprinted modification in the DNA, the replication fork stalls, and the result is a transient double strand break that initiates the recombination required for mating-type switching.

Mating types in filamentous fungi tend to be far more stable although unidirectional switching of mating type has been reported in some filamentous ascomycetes, though with no molecular details yet. Oddly enough, switching does not occur in any of the best-studied organisms like *Neurospora*, *Aspergillus*, or *Podospora*, but has been claimed in *Chromocrea spinulosa*, *Sclerotinia trifoliorum*, *Glomerella cingulata* and *Ophiostoma ulmi*.

2.7 Structure and function of mating type factors: mating types of *Neurospora*

Species of *Neurospora* exhibit four different mating strategies: (i) bipolar heterothallism with mating types *A* and *a* (in *N. crassa*, *N. sitophila*, *N. intermedia* and *N. discreta*) but mating type genes are present in a single copy per genome (unlike *Saccharomyces cerevisiae*); (ii) secondary homothallism (in *N. tetrasperma*) through the production of asci containing four ascospores each containing compatible nuclei; (iii) primary homothallism

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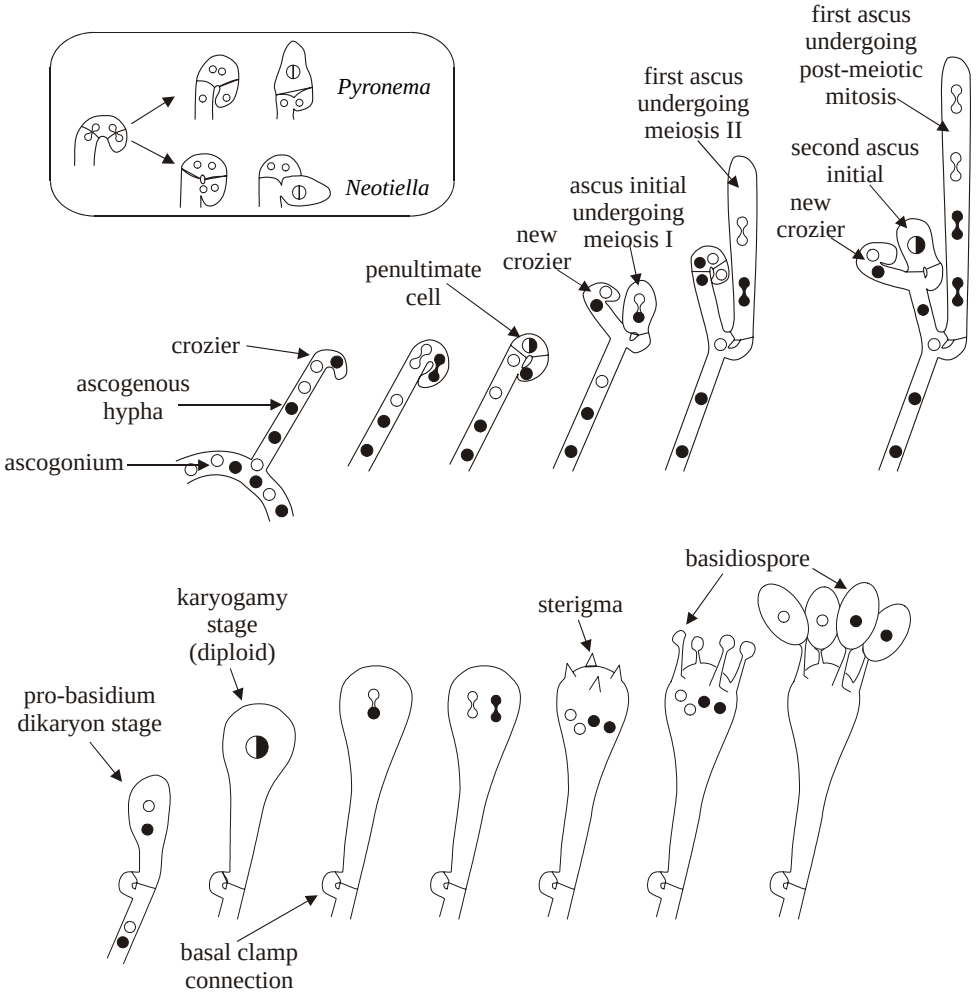

Fig. 2.8. Meiosis and sporulation in Ascomycota and Basidiomycota. The major panel of diagrams at the top shows ascus formation. Hyphal fusion or similar mating between male and female structures results in nuclei moving from the male into the female to form an ascogonium in which male and female nuclei may pair but do not fuse (transient dikaryon stage). 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. The cell at the bend of the crozier is binucleate but the other two cells are uninucleate. Generally, 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 (the octad). The boxed inset shows that karyogamy may occur in the penultimate cell of the crozier (*Pyronema*-type) or the terminal and stalk cell nuclei might fuse (*Neotiella*-type).
The panel of diagrams at the bottom shows basidium formation in a 'classic' mushroom-fungus. The basidium arises as the terminal cell of a dikaryotic hyphal branch that 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 these diagrams indicates how tempting it is to suggest some evolutionary relationship between crozier formation and the early stages of basidium and clamp connection formation. Figs 2.8 to 2.12 adapted and redrawn from Moore, D. (1998). *Fungal Morphogenesis*. Cambridge University Press: New York.

