Chapter 36

HETEROKARYOSIS

The term *heterokaryosis* (**hetero**=dissimilar, **karyons**=nuclei) has been defined as the coexistence of genetically-different nuclei in cytoplasmic continuity with one another. The phenomenon, discovered by Hansen and Smith (1932) in *Botrytis cinerea*, is a near-universal (if not universal), feature in fungi. Heterokaryosis plays the major role in bringing about variability and sexuality in fungi. It is the pre-requisite for parasexual cycle, in the same way as heterozygosis is for sexual reproduction.

Heterokaryosis has been given different dimensions by different authors. In the most limited sense, it may be applied to a single cell, but usually it is applied to the thallus. In the broader sense, a fungus population, consisting of genetically different individuals, is designated as heterokaryotic. Whitehouse (1949) regards the dikaryons of a heterothallic fungus as representing heterokaryosis, but Jinks (1952) feels that the term heterokaryosis should be restricted to conditions in which the heterokaryons lie in unrestricted ratios. Christian and De Vay (1955) plead that dikaryosis should be segregated from heterokaryosis. However, Parmeter *et al* (1963) are of the opinion that such special usage of the term can lead to confusion. According to them the dikaryotic cell, containing dissimilar nuclei e.g. the spores of rusts, is clearly heterokaryotic, representing only one of the several types of heterokaryotic associations.

FORMATION OF HETEROKARYONS

The heterokaryotic condition arises by: (1) mutation, (2) anastomosis,

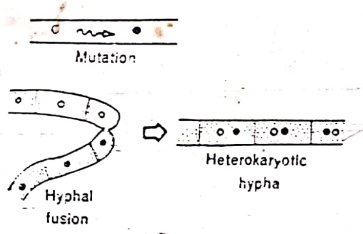

FIG. 36.1. Mutation and anastomosis render homokaryotic hyphae heterokaryotic.

and (3) inclusion of dissimilar nuclei in spores after meiosis, in heterothallic fungi (Fig. 36.1).

Mutation

A high frequency of mutation, which is characteristic of fungi, is the main source of variability. One who has experience of handling fungal cultures, especially species *Penicillium*, *Aspergillus*, *Verticillium*, *Fusarium* etc., knows how difficult it is to maintain a fungus in homokaryotic condition. Mutation frequently renders it heterokaryotic. It has been clearly demonstrated that new races of *Phytophthora* in USA, in absence of sexual reproduction, have arisen by mutation. Hansen (1938) observed that the single spore of *Fusarium* by frequent mutations produced variants of the wild type in cultures. To this Hansen gave the name "dual phenomenon".

Anastomosis (The Fusion of Hyphae)

This is a feature of common occurrence in fungi, and is certainly a big source of heterokaryosis. In general, fusion is mostly intra specific, though inter-specific and inter-generic fusions have been reported in unmistakable terms (Brierley 1929). Inter-specific anastomosis between *Botrytis cinerea* and *B. ricini* was reported by Hansen and Smith (1935) But, there are reports of cytoplasmic deterioration of fused cells, and antigen-like reactions are reported to be the cause of cytoplasmic incompatibility (Raper and Esser, 1961) Unfortunately, the physiological processes responsible for success or failure of anastomosis are inadequately studied.

Nuclear migration from the point of fusion to the remainder of the mycelia (involving daughter nuclei of the entrant nucleus) takes place and gives rise to a heterokaryotic mycelium. Alternatively, hyphal growth may initiate at the point of fusion, containing nuclei derived from both the parent hyphae. In nature, the most familiar example of anastomosis is the development of dikaryon in Basidiomycotina. Anastomosis is the method used for "making" heterokaryons in laboratory.

Inclusion of Dissimilar Nuclei in Same Spores

Meiosis results in the production of genetically different nuclei sharing common cytoplasm. In some fungi e.g. *Neurospora tetrasperma*, *Podospora anaserina* and other secondary homothallic fungi, dissimilar nuclei are contained in the same spore, which on germination gives rise to a heterokaryotic thallus. In the asexual phase, this occurs frequently in the multinucleate spores.

