2. FUNGAL GENETIC

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2.1 Introduction

2.1.1 Fungi as Pioneer Organisms for Genetic Analysis

Genetic manipulation of organisms implies the ability to direct the formation of new combinations of traits within an individual.

This process has historically been an important human endeavor, providing us with our breeds of domestic animals, economically important plants, and industrially important fungi. Genetic manipulation can be as simple as identifying and selecting, within a population, rare individuals that contain interesting traits, but is made more powerful by the ability to enhance the rate of individual variation, and more powerful still when traits identified in different individuals can be combined.

Thus, mutagenesis and genetic recombination underpin the process of genetic manipulation. Such genetic manipulation can be used directly for practical ends (new varieties of tomatoes for example), or for more academic aims directed at an understanding of life.

Fungi were among the first organisms to be studied scientifically through genetics.

Although peas and fruit flies provided the initial evidence for genes and for genetic linkage, some of the earliest fundamental insights into the genetic structure of organisms came from pioneering studies in fungal systems.

One exceptional insight developed from analysis of fungal systems,

In this case *Neurospora crassa*, was the recognition that individual enzymatic functions were encoded by the information from individual genes, a result rewarded by the 1958 Nobel Prize in Physiology or Medicine to G. Beadle, E. Tatum, and J. Lederberg.

Other fundamental advances based on the genetic analysis of fungi included the dissection of the cell cycle of the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and the analysis of *S. cerevisiae* telomeres, work that led to the 2001 Nobel Prize in Physiology or Medicine to L. Hart- well, P. Nurse, and T. Hunt, and the 2009 prize to J. Szostak, E. Blackburn, and C. Greider, respectively.

These awards, more than 40 years after that to Beadle, Tatum, and Lederberg, show that fungal systems have maintained their utility in uncovering important biological truths.

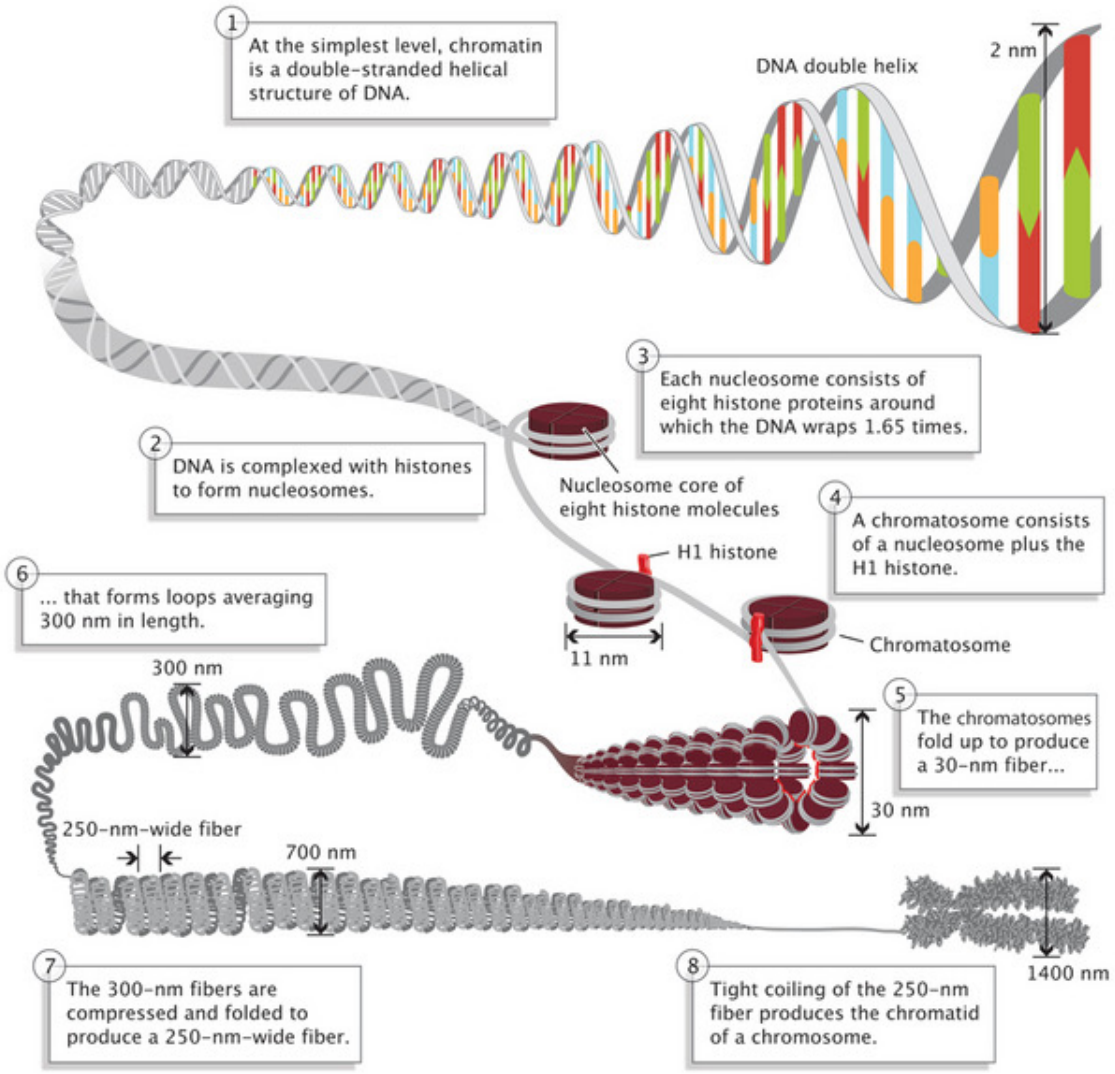
More recently, large-scale, semi-industrialized international efforts have had an enormous impact on the field of fungal genetics.

These efforts have been at the forefront of the development of the science of genomics.

Many of the primary successes of genomics have come through studies on fungi, in particular the baker's yeast *S. cerevisiae*.

Efforts directed at the analysis of the yeast genome have provided the first sequence of a eukaryotic chromosome, **the first sequence of an entire eukaryotic genome**, and the first development of a systematically created collection of null mutants of all the genes of an organism.

Building on this pioneering work, the sequences of many fungal genomes are now fully completed or available as un-annotated draft sequences.



https://www.nature.com/scitable/topicpage/eukaryotic-genome-complexity-437/

2.1.2 Significance/Advantages of Fungi as Model Organisms

Fungi have become among the pre-eminent models for the genetic investigation of basic cellular processes.

There are many intrinsic characteristics of fungal systems that make them ideal model organisms for genetic studies.

As unicellular organisms that can grow in simple defined media they are easy to culture.

Because they often contain a stable, propagatable haploid phase they are easy to mutate, and in those organisms with a well-characterized sexual cycle the mutants can be readily combined.

Finally, because they are eukaryotic cells they exhibit many of the properties and functions characteristic of human cells, and thus served as a better model for many cellular processes than the bacterial systems that had been investigated in depth previously.

2.2 Fungal Lifecycles

2.2.1 Ascomycete Yeast (*Saccharomyces cerevisiae*)

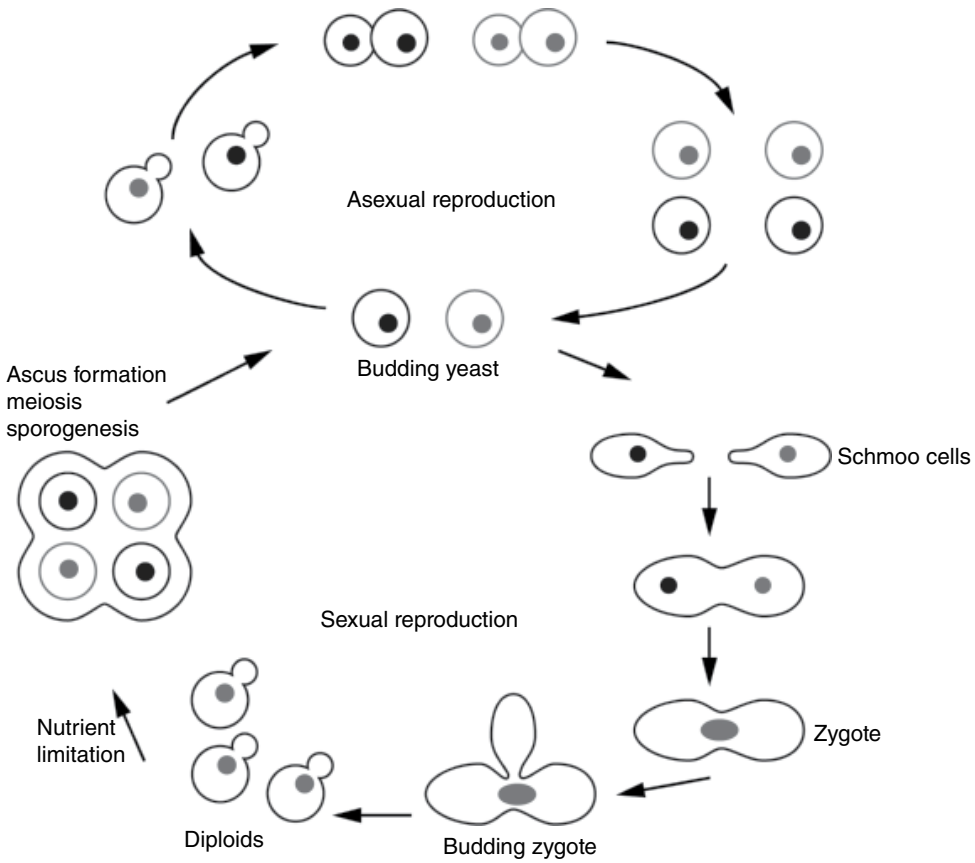
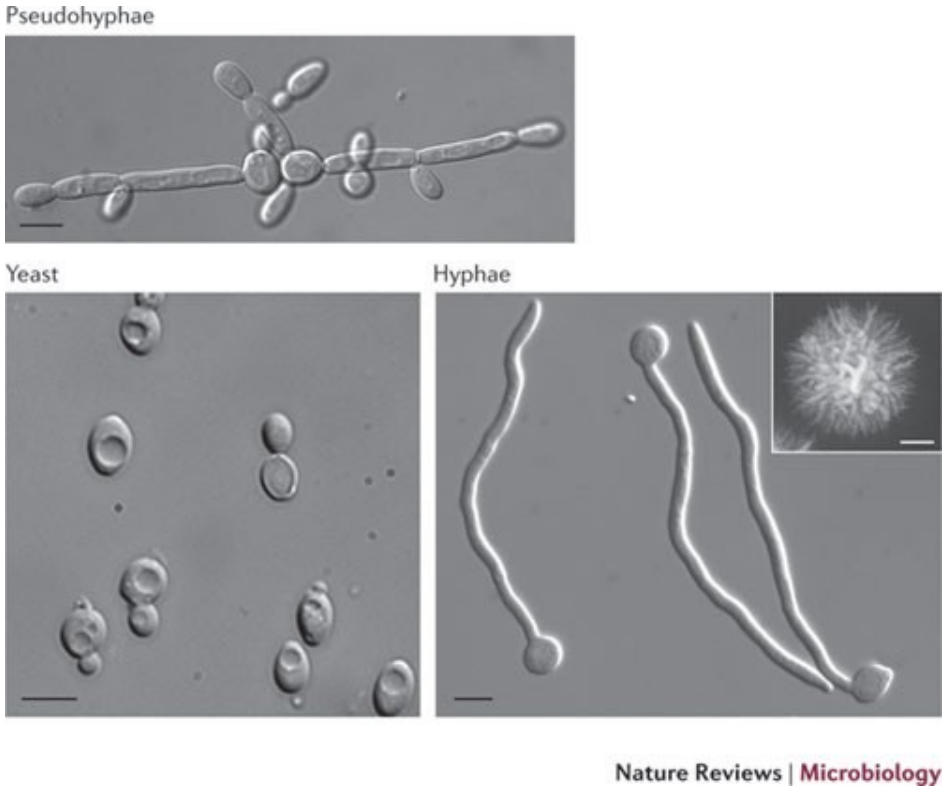


Figure 2.1 Lifecycle of *Saccharomyces cerevisiae.*

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

Mitotic growth of yeast cells involves budding. During this process, the growth of the cell is directed to a specific location on the surface of the mother cell, and a new cell is formed, somewhat like blowing up a balloon through a hole in the mother cell. This involves highly polarized growth of the developing daughter cell, implicating both the actin- and microtubule-based cytoskeletal networks, and is tightly coordinated with the cell cycle. This coordination ensures that the daughter cell receives a complete copy of the genetic material.

Both haploid and diploid cells divide by the budding process, although there are subtle differences in the choice of the sites of bud emergence between haploids and diploids. In addition, some diploid cells can modify the coordination of the cell cycle and polarized growth to switch to a **pseudohyphal** growth mode. In this growth pattern individual cells are more elongate, and the budding pattern leads to the formation of chains of cells rather than compact colonies characteristic of the true budding mode.



Sudbery, P. Growth of *Candida albicans* hyphae. *Nat Rev Microbiol* **9**, 737–748 (2011). https://doi.org/10.1038/nrmicro2636

Genetic analysis is highly developed in *S. cerevisiae*. When vegetatively growing haploid cells of opposite mating types are brought into proximity, they communicate with each other by diffusible pheromones, synchronize their cell cycles, conjugate, and then fuse their nuclei to create non-mating, meiosis proficient diploids.