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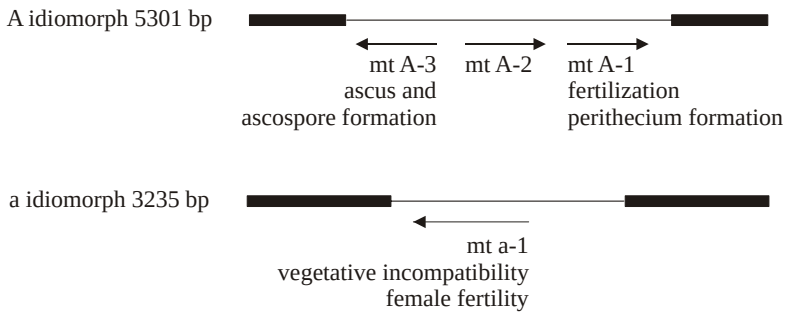

Fig. 2.9. Functional regions of mating type factors of *Neurospora crassa*. The arrows indicate direction of transcription and the legends beneath the arrows indicate functions of the gene products. The black bars represent the conserved DNA sequences either side of the idiomorphs, which are shown as lines. These diagrams are oriented so that the centromere is on the left; consequently the centromere-distal sequence is on the right.

in which each haploid genome carries genetic information of both mating types (*N. terricola*, *N. pannonica*); and (iv) primary homothallism, but in which genetic information for only one mating type can be detected (for example, *N. africana* possesses only an *A* idiomorph which shows 88% homology with the *A* idiomorph of *N. crassa*).

Species that show primary homothallism form linear eight-spored asci (octads) in which all progeny are self-fertile. In the bipolar heterothallic species, strains of both mating types develop female structures (protoperithecia and their receptive hyphae, the trichogynes) under nitrogen starvation, as well as asexual spores (macroconidia or microconidia). Since the spores can serve as the male in a sexual cross these mycelia are hermaphrodites. Migration of (the still haploid) nuclei into the *N. crassa* ascogonium (see Fig. 2.8) depends on mating type gene function. After the arrival in the ascogonium, a series of mitotic divisions occur. There must be some mechanism to sort nuclei, to ensure that meiosis only involves one *a* and one *A* nucleus, because mating type *always* segregates 1:1 in the progeny. It is likely that transient dikaryosis in the crozier involves a nuclear recognition mechanism. However, crozier abortion occurs even in normal ascosporogenesis, so an alternative process might be that a non-selective nuclear migration results in abortion of croziers that do not contain one *a* and one *A* nucleus.

Mating is followed by the formation of perithecia, within which as many as 200 asci are formed. Each ascus contains the products of a single meiosis. In many filamentous ascomycetes, a post-meiotic mitotic event, before ascospore formation, results in each ascus containing an octad comprised of four pairs of sister ascospores.

The best-characterized mating-type loci from filamentous ascomycetes are those of *Neurospora crassa*, *Podospora anserina* and *Cochliobolus heterostrophus*. *N. crassa* and *P. anserina* mating type loci contain essentially the same genes, but *C. heterostrophus* has a simpler arrangement. Mating type genes of *Magnaporthe grisea* are probably similar to those of *N. crassa*.

The first mating type genes to be cloned and sequenced in filamentous fungi were the *A* and *a* loci of *Neurospora crassa* (Fig. 2.9). In all the known *Neurospora A* and *a* idiomorphs the flanking regions are conserved, the region on the centromere side contains species-specific and/or mating type-specific DNA sequences. Immediately adjacent to these segments are regions that are very different between species. Next to these species-variable regions are the idiomorphs themselves. These 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 very similar between species and between the two mating types.

The *A* idiomorph is 5,301 bp in length and gives rise to at least three transcripts (MAT *A*-1, *A*-2 and *A*-3), the first two being transcribed in the same direction (Fig. 2.9). The 85 amino acids at the N-terminal region of the mating type *A* product are the minimum required for expression of female fertility. The region from position 1 to 111 determines the vegetative incompatibility activity of the mating type locus, and amino acids from position 1 to 227 are required for male-mating activity. Mating type-specific mRNA is expressed constitutively in vegetative cultures, and continues to be expressed after mating both before and after fertilization. Transcript MAT *A*-1 is very similar to *MAT*∀1 of *Saccharomyces cerevisiae* and is essential for fertilization and fruiting body formation.