SIGNIFICANCE OF HETEROKARYOSIS

1. Substitute for Heterozygocity and Variability

Heterokaryosis provides a substitute for heterozygocity rather than for sex. It is important for *maintaining* variability (by virtue of

the presence of geneticially dissimilar nuclei), rather than *creating* it, which occurs during sexual reproduction by genetic recombination. The significance of heterokaryosis as the dispenser of variability was first emphasized by Hansen and Smith (1932) in *Botrytis cinerea*, and later by Hansen (1938) and Hansen and Snyder (1943). They visualized the heterokaryons and the separation of the different nuclei during conidial formation as substitutes for syngamy and meiosis. Much of the variability assigned to heterokaryosis by Hansen and his co-workers, however, has been demonstrated, since then, to be due to cytoplasmic factors (Jinks, 1959). Thus, as said above, heterokaryosis is a substitute for heterozygocity, rather than for sex. The variability gained through heterokaryosis provides plasticity to the fungi to face the environment with greater success.

2. Heterokaryosis and Pathogenicity

Heterokaryosis plays an important role in the pathogenic activities of rusts and smuts. It is essential for (rather it conditions) pathogenicity in smuts. In heterocious rusts, heterokaryotic dikaryotic condition is essential for infection of one group of hosts.

3. Origin of New Races

Nelson *et al* (1955) inoculated a resistant wheat variety with different races of rust, which individually failed to cause infection. Lesions appeared after some time. It was made possible by anastomosis between the inoculated races, which resulted in the origin of a new pathogenic race. Similar origin of new races of rusts has been suggested in *Melampsora lini*. Fusion of urediniospore germ tubes has been seen in various rust fungi and has been taken as indication of the origin of new races. However, recent studies suggest that mechanisms other than simple nuclear exchanges may be frequently involved in the vegetative origin of new races.

4. An Initial Step in Parasexual Cycle

Heterokaryosis is an integral, first step of parasexual cycle. But all heterokaryotic fungi are not necessarily parasexual.

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— and Smith, R E. 1932. The mechanism of variation in the Fungi Imperfecti. *Phytopathology*. *22* : 953-964.

The parasexual cycle was defined as a cycle in which plasmogamy, karyogamy, & haploidization take place, but not at a specified time or at specified points in the life cycle of an organism.

Chapter 37

THE PARASEXUAL CYCLE

'If only one outstanding contribution of microbial genetics to biological thinking had to be singled out, it would be this: the realisation that transfer of genetic information from one individual or cell to another is not the monopoly of sexual reproduction.

—G. PONTECORVO (1958)

For a long time sexual reproduction was thought to be the only mechanism of achieving genetic recombination. The genetic systems of the myriads of asexual micro-organisms like bacteria and imperfact fungi, made no sense. However, when several 'alternatives' to sexual reproduction were discovered in bacteria in quick succession, biology got a new vision. J.B.S Haldane called these new discoveries as novel methods of genetic recombination". Biologists looking at evolution through neo-Darwinian field glasses had to reckon with the newly-discovered alternatives to sex: viz. transformation, conjugation, transduction, lysogeny and sexduction. The lesson that these discoveries taught was, as quoted above, that the transfer of genetic information from one individual to another is not the monopoly of sexual reproduction (Pontecorvo, 1958).

One such novel alternative to sexual reproduction was discovered in fungi (*Aspergillus nidulans*) by Pontecorvo and Roper (1952) of the University of Glasgow. This they named as the **parasexual cycle**. In this process the genetic recombination is achieved through "mitotic crossing over" and "haplodization". It is also called as **somatic recombination**. In *A. nidulans*, the parasexual cycle occurs in addition to the normal sexual reproduction. While the events of sexual reproduction are extremely uniform, having a fine coordination between recombination, segregation and reduction, there is no such coordination in the parasexual cycle, The karyogamy and haplodization are accidental events not bound by space and time. The parasexual phenomenon was next reported in *Aspergillus niger*, which, unlike *A. nidulans*, lacks a sexual cycle. The phenomenon is now reported in several fungi belonging to Asco-, and Basidiomycotina (see table 36.1). So far, it has not been reported in coenocytic fungi (Mastigo-and Zygomycotina). However, indications of parasexuality do occur in *Phytophthora cactorum* (Buddenhagen, 1958) and *Phycomyces blakesleeanus* (Park *et al*, 1968).