These diploids can be identified visually in their initial zygote form, and separated from the haploids by micromanipulation, or identified selectively because they contain a pattern of genetic traits not possessed by either haploid parent under **rich growth conditions**, such diploid cells themselves propagate vegetatively, but under conditions of nitrogen and fermentable carbon **limitation, the diploid cells are induced to initiate meiosis and sporulation**. The ability to propagate the diploid allows the amplification of the initial mating product, and provides an essentially unlimited source of potential meiotic events from a single mating.

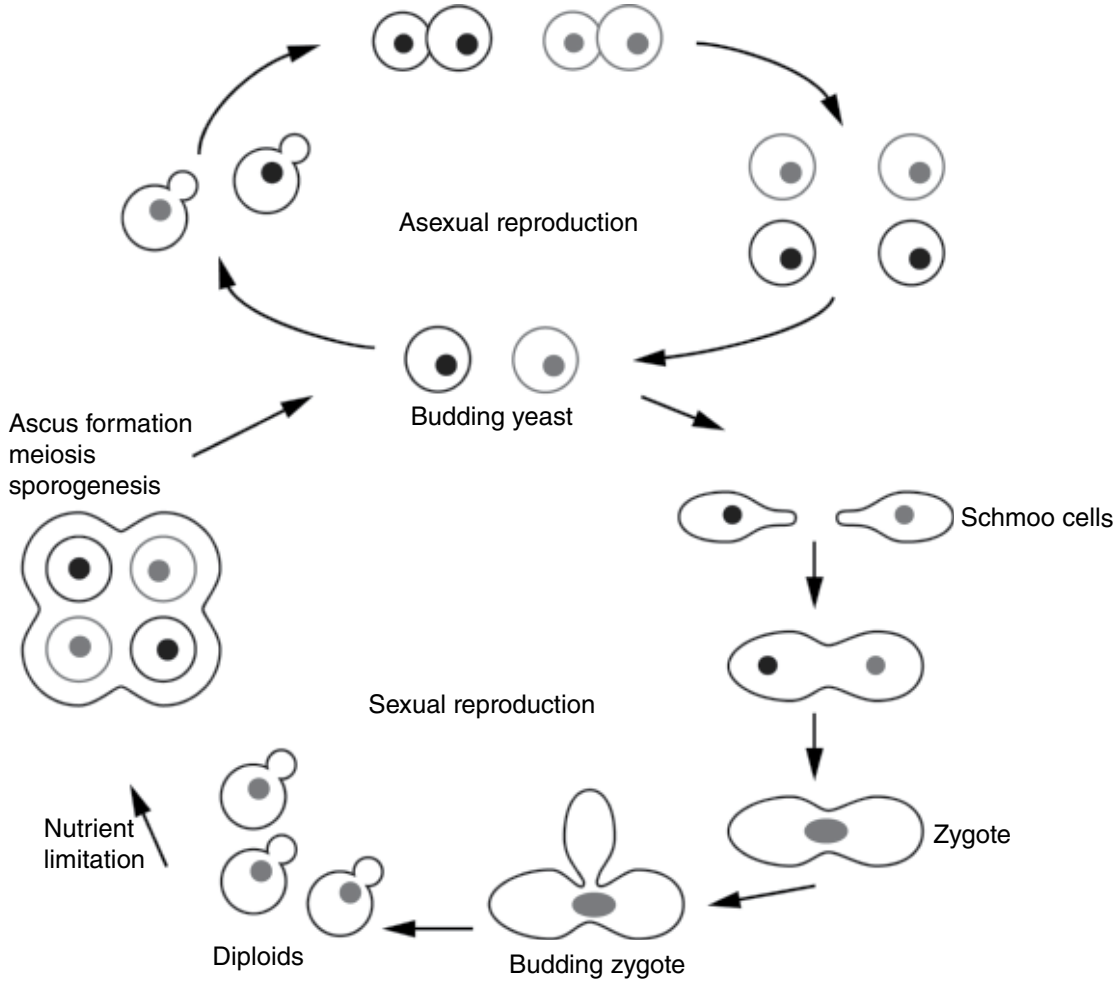
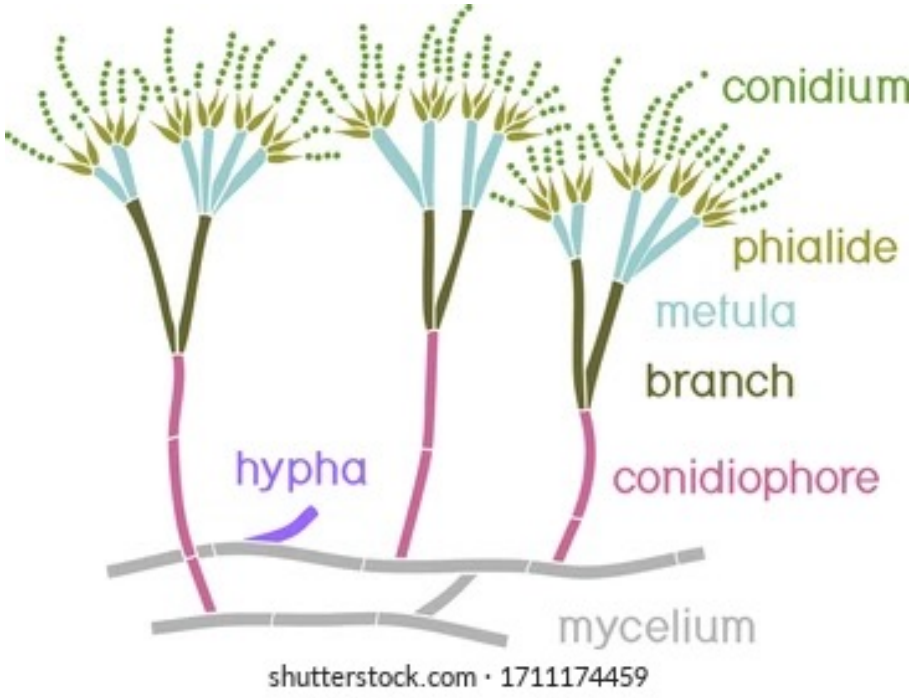
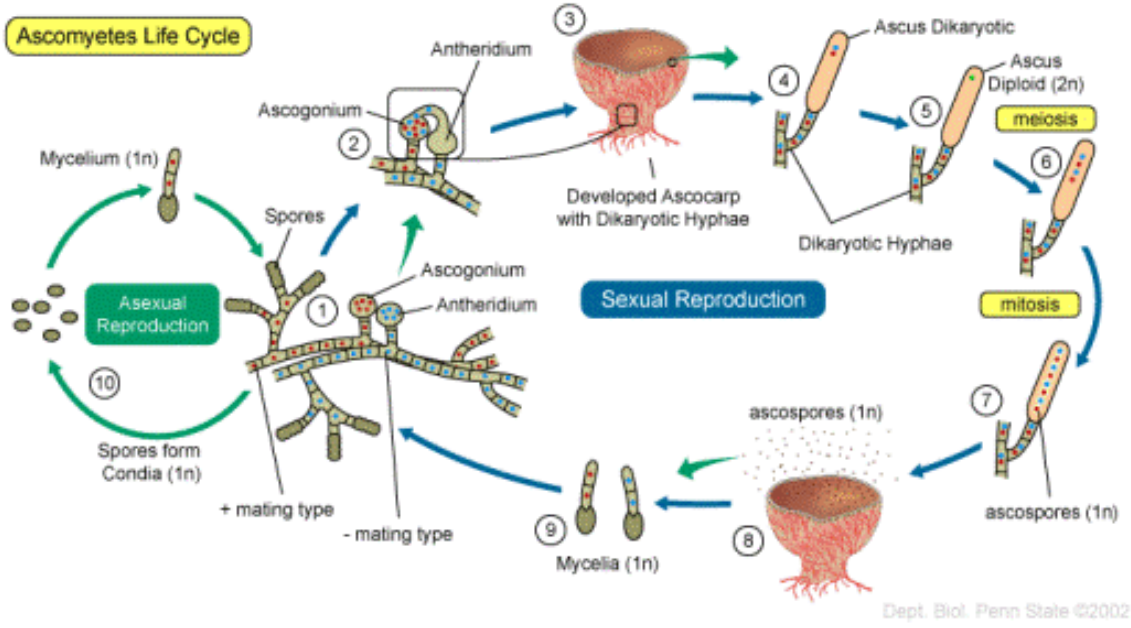


Figure 2.1 Lifecycle of *Saccharomyces cerevisiae*.

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

In filamentous fungi, vegetative hyphal growth initiates from a spore. Spores are products of either sexual (ascospores, basidiospores) or asexual (conidia) reproduction. **Conidia** are typically produced from a differentiated structure called a conidiophore, whereas ascospores and basidiospores are produced within an ascus or basidium, respectively, contained within the fruiting body called an ascocarp or basidiocarp.





During **asexual reproduction** in the ascomycetes, such as *A. nidulans*, a spore containing a single nucleus (monokaryotic) germinates into a multinucleate, homokaryotic hypha. The hypha grows and develops branches for a period of time, then initiates a specialized branch called the conidiophore.

The development of the conidiophore involves numerous different cell types and is investigated as a model developmental process. The nucleus divides mitotically within the conidiophore, allowing the ultimate production of asexual, haploid conidia upon release, conidia germinate into vegetatively growing hyphae, and the cycle continues.

The factors that trigger initial conidiophore development in *Aspergillus* are **not clear**, but they involve the supply of carbon and nitrogen. The process can normally only occur in cultures grown on solid media with an air interphase; conidiation does not occur in liquid.

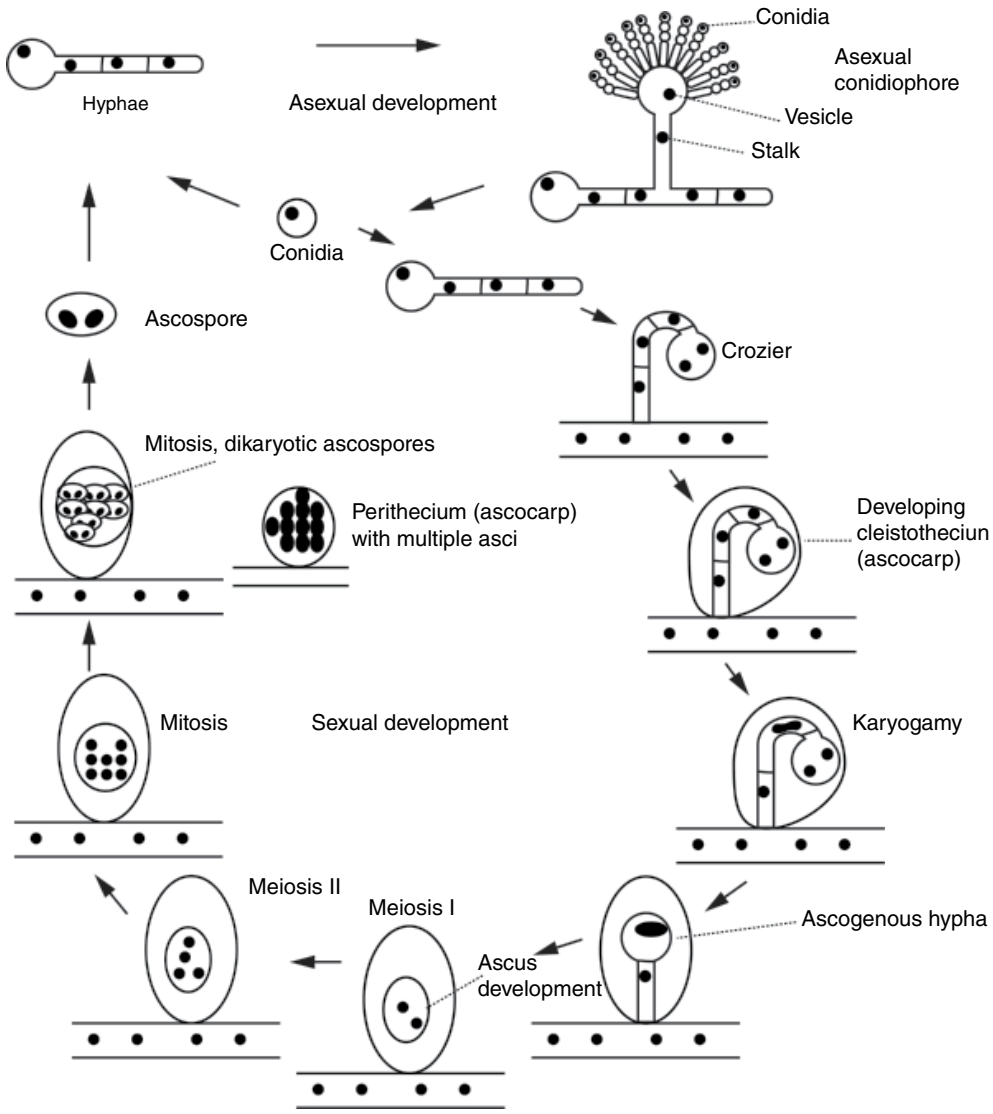


Figure 2.2 Lifecycle of *Aspergillus nidulans*.