The other two transcripts, MAT *A*-2 and MAT *A*-3, increase fertility and are essential in events after fertilization, including ascus and ascospore formation, but are not essential for sexual development, which is controlled by MAT *A*-1. The MAT *A*-3 transcript has DNA binding ability and might function as a transcription-

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regulating factor. The function of the MAT *A*-2 polypeptide is not known. Its sequence contains motifs that occur in transcription activator proteins, but there are no obvious relatives in sequence databases other than the *Podospora* mating type homologue, SMR1.

The *a* idiomorph of *N. crassa* is 3235 base pairs long and gives rise to a single transcript (called MAT *a*-1) which encodes one polypeptide of 288 amino acids with DNA-binding activity. MAT *a*-1 is the only gene essential to mating in the a idiomorph, and has a role in the dikaryon stage. Amino acids 216-220 of the MAT *a*-1 polypeptide act in vegetative incompatibility while the region with DNA-binding activity is responsible for the mating function, implying that vegetative incompatibility and mating work through different mechanisms. The DNA sequences bound by MAT *a*-1 center on CAAAG sequences, similar to 'high mobility group' (HMG) proteins that bind in the minor groove of the DNA helix and introduce a bend in the DNA molecule. DNA binding targets differ in different developmental stages, and the specificity may result from MAT *a*-1 interacting with unidentified protein factors. Mutations in either MAT *a*-1 or MAT *A*-1 cause mating defects.

N. crassa trichogynes respond to pheromones by orienting towards the pheromone source in a mating type-specific way. Mating type mutants do not orient their growth towards pheromones. DNA sequences of fungal pheromone receptors predict a product with seven transmembrane segments with the ability to interact with a heterotrimeric G-protein linked to a protein kinase cascade. Transcription of pheromone receptor genes is regulated by the mating type factors (in basidiomycetes, pheromone receptors are products of a mating type locus, see below). A G-protein encoding gene with mating function, called *gna-1*, has been characterized in *N. crassa*. Female fertility is lost if *gna-1* is disrupted. Female infertility also results if the *gna-1* homologue in *Cryphonectria parasitica*, which is called *cpg-1*, is disrupted. This suggests that this G-protein is a component of a female pheromone response pathway that has been conserved in these filamentous ascomycetes.

The *mat*+ mating type idiomorph of *P. anserina* contains a single gene (called *FPR*1), which is homologous to *Neurospora* MAT *a*-1. The *mat*- idiomorph encodes three genes (*FMR*1, *SMR*1, *SMR*2) similar to MAT *A*-1, *A*-2, and *A-3* of *Neurospora.* Functions of the *P. anserina* genes are very similar to those of their counterparts in *N. crassa*. The mating type idiomorphs of *C. heterostrophus* each contain only one gene. One of the idiomorphs contains a sequence homologous to the Mat-α1 of *S. cerevisiae*, and the other contains a sequence homologous to the *N. crassa* MAT *a*-1 gene.

2.8 Structure and function of mating type factors: mating type in *Ustilago maydis*

Ustilago maydis causes the smut disease of maize. It has a tetrapolar mating system comprising one multiallelic mating type factor and one with only two alleles. *Ustilago* produces unicellular, haploid sporidia that grow vegetatively by budding like a yeast phase; these can be cultured on synthetic media and are non-pathogenic for the host plant (Fig. 2.10). Conjugation tubes are formed when sporidia of opposite mating type are mixed, and fusion of these produces the dikaryon, which then grows as a filamentous fungus. The dikaryon is the pathogenic stage. Fusion of sporidia is controlled by the biallelic '*a*' mating type locus, the heterozygous *a*1/*a*2 genotype being required for conjugation and the transition between the yeast and filamentous forms. The multiallelic '*b*' locus stops diploid cells fusing, determines the true hyphal growth form, and pathogenicity.

The *a*1 idiomorph consists of 4.5 kb of DNA and the *a*2 idiomorph is 8 kb. Two genes have been identified in these regions: *mfa*1 (in *a*1) and *mfa*2 (in *a*2) code for pheromones, and *pra*1/*pra*2 encode pheromone receptors. The pheromones diffuse away from their producer cells and induce conjugation tubes after binding to pheromone receptors on cells of opposite mating type. Pheromone signaling is also necessary for the maintenance of the filamentous dikaryon after cell fusion. Pheromone induces all of the mating-type genes to levels 10 to 50 times higher than their basal level. The upstream control element responsible for this pheromone stimulation, which is called the pheromone response element (PRE), has the sequence ACAAAGGG. It is on the same DNA molecule as the pheromone genes so it is called a '*cis*-acting element', by analogy with chemical terminology which describes two substituents on the same side of an axis of symmetry in a molecule as the *cis*-configuration, and the alternative of two substituents on opposite sides of an axis as the *trans*-configuration.