TABLE 37.1

Showing Fungi in which Parasexuality has been Demonstrated, along with the Names of their Discoverers.

Organism	*Reported by*
Aspergillus nidulans	Pontecorvo and Roper (1952)
A. niger	Pontecorvo *et al* (1953)
A. fumigatus	Stromnaes and Garber (1963)
A. oryzae	Ishitani (1956)
A. sojae	Ishitani *et al* (1957)
Penicillium chrysogenum	Pontecorvo and Sermonti (1969)
P. expansum	Barron (1962)
P. italicum	Stromnaes *et al* (1964)
Cephalosporium mycophyllum	Tuveson and Coy (1961)
Cochliobolus sativus	Tinline (1962)
Fusarium oxysporum	
f. sp. *pisi*	Buxton (1956)
f. sp. *cubense*	Buxton (1962)
f. sp. *callistephi*	Hoffman (1967)
Coprinus fimetarius	Gans and Prud' Homme (1958)
Ustilago maydis	Holliday (1961)
U. violacea	Day and Jones (1968)
U. hordei	Dinoor and Person (1969)
Schizophyllum commune	Middleton (1964)
Piricularia oryzae	Yamasaki & Niizeki (1965)
Verticillium albo-atrum	Hastie (1962)
V. dahliae var. longisporum	Ingram (1968)

THE STEPS OF PARASEXUAL CYCLE

The various steps of the parasexual cycle have been worked out in detail for *Aspergillus nidulans*, *A. niger* and *Penicillium. chrysogenum.* In *A. nidulans*, which serves as the typical example, the parasexual cycle (see Fig. 37.1) occurs in the following four steps:

1. Establishment of heterokaryosis,
2. Formation of heterozygous diploids,
3. Occasional mitotic crossing-over during multiplication to the diploid nuclei, and
4. Occasional haplodization through aneuploidy.

Establishment of Heterokaryosis

The presence of haploid nuclei of dissimilar genotypes in the same cytoplasm is a pre-requisite for recombination. This is achieved by heterokaryosis, which is brought about by: (1) mutations, and (2)

anastomosis between hyphae of different origin (for details see chapter 36). This step is equal to plasmogamy of sexual reproduction.

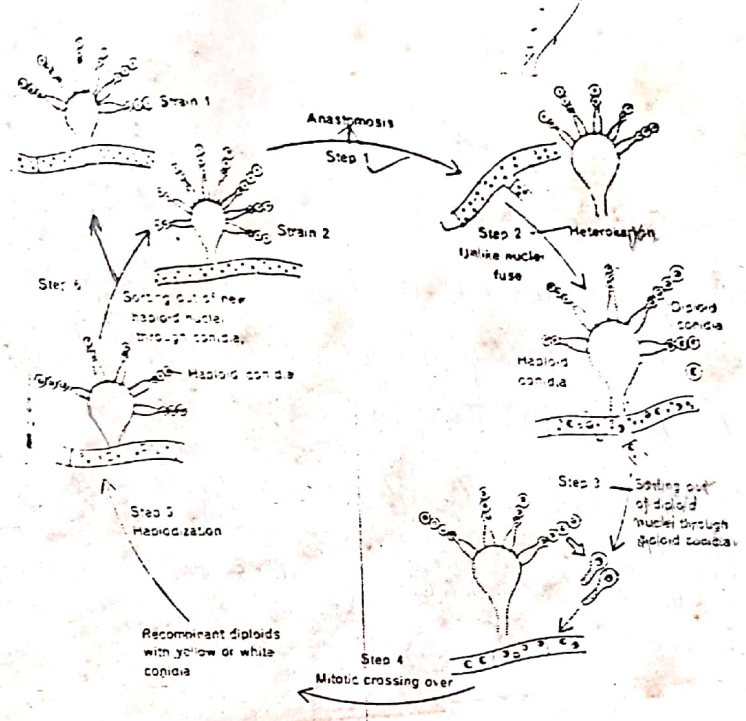


FIG. 37.1. Steps in the parasexual cycle (adapted from Pontecorvo 1958).