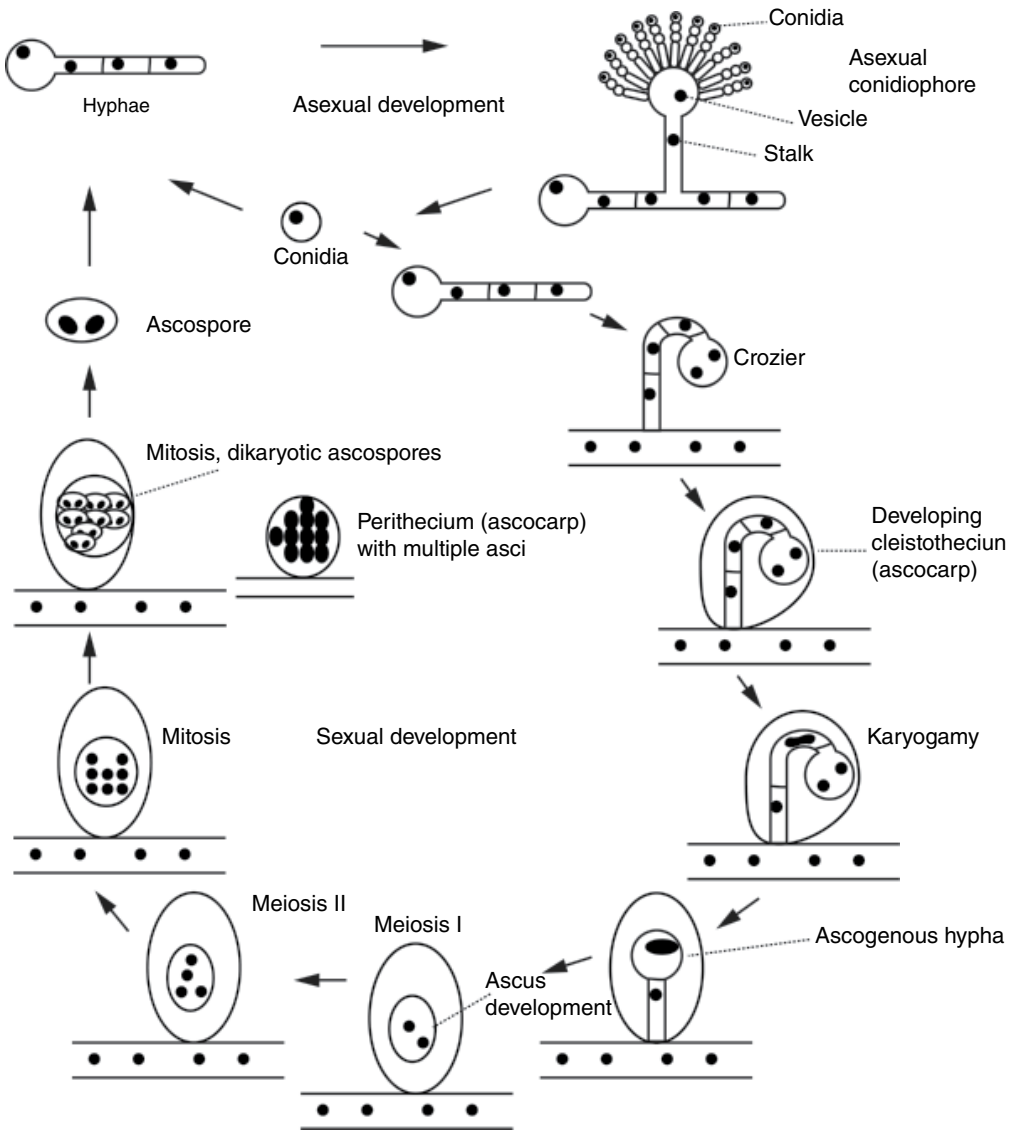


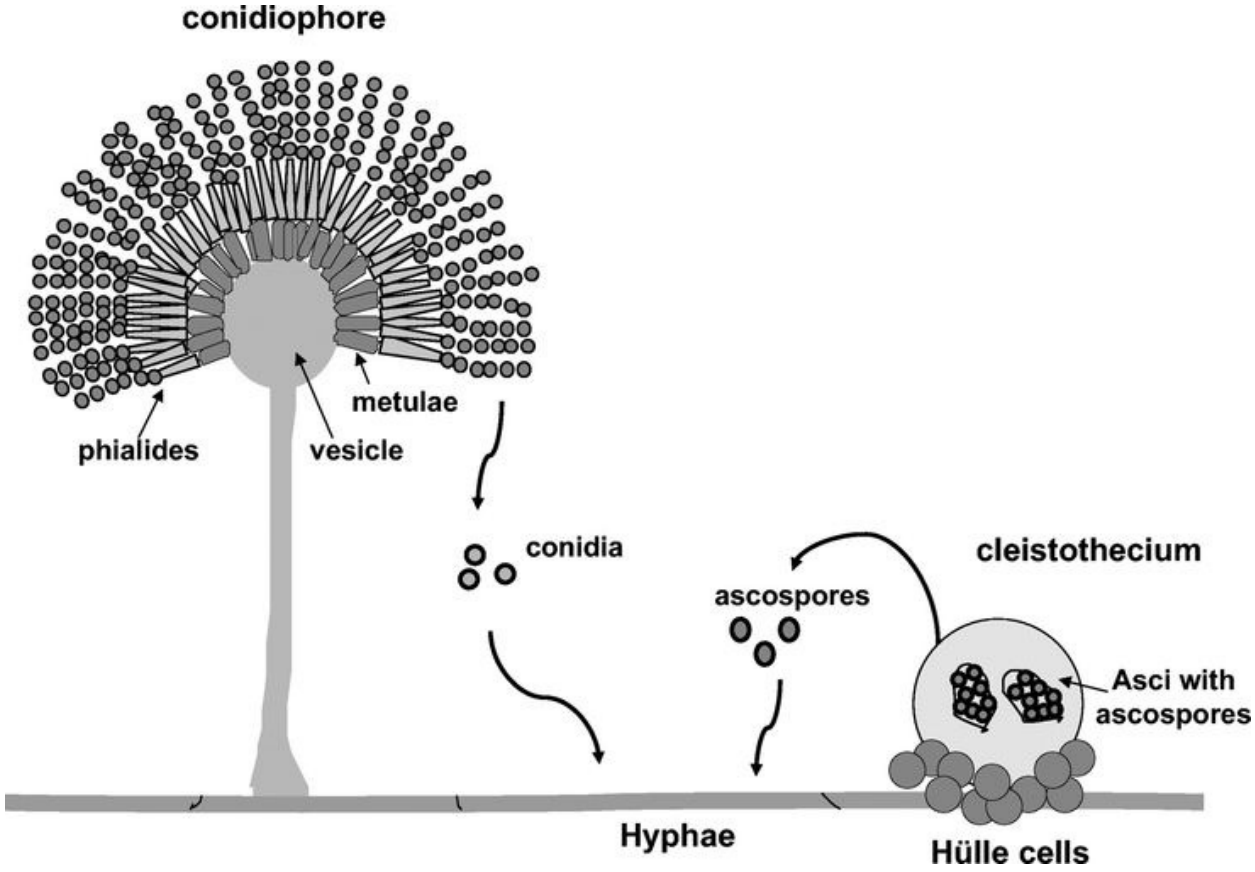
Figure 2.2 Lifecycle of *Aspergillus nidulans*.

Sexual reproduction in *A. nidulans* begins when vegetatively growing hyphae fuse to create a heterokaryon, or dikaryotic hypha (Figure 2.2). The dikaryotic hyphae differentiate into a developing fruiting body called a **cleistothecia**.

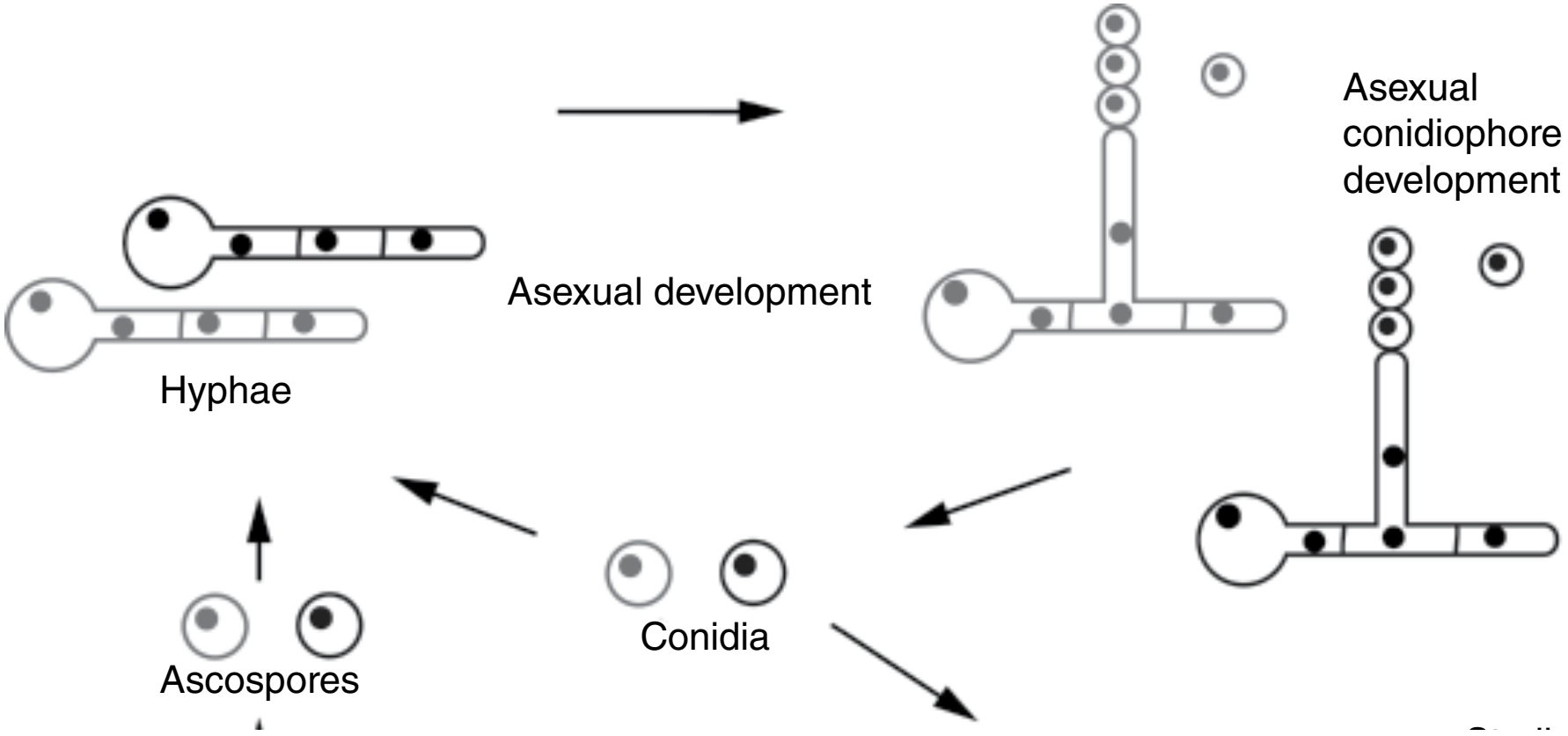
The fruiting body is a complex structure composed of many cell types, including both sterile and fertile hyphae. The dikaryotic fertile hyphae within the cleistothecium develop into hooked structures called croziers, which then differentiate into developing asci.

Karyogamy or nuclear fusion occurs within the **crozier**, creating a diploid. The diploid undergoes meiosis and the four meiotic products then undergo mitosis, creating **eight haploid ascospores**. The ascospores undergo another round of mitosis and are thus binucleate. **Thousands of asci are contained within a cleistothecium** and are fragile, hampering their individual isolation. upon release, the ascospores germinate into hyphae as described.

Aspergillus is homothallic, or self-fertile, and sexual reproduction can be initiated within one colony containing genetically identical nuclei. in the absence of heterokaryon formation with another strain, the individual strain differentiates a cleistothecium as described, into which the hypha develops into a crozier and an ascogenous hypha. unlike *S. cerevisiae*, *A. nidulans* does not undergo any mating-type switching. *Aspergillus nidulans* hyphae can also grow as heterokaryons and diploids as part of a parasexual cycle.



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



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

A polarized structure called a **trichogyne** grows from the protoperithecium of one mating-type female and fuses with the male conidia of the opposite mating type. The nucleus from the latter moves through the **trichogyne** into the ascogonium within the protoperithecium, which is then referred to as the perithecium.

Nuclei from both mating partners divide within a developing dikaryotic ascogenous hyphal structure. The ascogenous hypha develops a **crozier**, where nuclear fusion or karyogamy takes place, followed quickly by meiosis within the developing ascus. Mitosis and subsequent ascosporogenesis results in **eight spores within an ascus** within the perithecium. Acsi are long and slender in *Neurospora*, allowing for individual dissection and separation of ordered ascospores.