The PRE sequence is similar to the consensus sequence recognized by HMG polypeptides, including MAT *a*-1 of *N. crassa* (see section 2.7 above). A gene called *pfr1* (*p*heromone *r*esponse *f*actor), the product of which binds to the PRE sequences found in both *a*1 and *a*2 idiomorphs, encodes the controlling transcription factor. The downstream pheromone response pathway includes at least one MAP kinase encoded by a gene called *fuz7*, which is homologous to the 'archetypal' MAP kinase gene of *Saccharomyces cerevisiae*, *STE7* (a MAP kinase is a 'mitogen activated protein kinase', where a mitogen is any agent that induces mitosis; see section 10.11). Disruption of *fuz7* results in phenotypes that show that *fuz7* is involved in *a*-dependent mating events like conjugation tube formation, conjugation and establishment and maintenance of filamentous growth. Other components of the pheromone response pathway that have been found are four genes encoding G-proteins (*gpa1* to *4*). There are indications that *fuz7* and *gpa3* do not belong to the same pathway, so there may be several pheromone responses in *U. maydis*, either in parallel or in series.

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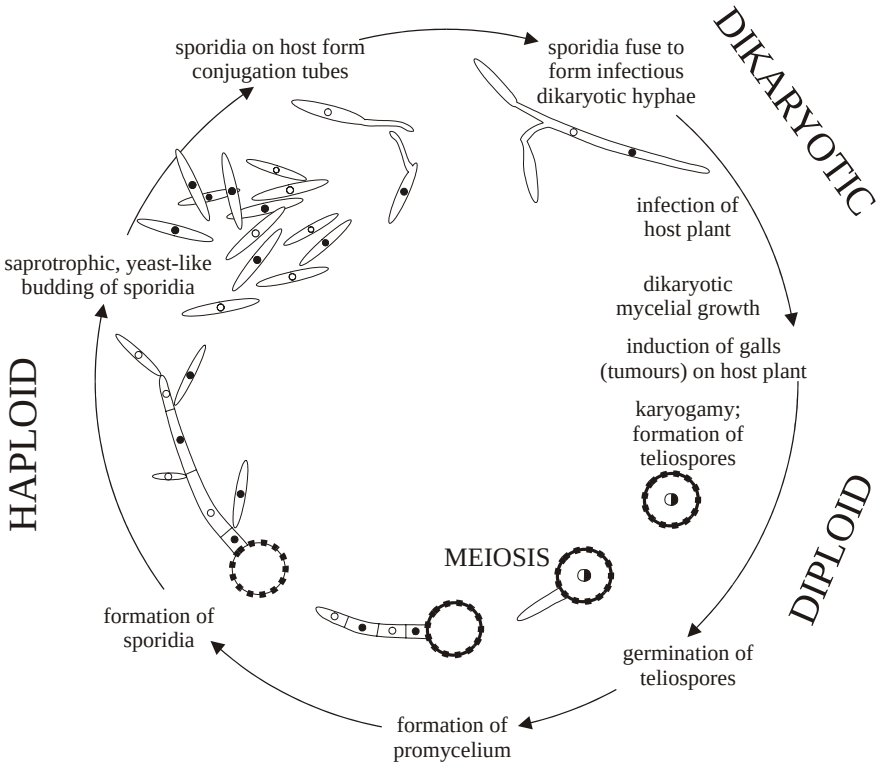

Fig. 2.10. Diagram of the life cycle of *Ustilago maydis*.

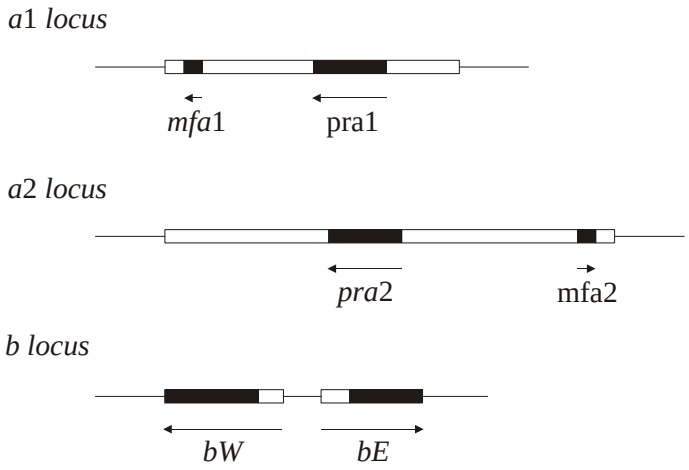

Fig. 2.11. Schematic representations of the structures of the a and b mating type loci of *Ustilago maydis*. Idiomorphs of the a locus consist of mating-type specific (i.e. variable) DNA sequences (4500 base pairs in a1, 8000 base pairs in a2), here shown as open boxes, within which are the genes for mating (*mfa* and *pra*). The b locus has two reading frames, bW and bE, which produce polypeptides containing domains of more than 90% sequence identity (shown as black boxes) and variable domains (open boxes) which show 60 to 90% identity. Arrows indicate the direction of transcription. The PRE sequence mentioned in the text is a very short control site upstream of the pheromone genes, *mfa*1 and *mfa*2.