Formation of Heterozygous Diploids

Nuclear fusion in heterokaryotic vegetative cells was first noted by Roper (1952) in *Aspergillus nidulans*. The nuclear fusions between dissimilar nuclei result in the formation of heterozygous diploid nuclei or "zygotes"—a rare event, occurring at the rate of one in a million. Fusions between identical nuclei too occur. But since the homozygous diploid nuclei, thus formed, ultimately give rise to non-recombinant haploid nuclei, such fusions have no genetic relevance.

The heterozygous diploid nuclei, which are fairly stable, by multiplication and sorting out through conidia, give rise to diploid thalli. The diploid colonies are recognized by : (1) higher DNA content of their nuclei, (2) the bigger size (1.3 times) of their conidia, and (3) certain phenotypic characteristics of the colony. This is typical of parasexual phenomenon, and, obviously, the prolonged diploid phase, involving repeated nuclear divisions enhance the chances of "mitotic crossing-over".

Occasional Mitotic Crossing-over during Multiplication of Diploid Nuclei

This is the most important or 'key event' in parasexual cycle. It is during this step that genetic recombination takes place. Crossing-over during mitosis is a rare phenomenon, first noticed in 'fruit fly' (*Drosophila melanogaster*) by Stern in 1936. In *Aspergillus nidulans*, mitotic crossing-over occurs only rarely, with a frequency of 10^{-4} per nuclear division. Interestingly enough, in some fungi, like *Penicillium chrysogenum* and *Aspergillus niger*, it is as frequent as during meiosis. Pontecorvo and Roper (1952) invoked mitotic crossing-over to explain the development of new heterozygous, recombinant haploid nuclei, which was subsequently also proved cytologically. Reciprocal exchanges between homologous chromosomes at the four-strand stage were observed by Roper and Pritchard (1955) and Kafer (1961). It is a characteristic feature of mitotic crossing-over that the exchange, or chiasmata formation, is confined to a single chromosome pair out of the whole complement of chromosomes. In meiosis, the crossing-over occurs simultaneously in all the chromosomes. The subsequent splitting of chromosomes and segregation of strands is as it occurs in mitosis.

Crossing-over followed by segregation of strands, gives homozygosis for all markers distal to the point of chiasma. Markers proximal to the point of exchange and markers on other chromosomes remain heterozygous (Fig. 37.2).

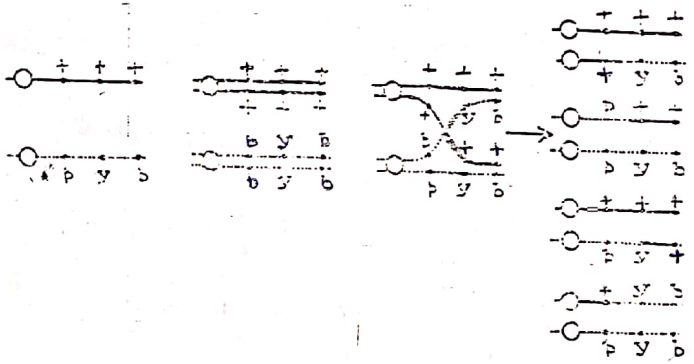


FIG. 37.2. Showing mitotic recombination.

Occasional Haplodization through Aneuploidy

The diploid nuclei give rise haploid to nuclei by gradual loss of chromosomes during successive mitotic divisions (Aneuploidy—Fig. 37.3). This is called **haplodization**. Meiosis is not involved. The haplodization, which occurs at a constant frequency of 10^{-3} per nuclear division, is the result of aneuploidy During mitotic divisions, non-disjunction of the chromatids of one chromosome pair results in

aneuploid nuclei which can be represented as (*2n-1*) at the beginning and (*2n-n*) i.e. (*n*) or haploid, at the end. The aneuploids are genetically unstable, and once the loss of chromosomes has started, the selection favours the development of the fully balanced haploid nuclei.

Parasexual cycle closely simulates the events of the sexual cycle. It involves everything : plasmogamy, karyogamy and haplodization but in a modified form and without any fixed plan with regard to time and space. The two processes, nevertheless, end up with similar

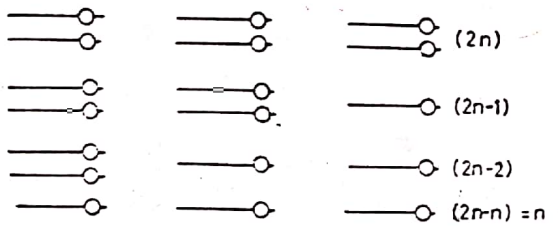


FIG. 37.3. Steps in the process of haplodization.

results i.e. give rise to recombinant haploid nuclei. A summarized comparison of the sexual and parasexual cycles has been made in Table 37.2.