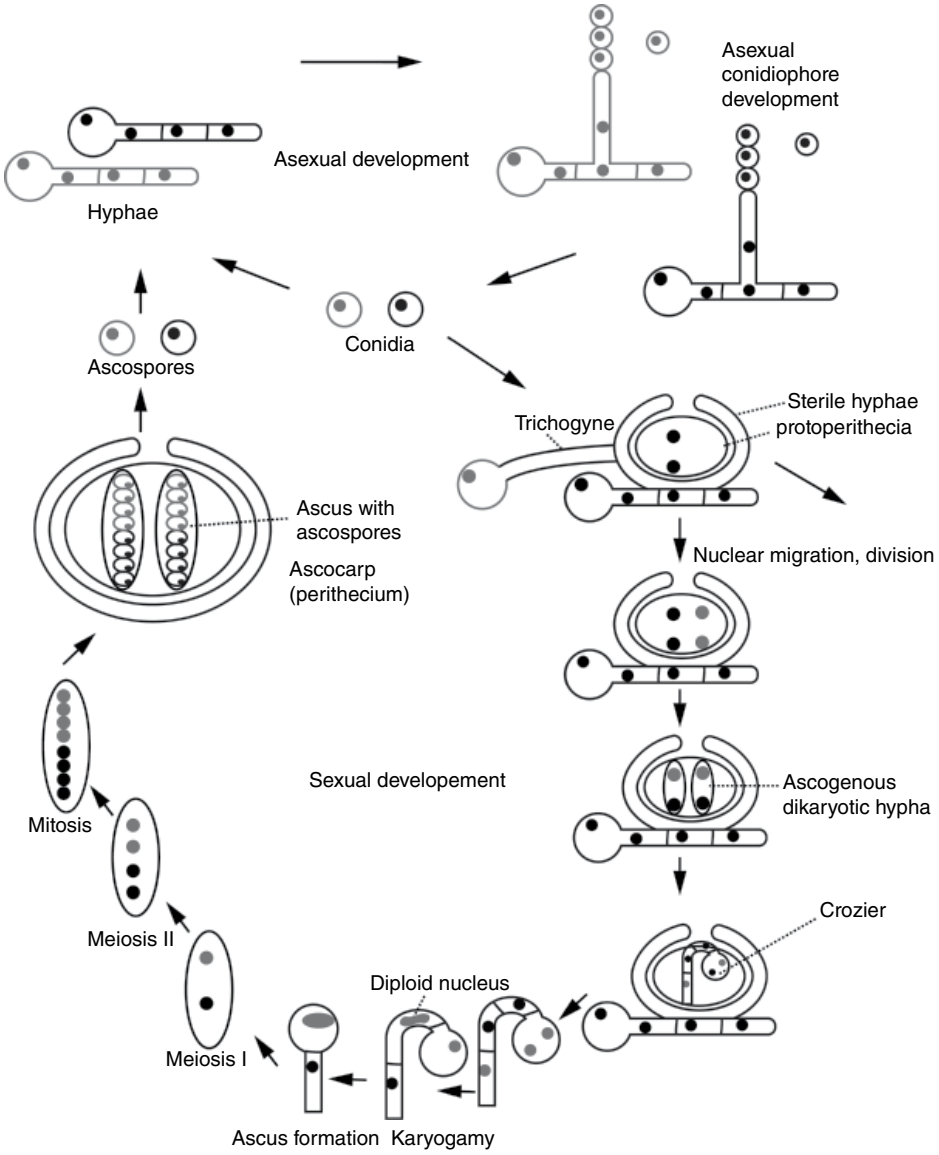


Figure 2.3 Lifecycle of *Neurospora crassa.*

2.2.3 Basidiomycete Filamentous Fungi (*Coprinus cinereus*)

The basidiomycete lifecycle is typically similar to the ascomycetes with a few exceptions. in *Coprinus cinereus* a typical mushroom fungus, monokaryotic hyphae produce **asexual spores** called **oidia**, which germinate and form hyphae.

To initiate **sexual reproduction**, monokaryotic hyphae fuse at their tips (**anastomosis**) to create a dikaryotic hypha. The dikaryotic hypha grows vegetatively, and is distinguished from hyphae of ascomycetes by the presence of hooked cells or clamp connections, which connect septated compartments of the hypha.

Changes in temperature and light can trigger the hypha to undergo differentiation into the fruiting structure, or **basidiocarp**. Within the basidiocarp, basidium formation (the equivalent of an ascus) and karyogamy take place. Meiosis produces the basidiospores, which hang off the basidium contained within the **gills** of the mushroom cap, as opposed to being encased as in the asci of ascomycetes. Haploid basidiospores are then released, and germinate into monokaryotic hyphae and continue the cycle.

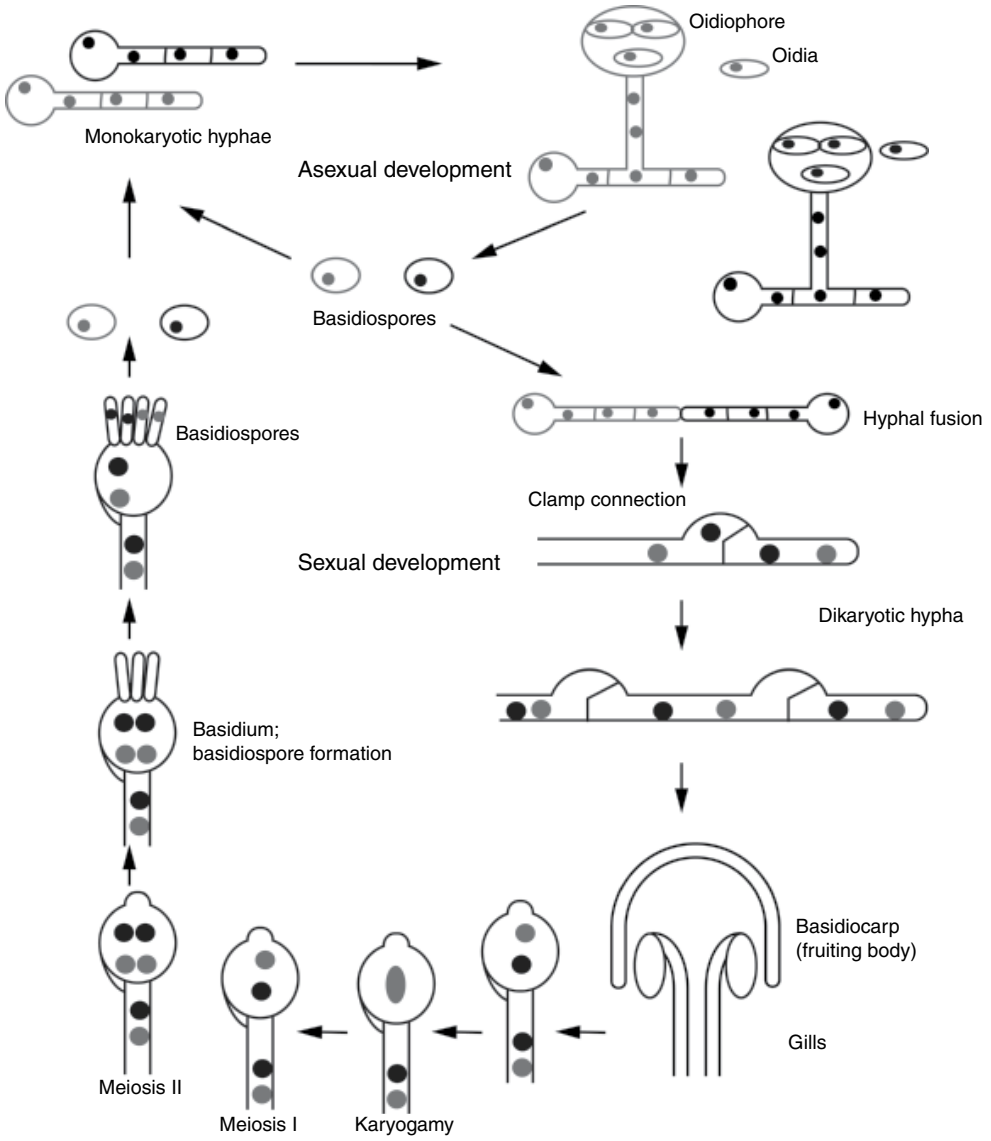


Figure 2.4 Lifecycle of *Coprinus cinerus.*

In dimorphic basidiomycetes that exist in both yeast and hyphal forms, such as the **human pathogen** *Cryptococcus neoformans*, vegetative growth occurs via budding yeast.

Sexual reproduction involves the differentiation of yeast cells into hyphae upon exposure to pheromone, resulting in dikaryotic hyphae. Basidia differentiate from the ends of the hyphae, in which karyogamy followed by meiosis and mitosis occurs, producing haploid basidiospores. upon release, the spores grow as budding yeast.

The yeast cells can also undergo asexual sporulation in response to nitrogen limitation or desiccation. Under these conditions, yeast cells differentiate into hyphae, which differentiate monokaryotic basidia at their tips. Mitosis occurs within the basidia, producing haploid spores, which then germinate into yeast cells.

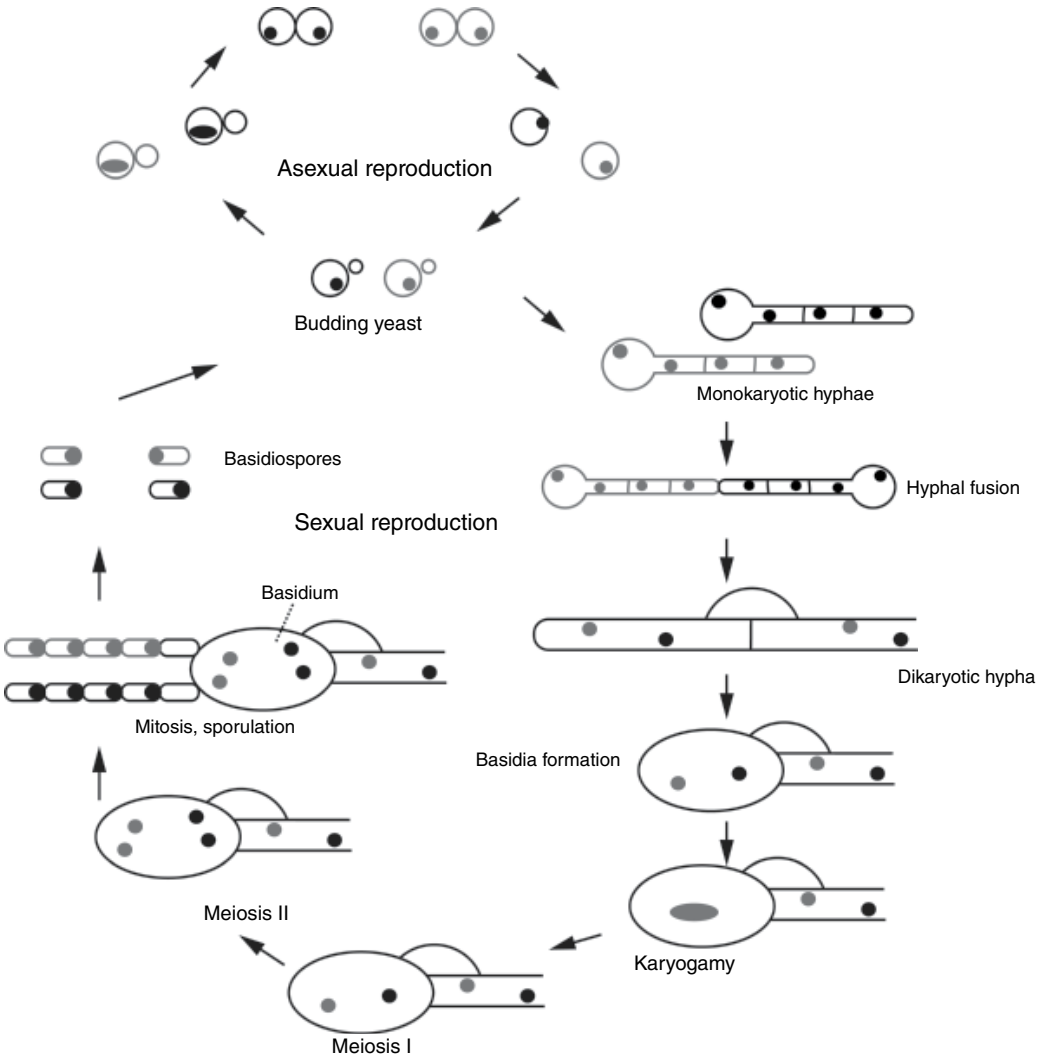


Figure 2.5 Lifecycle of *Cryptococcus neoformans*.

Filamentous fungi exhibit many variations in their lifecycles, and some do not exhibit a known sexual phase (the deuteromycetes). Others exploit parts of the lifecycle for pathogenesis, particularly in the pathogenic basidiomycetes. For example, the dimorphic corn smut fungus *Ustilago maydis* exists as a nonpathogenic yeast form, but upon mating yeast cells differentiate into dikaryotic hyphae, which are associated with virulence.