The *b* mating type factor contains two genes which are transcribed in opposite directions (Fig. 2.11): *bE* and *bW* (= East and West) with coding sequences equivalent to polypeptides of 473 and 629 amino acids, respectively. The amino terminal end of the coding sequence is highly variable, whereas the carboxy-terminal end is conserved in different *b* idiomorphs. The bE and bW proteins are, respectively, the HD1 and HD2 homeodomain proteins that form a dimer that is a transcription activator of genes required for the sexual cycle

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and/or repressor of haploid-specific genes (interaction of the equivalent *Coprinus* homeodomain proteins is illustrated in Fig. 2.14). Dimers comprised of bE & bW from the same idiomorph are inactive; the heterodimer functions properly only when the proteins come from different idiomorphs. The proteins encoded by these genes contain sequences homologous with DNA-binding homeodomain regions of known transcription-regulating factors, which is why they are called HD1 and HD2, and HD1 and HD2 may themselves encode transcription factors. Significantly, the homeodomain is an extended helix-turn-helix DNA-binding motif (see section 10.3), which is encoded by a conserved DNA sequence of about 180 bp called the homeobox. This sequence is particularly associated with the transcriptional regulators of homeotic, or *Hox*, genes that were found originally in the fruit fly *Drosophila*, and are involved in orchestrating development in higher eukaryotes. Mutations in animal *Hox* genes convert one body part into another. *Drosophila* has two *Hox* clusters, but vertebrates have four clusters of 9 to 11 genes each on different chromosomes. Vertebrate *Hox* genes are expressed in different patterns and at specific embryological stages. There is a compelling comparability between these developmental regulators and the HD1/HD2 genes of the fungal mating type factors in *Schizophyllum* and *Coprinus*, as well as *Ustilago*.

2.9 Structure and function of mating type factors: mating types in *Coprinus cinereus* and *Schizophyllum commune*

C. cinereus and *S. commune* exhibit tetrapolar heterothallism, determined by two mating type factors, called *A* and *B*. The natural population contains many different mating types. In crosses these behave like multiple alleles of the two mating type loci. Molecular analysis has revealed that each mating type locus is a very complex region containing several or even many genes (which is why we refer to mating type *factors*). The genes at *A* encode transcription factor homeodomain proteins and genes at *B* encode lipopeptide pheromones and pheromone receptors. Mating type factors are located on different chromosomes, and even conventional genetic analysis has demonstrated internal structure, identifying subloci that are called *A*α, *A*β, *B*α and *B*β. In *S. commune* these subloci are relatively far apart, in terms of linkage distance, but they are much closer in *C. cinereus* (Fig. 2.12). The α and β subloci are functionally redundant in the sense that a difference need exist at only one of them for compatibility. Nine versions of *A*α, and 32 of *A*β results in 288 different *A* mating type specificities in *S. commune*. In *C. cinereus*, there are an estimated 160 *A* mating type specificities in the natural population.

A compatible mating in both fungi, characterized by clamp connections and conjugate nuclear divisions in the mated hyphae, requires that both *A* and *B* are different. In the belief that in this state the mating type factors are fully active, it is called *A*-on, *B*-on (Fig. 2.3). Cytological observations indicate that mating type factor *A* controls nuclear pairing, clamp cell formation and the synchronized (conjugate) mitosis of nuclei, while mating type locus *B* controls nuclear migration and clamp cell fusion.

Dikaryons arise when both *A* and *B* are different but heterokaryons can also be formed in matings in which one of the mating type factors is homozygous. When *A* factors are the same (called common-*A* or *A*-off, *B*-on), nuclear migration takes place but no clamp connections form. Mating strains carrying the same *B* factor forms a heterokaryon (called common-*B* or *A*-on, *B*-off) only where the mated monokaryons meet because nuclear migration is blocked. In this case, apical cells of heterokaryotic hyphae start to make clamp connections and the nuclei divide but the clamp (hook) cell fails to fuse with the adjacent cell and the nucleus in the clamp remains trapped.

Detailed molecular analysis of the *A* mating type factors show that they contain many more genes than classical genetic analysis could reveal (Fig. 2.13). In fact, each *A* locus of *C. cinereus* contains a variable number of genes, which are arranged in pairs like the *bE-bW* pair in the *U. maydis b* locus. The *C. cinereus* gene pairs were originally designated *a*, *b*, and *d*. Unfortunately, the first locus sequenced had a nonfunctional gene (which was labeled *c*) between the *b* and *d* pairs; so the *c* genes do not exist. Recently, the gene pairs were redesignated and they are now called groups 1, 2, and 3. Each group within the *A* locus encodes two dissimilar homeodomain proteins (HD1 and HD2) which are homologous to the *S. cerevisiae MAT α2* and *MAT a1* mating proteins, respectively.