2.3 Sexual Analysis: Regulation of Mating

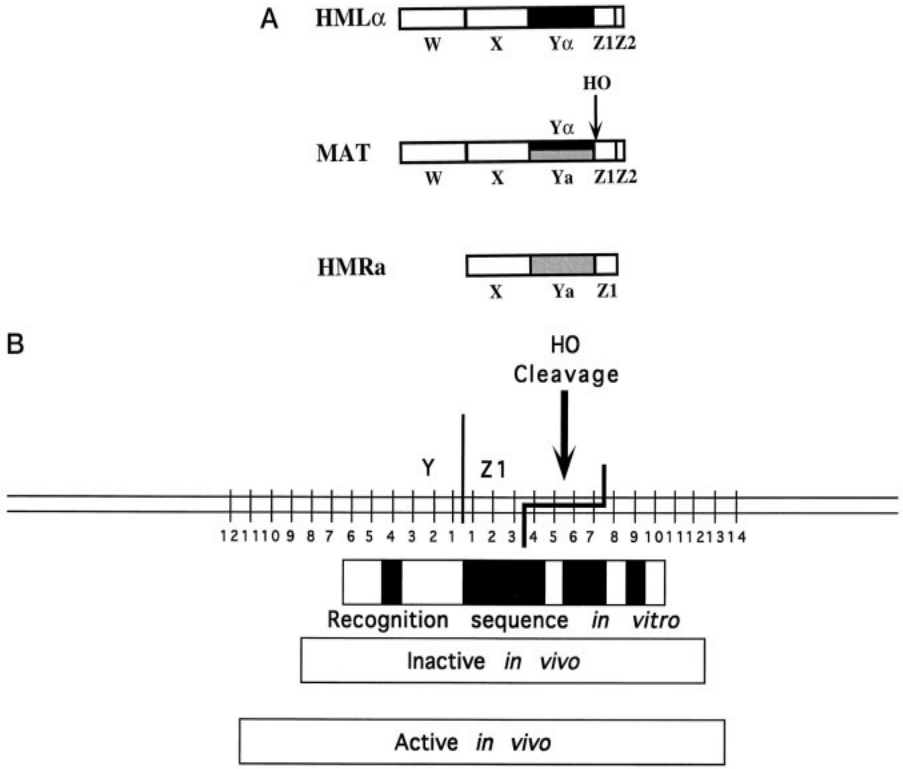
2.3.1 Ascomycete Yeast

The yeast *S. cerevisiae* has a simple mating system, with cells of two haploid mating types termed **a** and α. These cells can conjugate to form a diploid cell containing both **a** and α information. The **a**/α diploid is not capable of mating but can initiate meiosis to form four haploid products, two of which are mating type **a** and two of which are mating type α. Laboratory strains typically have stable mating types, and are termed heterothallic.

In contrast, most wild strains are homothallic, and do not have stable mating types. instead, during mitotic growth the cells are capable of switching their mating types, and thus a growing spore colony develops cells of both **a** and α. These cells mate with each other, resulting in a colony that grows up as an **a**/α diploid.

The regulation of the stability of the mating type is controlled by a single locus, termed **the homothallism locus (*HO*)**. The *HO* locus contains a functional endonuclease, while the stable mating types of the heterothallic strains result from a defective allele of this locus, designated ***HO***.

The reason that an endonuclease controls the stability of the mating type is the result of a sophisticated genetic exchange system involving expressed and unexpressed cassettes of information.



In the presence of a functional *HO* endonuclease the information is exchanged between the *HMR* or *HML* loci and the *MAT* locus as often as once per cell division. This exchange is recombinational; the *HO* endonucleases make a double strand cut at the *MAT* locus, and gene conversion then transfers the information from one of the silent loci to the *MAT* locus. The same machinery that keeps the information at *HML* and *HMR* unexpressed blocks the cutting of these DNA sequences by *HO*, so the informational exchange is typically unidirectional.

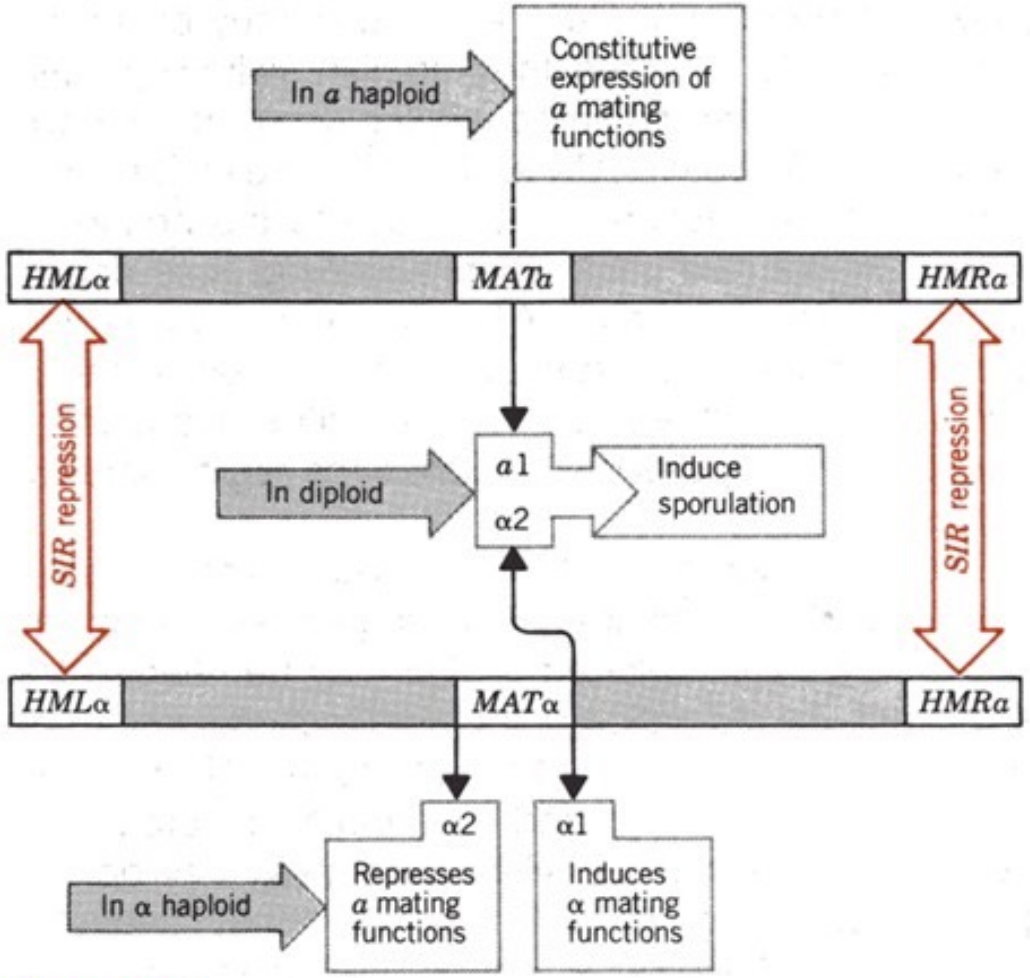


Figure 36.20

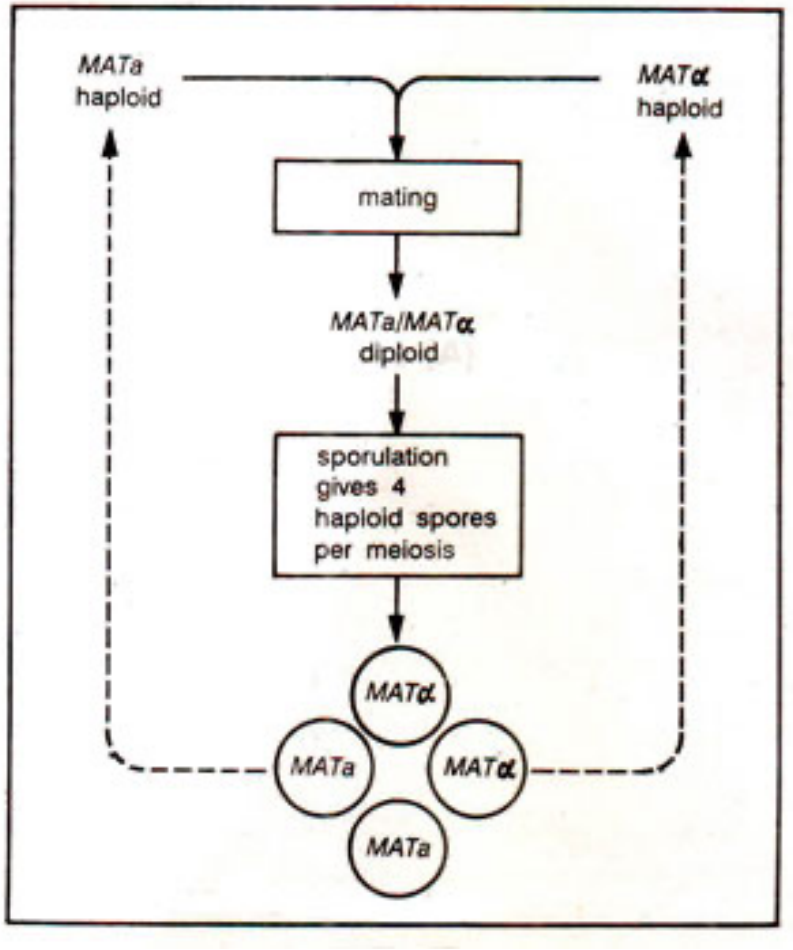
The a1 and α2 functions cooperate to induce sporulation in diploids. In haploids, the α2 function represses a mating functions; α1 induces α mating functions.

The mating type of the cell is controlled by a single locus termed ***MAT*** close to the centromere of chromosome 3.

When this locus contains the *MAT***a** allele the cells are of the **a** mating type;
when the locus contains the *MAT*α allele the cells are mating type α.

if there is no information at *MAT* the cells select **a** as the default mating type.

However, the typical *S. cerevisiae* cell also contains an extra copy of both mating-type genes, typically **a** information near the right telomere of chromosome 3 at a locus called *HMR*, and α information at the left telomere of chromosome 3 at locus *HML*. The sequences at *HML* and *HMR*, although structurally identical to the sequences for **a** and α information at *MAT*, are not expressed due to the action of a series of proteins designated silent information regulators.



The *MAT* locus defines the mating type of the cell through direct transcriptional control. Each allele, *MAT***a** and *MAT*α, expresses transcription factors, and these transcription factors control the expression of blocks of genes defining the two cell types.

*MAT*α encodes two transcription factors, α1 and α2. The role of α1 is to stimulate the expression of α-specific genes such as

- *STE3*, encoding the receptor for the **a**-factor mating pheromone,
- *Mfα1* and *Mfα2*, the genes specifying the α-factor mating pheromone.

The α2 transcription factor is a repressor, serving to repress **a**-specific gene expression in the *MAT*α cells and, together with the **a**1 factor, to shut off haploid gene expression in *MAT****a****/MAT*α diploid cells.

Among the key genes shut off in the diploid cell is *RME1*, which encodes a repressor of meiosis; this regulatory circuit ensures that it is the **a**/α diploid cell that is uniquely capable of meiosis and sporulation.

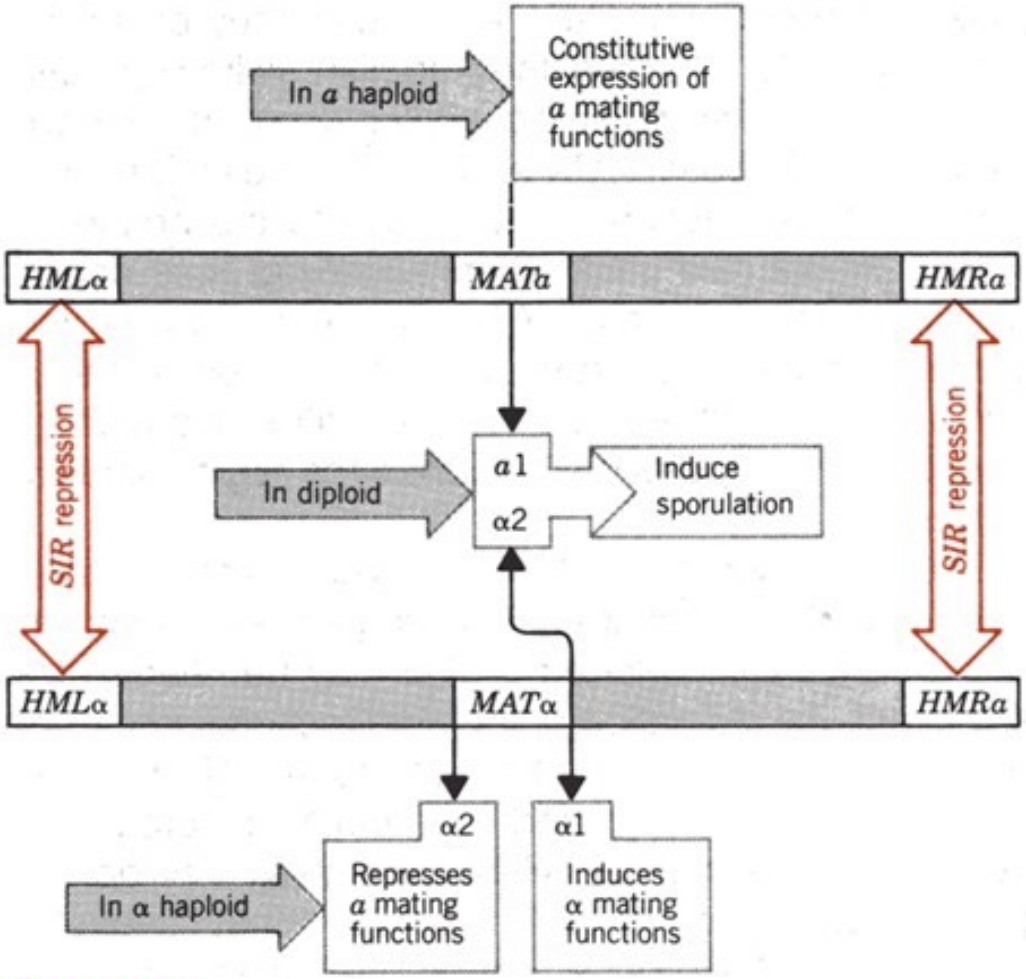


Figure 36.20

The a1 and α2 functions cooperate to induce sporulation in diploids. In haploids, the α2 function represses a mating functions; α1 induces α mating functions.

2.3.2 Filamentous Ascomycetes

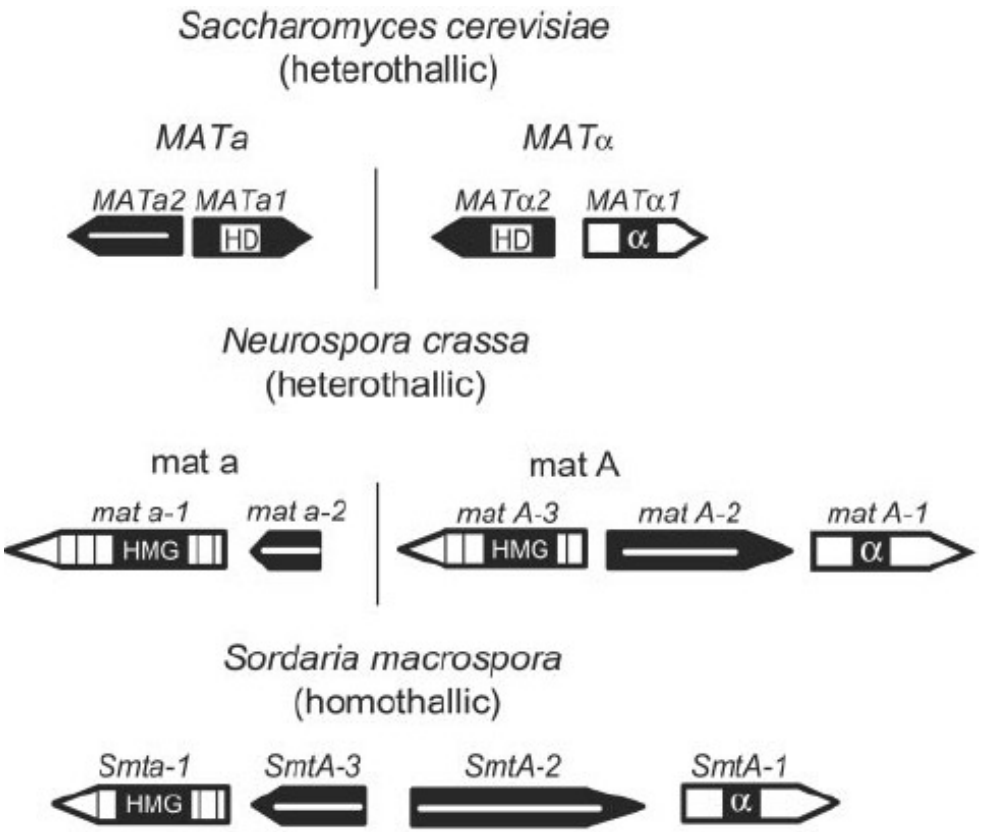
Neurospora crassa is heterothallic and requires two mating types, A and a, for sexual reproduction. The mating-type loci, mat A and mat a, control gene expression required for the mating process. The DNA sequences at the mating loci of opposite mating types are very different, and are therefore regarded as "**idiomorphs**" as opposed to alleles.

The mat a idiomorph is 3.2kb in length, and encodes one gene called mat a-1,

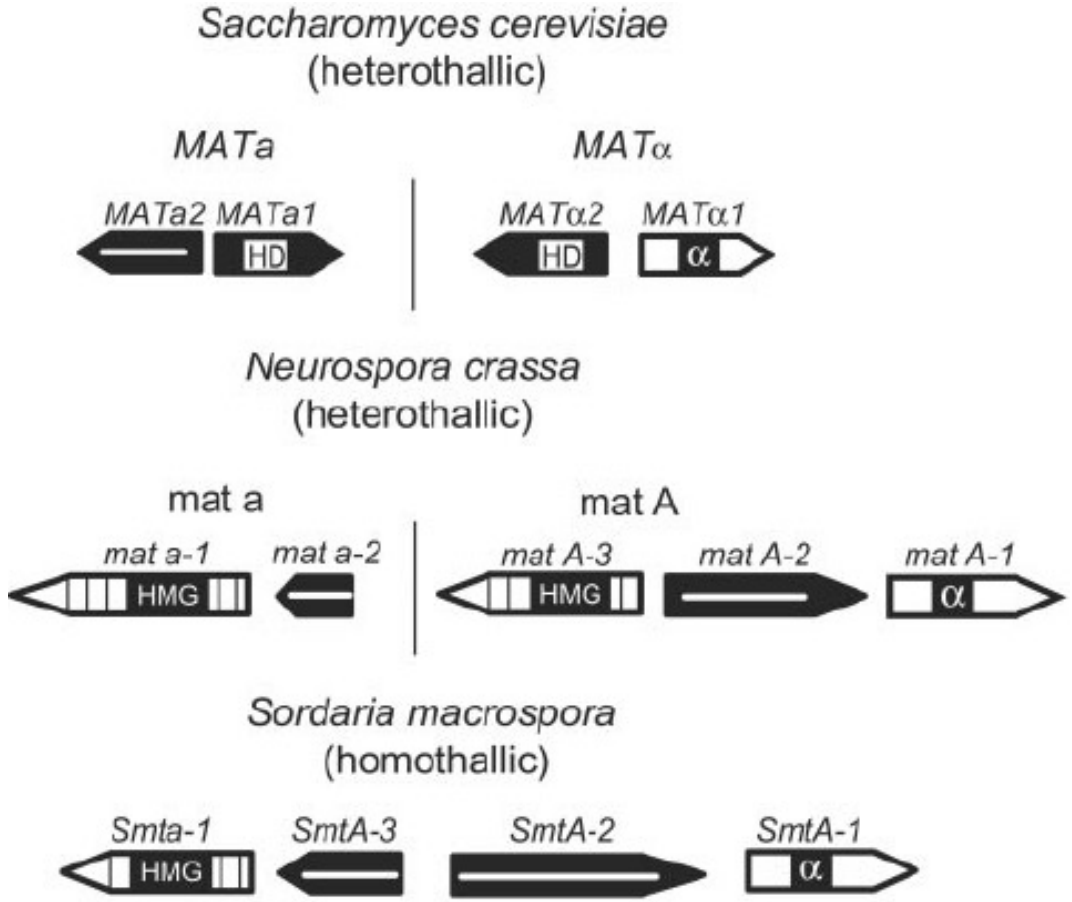
while the MAT A idiomorph is 5.3 kb long and encodes three genes, including mat A-1, A-2, and A-3.

The mat A-1 and mat A-3 genes encode for HMG box-containing **DNA-binding proteins**, and are the major regulators of mating in both strains. Homologs of such factors are required for mating in other organisms, including some filamentous fungi and the fission yeast *Schiz. pombe*.

The mat A-1 product is also a DNA-binding protein and similar to α1p from *S. cerevisiae*. mat A-3 encodes a protein with little homology in other organisms and of unknown function.



But, together with mat A-2, is required for **ascosporogenesis**. mat A-2 and A-3 are expressed constitutively during both vegetative growth and sexual development. in contrast to the situation in *S. cerevisiae*, the downstream targets of the mating regulatory genes are unknown. They presumably control expression of the mating pheromone, but other processes must also be regulated, including nuclear migration, nuclear compatibility, and fruiting body development. Since filamentous fungi utilize multiple different cell types for sexual reproduction and contain complex fruiting body structures, including different thalli for male and female mating partners, the regulation and function of the mating-type loci must be more complex than in yeast. in contrast to *S. cerevisiae*, mating-type switching does not occur in *N. crassa*.



The mating loci in *Neurospora* regulate additional processes, including vegetative, heterokaryon compatibility. Vegetative hyphae can fuse to form a **heterokaryon**, but only if they arise from opposite mating-type strains.

If hyphal fusion occurs **between incompatible cells**, the fused hyphal compartment seals off from the rest of the hyphae through deposition of cross-walls, and the compartment undergoes a type of **programed cell death** characterized by DNA fragmentation, organelle and cytoplasmic breakdown, and vacuole production. Mutants of the *mat a* strain, which lost the incompatibility response, were affected in the *mat a-1* orf, and analysis of the *mat a-1* protein identified domains important for mating versus vegetative incompatibility.

Several filamentous fungi, including *A. nidulans*, are homothallic or self-fertile, where a colony derived from a single spore is able to undergo sexual reproduction. Although the genetic basis of homothallism is not fully understood, recent completion and analysis of the genome from *A. nidulans* has uncovered the presence of many conserved elements of mating from heterothallic species, suggesting that sexual reproduction may be regulated by similar genes in “selfing” fungi.

For example, MAT-2 and MAT-1 genes encoding an HMG box-containing DNA-binding protein and an α1p domain homolog, respectively, have now been identified. In addition, genes encoding homologs to the hydrophilic pheromone alpha factor in *Saccharomyces*, the mating pheromone protease *KEX2*, and pheromone receptors *STE2* and *STE3* are present in the genome. However, no homolog to **a** factor mating pheromone was detected, despite the fact that its receptor was present. The MAT genes and factors associated with a pheromone response MAPK pathway were shown to be important for sexual development, suggesting that similar pathways underlie self and nonself mating.

2.3.3 Filamentous Ascomycete Dimorphic Fungi

Candida albicans is an important human pathogen and has been extensively studied for this reason. *Candida albicans* had been classified as an asexual **Deuteromycete**, but recent genomic studies have provided convincing evidence for the potential for a sexual cycle. However, although a well-defined mating system has been identified that allows the conjugation of mating-type locus homozygous diploid cells, there is currently no evidence for a functional meiotic pathway that allows reductional division and a return to the diploid state from the tetraploid.

The detection of the potential mating ability of *C. albicans* arose through analysis of the genome sequence. A region of the genome was detected that encoded genes similar to those found at the mating-type locus of *S. cerevisiae*. Further analysis of this region uncovered a more complex locus than that found in *S. cerevisiae*; in addition to the candidate cell-type regulating transcription factors, there were other genes whose function implied no obvious link to the mating process.

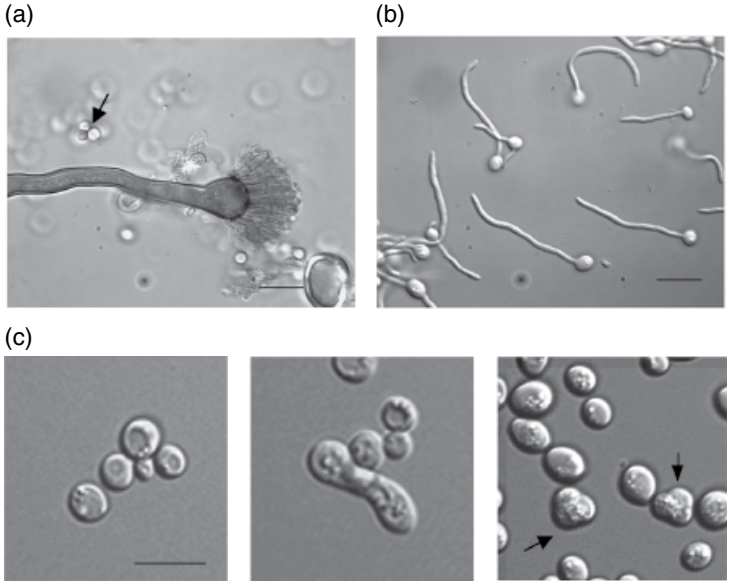
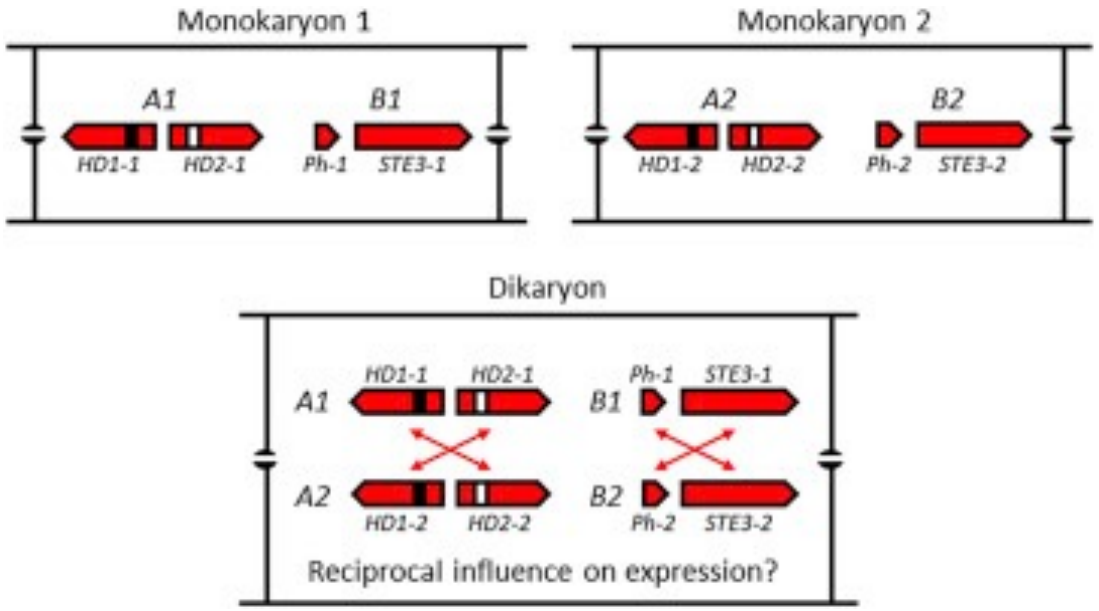


Figure 2.6 (a) Developing conidiophore composed of a vesicle giving rise to phialides and conidia in the filamentous fungus *A. nidulans*. Released conidia are indicated by an arrow. (b) Hyphae growing from yeast cells of the dimorphic fungus *C. albicans*. (c) Various stages in the lifecycle of the yeast *S. cerevisiae*. The first panel demonstrates vegetatively growing yeast, the second shows a zygote, and the third asci (arrows). Bars: 10μm.