Several features combine to ensure that there is no intragenic recombination within the A locus, which is likely to disturb its regular 'two-by-two' structure. The groups are organized into cassettes so that they act as a single unit and the DNA sequences are sufficiently different between groups 1, 2 and 3 to avoid homologous recombination. In addition, the paired genes are transcribed in opposite directions (Figure 2.13). The *A* locus is bounded by DNA sequences that ***are*** homologous in all *A* mating type specificities, called α-*fg* and β-*fg*; also, the group 1 gene pair is separated from groups 2 and 3 by a 7 kbp DNA sequence that is homologous in all *A* loci (known as the 'homologous hole'). The group 1 gene pair corresponds to the *A*α sublocus defined by conventional linkage analysis, while the group 2 and group 3 gene pairs comprise the *A*β sublocus; 7 kbp is approximately equivalent to the 0.1% recombination observed between these subloci (Fig. 2.12). These short homologous sequences limit recombination to the regions between the homeodomain loci.

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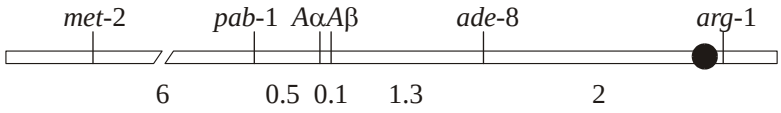
Coprinus cinereus



Schizophyllum commune

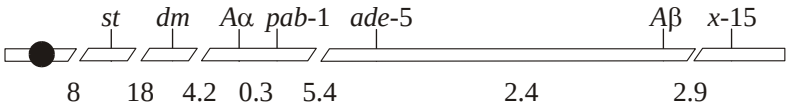
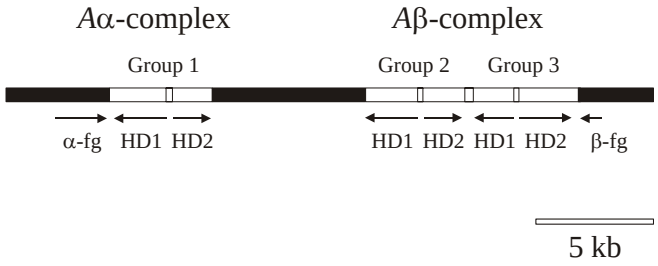


Fig. 2.12. Linkage maps of the chromosomes which carry the *A* mating type factor in *Coprinus cinereus* and *Schizophyllum commune*. Conventional genetic analysis reveals the subloci called *Ą* and *Ą*. In *S. commune* these subloci are relatively far apart but they are much closer in *C. cinereus*. The closed circles indicate the positions of the centromeres.

Coprinus cinereus
A factor archetype



Schizophyllum commune
*A*α sublocus

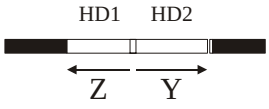


Fig. 2.13. Diagrams of parts of the *A* mating type factors in *Coprinus cinereus* and *Schizophyllum commune*. Arrows show the direction of transcription. The (predicted) archetypal *A* factor from *Coprinus cinereus* has three pairs of functionally redundant genes (group 1, group 2, & group 3) which encode the homeodomain proteins (HD1 & HD2). The α-*fg* and β-*fg* sequences are homologous in all *A* mating type specificities in *C. cinereus*. Interaction between HD1 and HD2 proteins is the basis of the compatible reaction (see Fig. 2.14). *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 Z and Y and encode HD1 and HD2 respectively. Again, different idiomorphs are found in different natural mating types; indeed, the Z gene is absent in the *Ą*1 mating type. Figs 2.13 & 2.14 based on illustrations in Brown, A. J. & Casselton, L. A. (2001). Mating in mushrooms: increasing the chances but prolonging the affair. *Trends in Genetics* **17**, 393-400.

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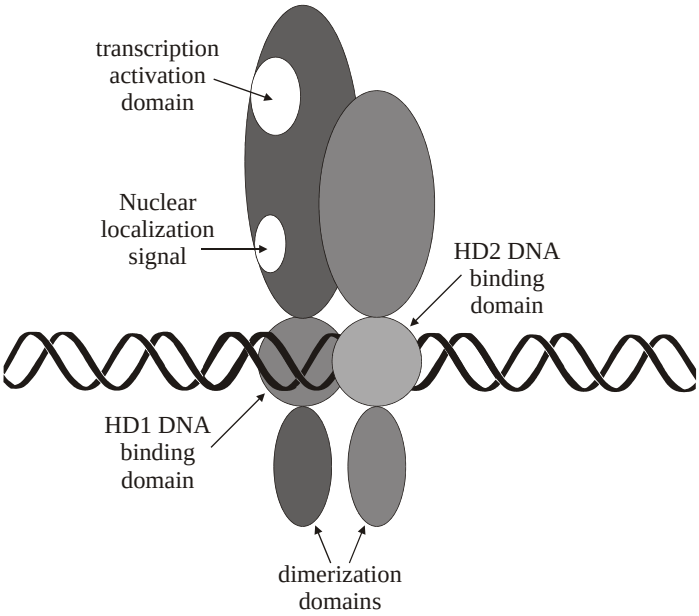

Fig. 2.14. Schematic diagram showing a model of homeodomain protein interactions involved in *A* mating type factor activity in *Coprinus*.