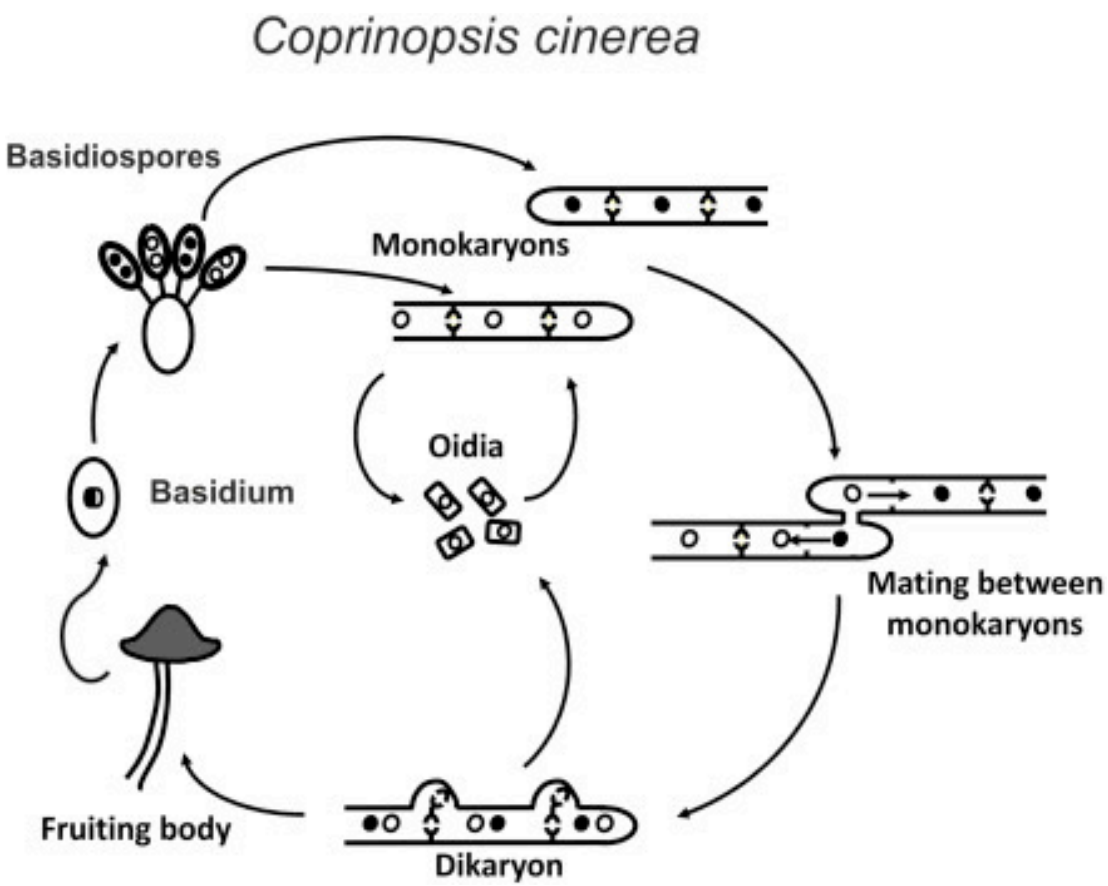
2.3.4 Filamentous Basidiomycetes

The basidiomycetes are novel in that they have multi-allelic mating-type genes. In *Coprinus cinereus*, for example, more than 12,000 mating types exist, demonstrating the diversity in mating systems and complex levels of control within the filamentous fungi. *Coprinus* has two unlinked mating-type loci, A and B, which are polymorphic and contain subloci called α and β. The mating system is therefore described as being tetrapolar. The α and β loci are redundant, but recombination occurs between them. Together these loci contribute hundreds of specificities to A and B loci, creating thousands of different mating types.



The A locus encodes genes for homeodomain proteins, and regulates nuclear pairing, clamp connection formation, and septation. At Aα, two classes of homeodomain proteins are produced. The HD1 and HD2 classes contain homologs to α2p and **a**1p, respectively, from *S. cerevisiae*. HD1 and HD2 proteins from different specificity loci dimerize and subsequently regulate the dikaryon. The B locus encodes six pheromone genes and three pheromone receptor genes.

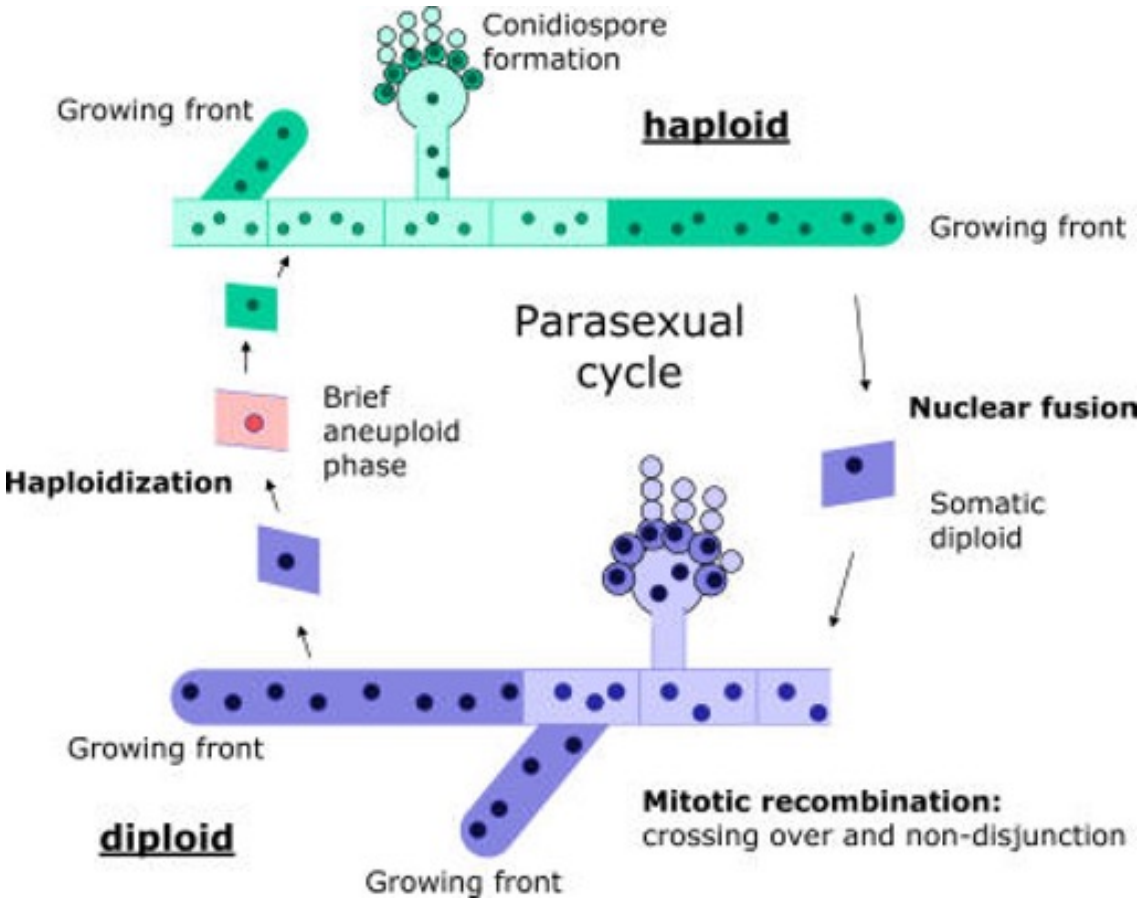
In contrast to ascomycetes where pheromone signaling stimulates the formation of mating-specific morphological shapes, pheromone signaling in *Coprinus* stimulates fusion of the monokaryotic hyphae to initiate the sexual cycle. The B locus also regulates nuclear migration and attachment between the clamp connection and the corresponding subapical hyphal compartment. As in the filamentous ascomycete *N. crassa*, the targets of the mating loci genes are currently not well characterized.



2.4 Unique Characteristics of Filamentous Fungi that Are Advantageous for Genetic Analysis

2.4.1 Parasexual Analysis

- Parasexual cycle was reported in ***Aspergillus nidulans,***the imperfect stage of **Emericella nidulans**.
- Since then parasexual cycle has been discovered not only in several members of **Deutromycetes** but also in fungi belonging to **Ascomycetes** and **Basidiomycetes.**
- So,far it has not been reported in **Phycomycetes fungi**.



Sr. No.	Sexual cycle	Parasexual Cycle
1.	Nuclear fusion takes place in the specialized structure	Nuclear fusion takes place rarely in vegetative cells.
2.	Zygote usually persists one nuclear generation.	Zygote persists through many mitosis.
3.	Recombination by meiosis, crossing over in all chromosomes pair, of reduction of chromosome number and random assortment of numbers of each chromosome pair are characteristics of the cycle.	Recombination by rare accidents of mitosis; mitotic crossing over at event usually confined to one exchange in a single chromosome arm and Haplodization independent of crossing over are the features of this cycle.
4.	Products of meiosis can easily be recognized and isolated.	Products can be recognized by the application of suitable genetic markers.

2.4.2 Gene Silencing

A unique feature that has greatly facilitated genetic/molecular analysis in the filamentous fungus *N. crassa* is the process of repeat-induced point mutation (RiP). if more than one copy of a gene is introduced in tandem into the haploid strain prior to sexual reproduction, the tandem copies are inactivated through Gc to AT mutations when passed through the sexual cycle.

Therefore, gene inactivation can be achieved by simply introducing additional copies, and allowing the strain to undergo sexual reproduction. *Neurospora crassa* was one of the first eukaryotic systems in which a form of RNA interference (RNAi) was investigated. The fungus demonstrates the process called quelling, where genes that are introduced at heterologous locations are silenced. This silencing involves degradation of mRNA through several factors including homologs of Argonaute and Dicer which are involved in RNAi in other systems. RiPing and quelling are therefore very useful for investigating gene function in *N. crassa*, and for understanding the related mechanisms of gene silencing in other eukaryotes, including plants and worms. Because of the utility of RNAi, recent efforts have been made to transfer the molecular machinery to organisms like *S. cerevisiae* and *C. albicans* that do not naturally possess the capacity.

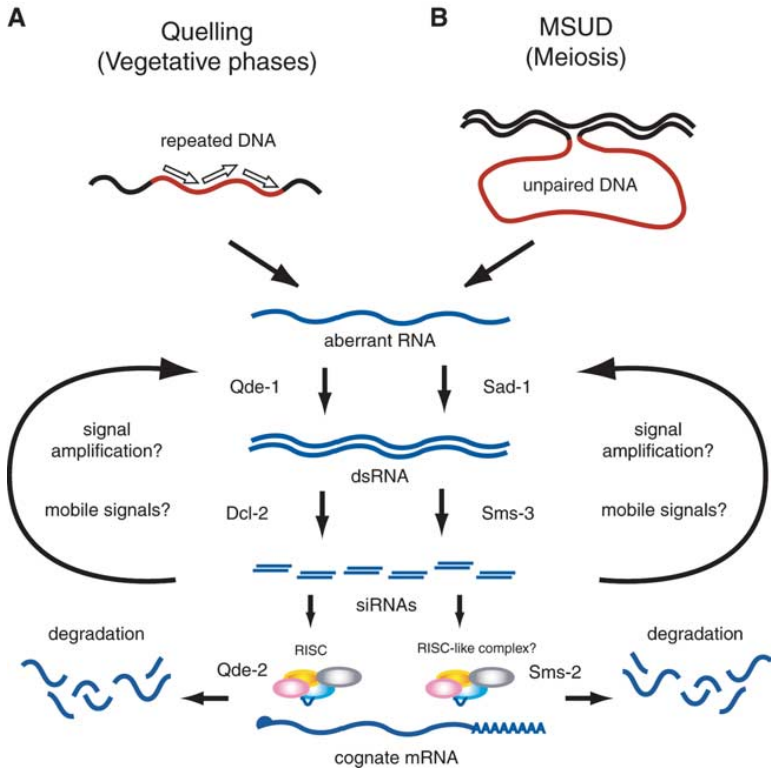


Fig. 2. A proposed model of two RNA silencing pathways in *N. crassa*. (A) During the vegetative phases of the *N. crassa* life cycle, repeated sequences in the genome can induce quelling. In this pathway, dsRNA produced by Qde-1 RNA-dependent RNA polymerase is diced into siRNAs mainly by the action of Dcl-2. The siRNAs guide degradation of cognate mRNA after their incorporation into RNA-induced silencing complex (RISC) where Qde-2 is one of the components. (B) During meiosis, a DNA fragment that has failed in pairing (unpaired DNA) triggers the second RNA silencing pathway in *N. crassa*, called MSUD. Mechanisms of silencing in MSUD are supposed to be quite similar to those in quelling except that MSUD uses a different set of silencing protein components (paralogs) from those in the quelling pathway.