The three pairs of homeodomain genes are thought to have arisen by duplication but are now functionally independent, and redundant in the sense that only one compatible HD1/HD2 gene combination is required to promote sexual development. However, compatible pairs must come from the same subset of genes (that is, group 1 genes work only with group 1 genes, group 2 genes only work with group 2 genes, etc.), and providing HD1 and HD2 proteins come from different mating type loci. The many *A* mating-type specificities of *C. cinereus* are derived from different combinations of alleles of the group 1, 2, and 3 genes. Only five or six alleles of each gene pair are needed to generate the estimated 160 specificities of mating type factor *A* found in nature. However, there are so many functionally redundant genes in the *A* locus that some A mating type specificities in nature have lost one or more of them. Nine loci have been examined so far; only one (A_{44}) has all six genes, in mating type A_6, group 1-1 and group 3-2 are missing, and in mating type A_5, group 3-1 is missing.

Basidiomycete sexual development is triggered by a dimerization between HD1 and HD2 proteins from the different *A* mating type factors of compatible individuals (Fig. 2.14). The N-terminal regions of these proteins are essential for choosing a compatible partner but not for regulating gene transcription. Sequences of different idiomorphs of the mating type genes are dissimilar and have been interpreted as being equivalent to the highly variable region in major histocompatibility loci in mammals that forms a self/nonself recognition system. The N-terminal region of the mating type protein product is the part that is essential for such self/nonself recognition. It ensures that monomers from the same mating type idiomorph are not compatible, and that only the ***hetero***dimers made between the products of the two compatible mating type factors present in the cell are able to form the DNA-binding transcription regulators. It has also been shown that compatible protein-protein interactions (heterodimerization) is more important to compatibility at the *A* locus than is the occurrence of two homeodomains. The HD2 homeodomain is crucial to DNA binding, but the homeodomains of HD1 proteins are relatively dispensable.

The *A* locus of *S. commune* also controls nuclear pairing, clamp connection formation, conjugate nuclear division and clamp septation. The *A*α locus contains the *Y* gene (which has alleles *Y1, Y*3, and *Y*4) and the *Z* gene (with alleles *Z3* and *Z*4), which encode the homeodomain proteins HD2 and HD1, respectively. The *S. commune A*α locus corresponds to a single gene pair from the *C. cinereus* complex. The *A*β locus also encodes a polypeptide with a homeodomain. *Y* and *Z* are the only determinants of *A*α activity and *A*α and *A*β function independently of each other. Interactions of the Y and Z proteins have been demonstrated experimentally for nonself combinations (for example, Y4 with Z5) proteins, but no interaction occurs between Y and Z proteins encoded by the same *A* factor (for example Y4 and Z4).

The search is under way for the genes that are regulatory targets of homeodomain proteins. Several mutants defective in mating type-regulated events have been isolated in *S. commune* and *Ustilago* but a consistent story has not yet emerged.

Most of the initial work on mating type factors in *S. commune* and *C. cinereus* was aimed at determining the structure of the *A* loci. Cloning the *B* sequences revealed that the multiallelic *B* mating type

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factor codes for several pheromone and receptor genes. In the smut fungi, pheromone signaling is important in cell fusion, in establishing the dikaryon, and in maintaining filamentous growth. However, hyphal anastomosis (= hyphal fusion) of monokaryotic vegetative cells in *S. commune* and *C. cinereus* does not depend on pheromone-based recognition. As we have discussed in section 2.1, in the saprotrophic basidiomycetes hyphal anastomoses occur readily as part of the maturation process of the mycelium, and anastomosis is independent of the mating type factors. On the other hand, it is now quite clear that pheromone signaling controls the *B*-regulated events of reciprocal nuclear migration and clamp cell fusion. Sequencing the *S. commune Bα1* region revealed a pheromone receptor gene (called *bar1*, standing for B-alpha-receptor-1) and three pheromone genes, *bap1*, *bap2* and *bap3* (*bap* stands for B-alpha-pheromone). The Bβ1 locus also contains a receptor gene (*bbr1*) and genes for pheromones, *bbp1*(*1*) and *bbp1*(*2*). The *B* factor of *C. cinereus* contains 3 groups (called 1, 2, 3) of genes which each code for a pheromone receptor and two pheromones. The *B* pheromone genes are all predicted to encode for lipopeptides similar to the *S. cerevisiae a* factor (see Fig. 2.5) while the *B* pheromone receptors are homologous to the *S. cerevisiae a* factor receptor, which is a typical G protein coupled receptor. It is worthy of note that only the *a* factor-type pheromones have been found in basidiomycetes whereas both *a*- and α-type ***receptors*** are present.

The model of pheromone function that has been developed for these filamentous basidiomycetes is that after anastomosis, the pheromones produced by the invading nucleus diffuse ahead and act as advance signals of nuclear migration. This activates receptors encoded by resident nuclei in nearby cells and the interactions prepare the cells for nuclear migration by initiating septal dissolution to allow nuclei to pass. *A*-factor functions then establish the dikaryotic state and clamp connection formation. Pheromone signaling is then further involved in clamp cell fusion. There are nine mating type specificities at the *B*α factor of *S. commune*, each encoding a receptor and one or more pheromones. Hence, each receptor must distinguish at least eight nonself pheromones. Individual pheromones may also activate more than one receptor. Work with mutants in *B*-regulated functions is beginning to identify the genes subject to pheromone signaling. Some of these mutations map to the *B* loci and affect mating specificity and could be modifiers of pheromone or pheromone receptor gene specificity. Others include nine genes that influence nuclear migration. Many of these genes are linked to *B*, suggesting that related functions are clustered.

2.10 Overview: biology of incompatibility factors

Mating type factors regulate pheromone and pheromone receptors involved in mating, and this might be interpreted as ranging from recognition between sexually competent cells in yeast to governing growth of clamp connections in basidiomycetes. It has also been suggested that *B*-mating type pheromone signalling is involved in internuclear recognition, and, in particular, regulation of the distance between the two nuclei in the dikaryon. Furthermore, heterodimerization of homeodomains from different idiomorphs is employed to transcriptionally regulate further aspects of sexual development. In the highest expression of this activity, compatible mating type factors permit the development of complex mushroom fruit bodies, which contain several different interacting tissues and, in some tropical genera, may have caps approaching one meter in diameter. However, not all fungi possess mating type factors. In *Podospora*, the progress of meiosis and sporulation does not require heterozygous mating type factors. Indeed, apparently normal fruiting bodies can be formed by haploid cultures, and fruit body formation can usually be separated from other parts of the sexual pathway by mutation, even in species that have a well-developed mating type system. Therefore, the significance of mating type factors in regulating events beyond the initial mating reaction is difficult to judge.

Vegetative compatibility genes define the individuals of fungal populations, and mating type factors are usually interpreted as favoring the outbreeding of a fungal population. Only 25% of siblings are able to mate with a bifactorial mating type system, so it favors outbreeding. Consequently, mating type genes contribute to management of the genetics of the population as well as to the sexual development of the individual. Sexual reproduction generates genetic variation, offers an escape from DNA parasites, and provides a means to repair DNA damage. Sexual reproduction is an important way of enhancing the overall rate of adaptation of the species. This is despite the fact that most fungi produce asexual spores that are extremely effective in dispersing the organism. These are usually produced in such very large numbers that even small quantities of substrate might be expected to produce a sufficient number for mutation alone to provide the variation on which selection might operate. If this can be the case, then we have to ask why so many fungi invest more resources in sexual reproduction.

Admittedly, there are many fungi that only reproduce asexually, but the majority do have a sexual cycle and this must have a selective advantage. Otherwise, asexual stages would replace sexual ones entirely. The crucial step in sexual reproduction, which provides the contrast with asexual reproduction, is the ‘fusion of nuclei derived from different individuals’. For, in asexual reproduction, mitotic nuclear divisions multiply the genetic constitution of only one individual. If the individuals differ in genotype the fusion nucleus will become heterozygous and the products of the meiotic division (= the progeny if the organism is haploid, or the gametes

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if the organism is diploid) can have recombinant genotypes. Thus, in one sexual cycle, new combinations of characters can be created in the next generation for selection. 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. There is plenty of evidence to show that asexual lineages change little in time and that outcrossing certainly does promote variability in a population, which enables the organism to survive ecological and environmental challenges.

This, though, is a 'group selectionist' interpretation. It argues that variation generated in an *individual* meiosis benefits the *group* or population to which the individual belongs. Yet current theory emphasizes, instead, that selection acts on individuals so any feature that is advantageous in selection must be so because it benefits either the individual itself or its immediate progeny. An alternative interpretation suggests that repair of damaged DNA is the crucial advantage of the meiotic sexual cycle. DNA damage in one chromosome, caused by mutation or faulty replication, can be repaired by comparison and recombination with the normal chromosome provided by the other parent. Genetic fitness would be increased but only when out-crossing ensures heterozygosis. Even an incomplete sexual cycle might be of advantage in this case.

Mutations can be recessive and damaging, and different mutations will occur in different mitotically generated cell lines. Merely the formation of the diploid (or heterokaryon or dikaryon) by out-crossing will benefit the mated individual if recessive adverse mutations are masked by non-mutant alleles in the nuclei of 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 *Saccharomyces cerevisiae*. These alleged advantages of the sexual cycle are not mutually exclusive, nor of equal value; rather, they are themselves phenotypic characters that 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. Life is a rich tapestry.

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