2.5 Genetics as a Tool

2.5.1 Tetrad Analysis

• Linear Asci (arrangement of ascospores):

I. The linear arrangement of ascospores within an ascus allows for precise mapping of genetic loci.

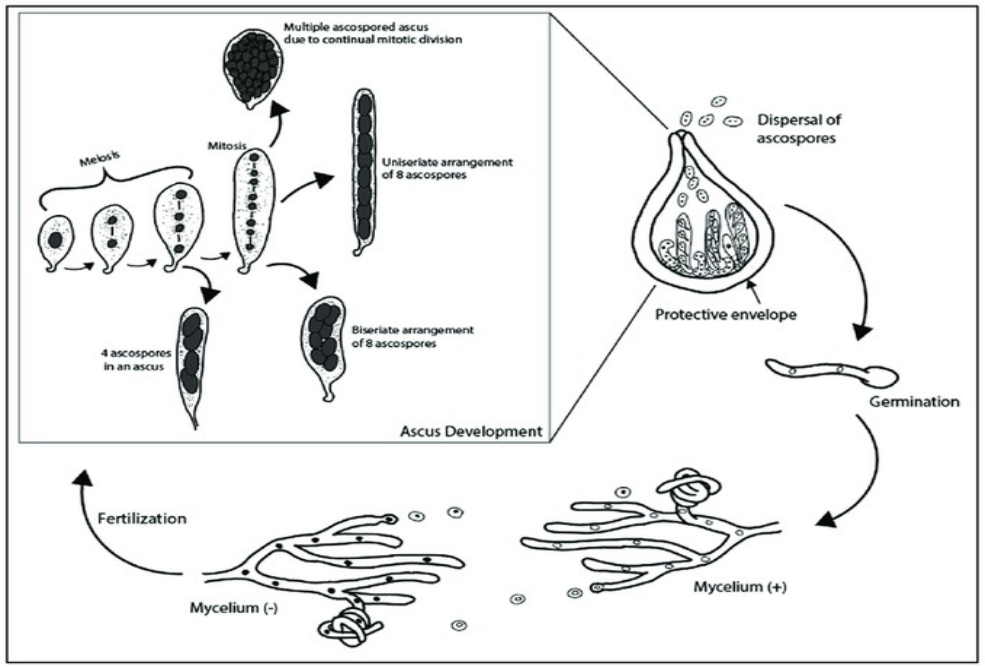
*A locus (or loci) is the actual location of the gene on a region of a chromosome.

*Ascospores are sexual spores.

*(Ascus is the largest cell in the entire life cycle of Neurospora ; it is where the transient diploid nucleus undergoes meiosis and a postmeiotic mitosis.The eight haploid nuclei are then sequestered into eight linearly ordered ascospores.)

II. During meiosis, crossing over can occur, leading to recombination between homologous chromosomes.

This results in different combinations of alleles among the ascospores.



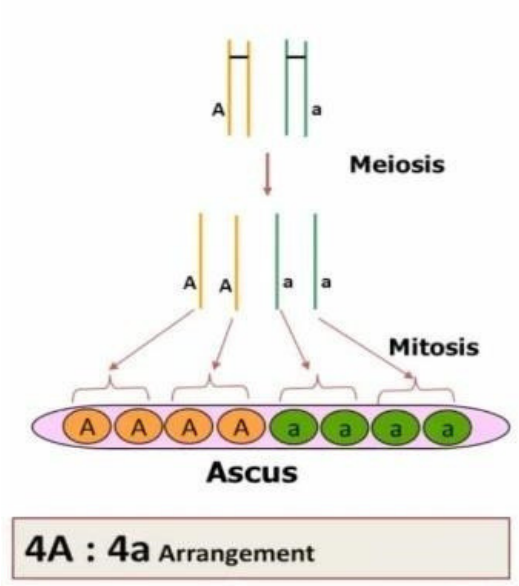
- **First and Second Division Segregation:**

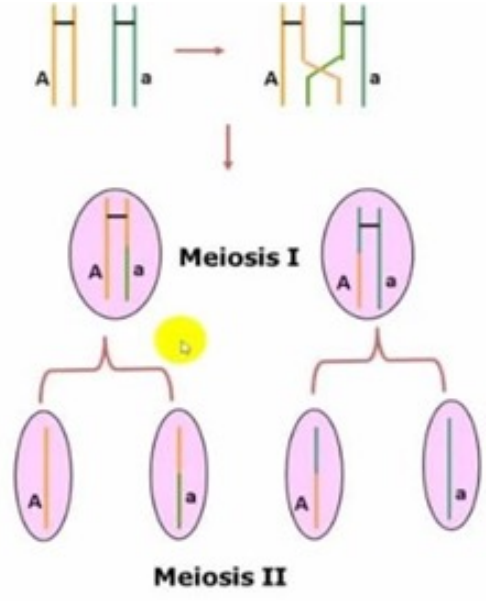
i) 1st Division segregation

I. No crossing-over

II. Segregation of alleles occurs during the first division of meiosis III. Ascospores are arranged in 4:4 pattern

III. Ascus with this type of arrangement is known a Parental Ditype





ii) 2nd Division segregation

I. Crossing-over occurs

II. Segregation of alleles occurs during the 2nd division of meiosis

III. Ascospores are arranged in 2:2:2:2 pattern ,Such ascus is known as tetratype

IV. Four different segregation pattern occurs

V. It depends upon the orientaion of the recombinant chromatids during anaphase II.

This information is used to calculate the distance between genes and the centromere, an essential aspect of genetic mapping.

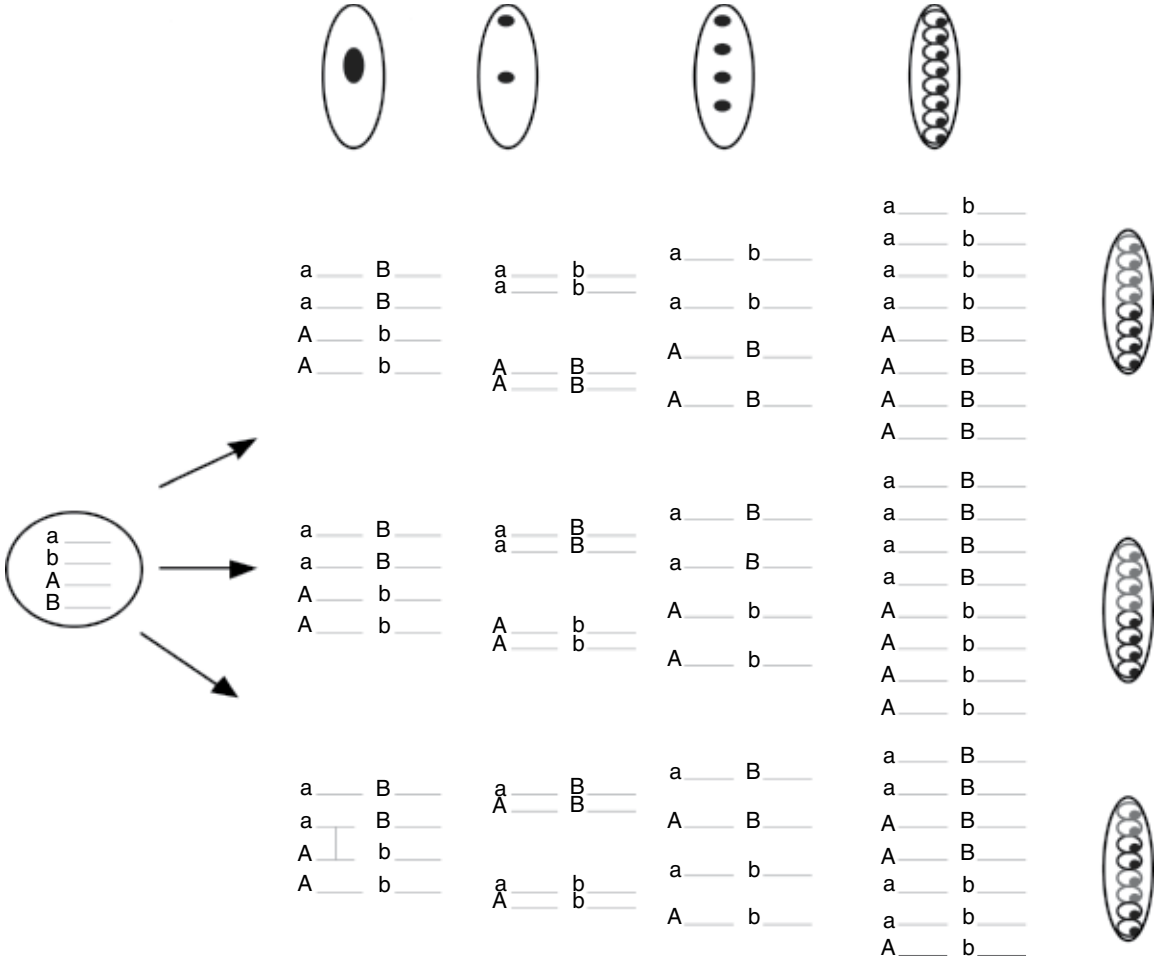


Figure 2.7 Chromosome assortment in *N. crassa*. (Adapted from Davis and De Serres (1970).)

2.5.2 Molecular Methods for Genetic Screens

2.5.2.1 Transformation

2.5.2.2 Plasmids, Transforming DNA

2.5.2.3 Genetic Screens

2.5.2.4 CRISPR Gene Editing in Fungi

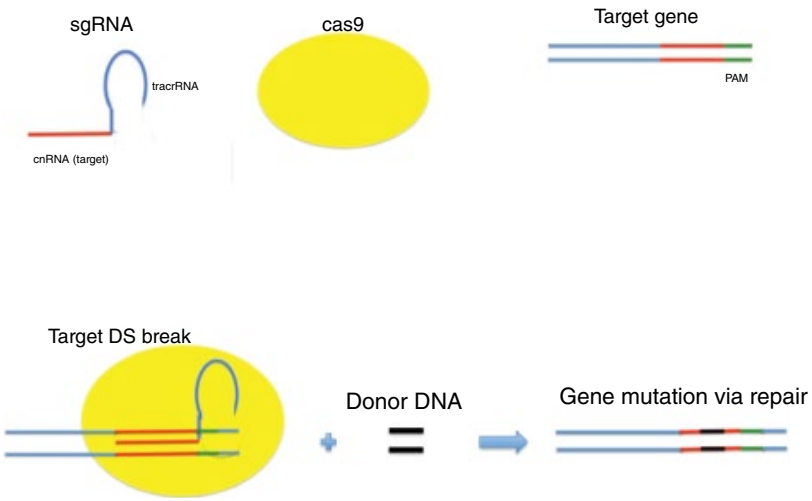


Figure 2.10 CRISPR/Cas9 for gene editing. (a) CRISPR components sgRNA and Cas9, and the target gene of interest containing a PAM sequence. (b) Cas9 interaction with the sgRNA and target locus, resulting in double-strand (DS) DNA breaks and introduction of mutation during repair.

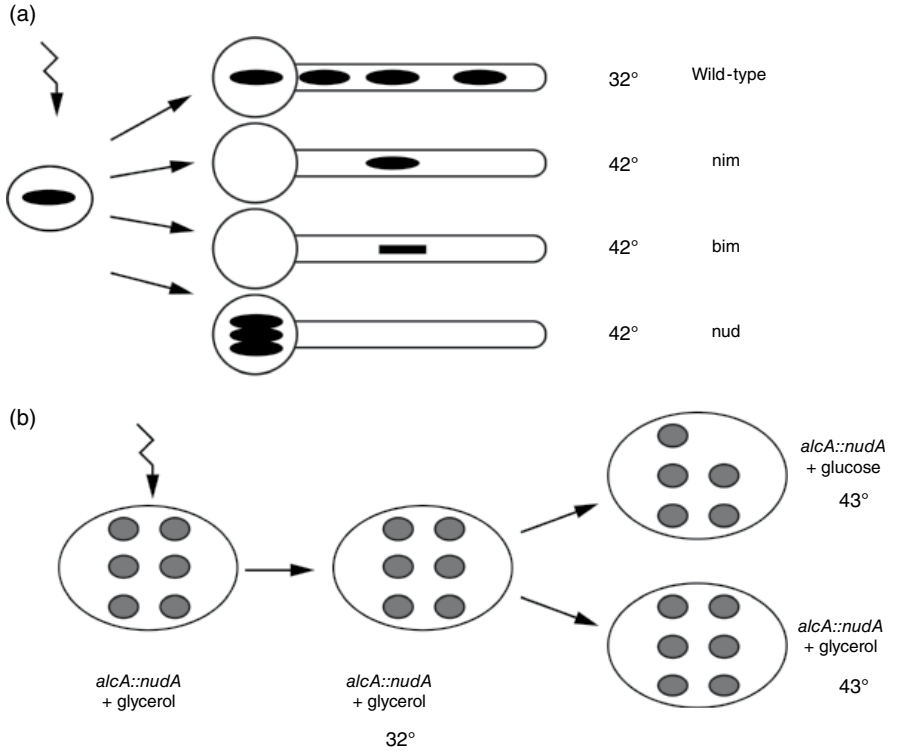


Figure 2.9 Selected genetic screens identifying cell cycle mutants in *A. nidulans*. (Adapted from Casselton and Zolan (2002).)

2.6 Conclusion