TABLE 37.2

Comparison between Sexual and Parasexual Cycles of Fungi

Sexual Cycle	*Parasexual Cycle*
1. Nuclear fusion in specialized structures.	1. Rare nuclear fusions in vegetative cells.
2. Zygote usually persists one nuclear generation only.	2. Zygote persists through many mitoses.
3. Recombination by meiosis : crossing-over in all chromosome pairs, reduction of chromosome number, random assortment of members of each chromosome pair.	3. Recombination by rare "accidents" of mitosis: (*a*) mitotic crossing-over, at each event usually confined to one exchange in single chromosome arm, (*b*) haplodization independent of crossing-over, random assortment of members of each chromosome pair.
4. Products of meiosis readily recognised and isolated.	4. Recombinants occur among vegetative cells, recognised only by use of suitable genetic markers.

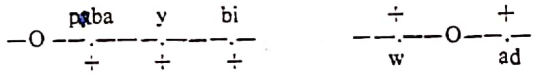
PARASEXUAL CYCLE IN *ASPERGILLUS NIDULANS*

Let us consider a typical example of parasexual cycle. Cytological evidences for the various steps are difficult to make out because of two main reasons : (1) the fungal nuclei are extremely small (lie at the border line of resolution by light microscope), and (2) the chromosomes have low affinity for stains and, are extremely small. Notwithstanding these difficulties, the various steps were elegantly

tracked with the help of suitable "markers" like colour of the conidia, deficiency of certain vitamins, growth factors etc.

Roper (1952) 'prepared' two homokaryotic, mutant strains of *Aspergillus nidulans*. Strain 1 had the genotype, ad⁻, w (ad⁻= requirement for exogenous adenine, w=white conidia, against green conidia of the wild type). Strain 2 had the genotype, **paba⁻ y, bi⁻** (paba⁻= requirement for exogenous para aminobenzoic acid: y=yellow conidia, and bi⁻= requirement for exogenous biotin). The conidia of *A. nidulans* are uninucleate and the condial colour alleles *w* and *y* were autonomous. Also, *w* is epistatic to colour, i.e. it prevents expression of any colour gene at another locus.

Roper 'synthesized' a 'balanced' heterokaryon from these two strains, which resembled the wild type in having no requirement for exogenous vitamins. For this he allowed a mixture of the conidia of the two strains to grow together on a minimal medium, to support initial growth and to enable anastomosis. The heterokaryotic hyphae formed, grew on the minimal medium without any addition of vitamins. The heterokaryon formed both yellow and white conidia on same conidial head. Occasionally, green conidia appeared in the culture, which produced the wild type colony on minimal medium i.e. prototrophic fungus producing green conidia. The sudden appearance of prototrophic individuals producing green conidia was the result of accidental nuclear fusions in the heterokaryotic mycelium. Conidia containing heterozygous diploid nuclei gave rise to these prototrophic individuals with green conidia. The diploid nature of these individuals was proved by the fact that : (1) they had the phenotype expected of the heterozygous diploid, (2) the conidia were bigger than the haploid conidia (the volume being in the ratio of 2 : 1), and (3) the nuclei contained DNA which was double the amount present in the haploid nuclei. Mutation, as the cause of origin of green conidia, was ruled out on the ground that it involved several mutations in one stroke. The second step, viz. the formation of heterozygous diploids was thus accomplished. The full genotype of the diploid can be written as shown below. The linkages and the positions of the centromere were based on studies of standard meiotic analyses.



The diploid individuals were stable which multiplied through diploid conidia. Nevertheless, occasionally, sectors forming yellow and white conidia re-appeared. Unmistakably, these were recombinants, and in tests for ploidy proved to be diploid. These were the products of accidental mitotic crossing-over during divisions of the diploid nuclei.

The diploid recombiant colonies were of various types. All white-conidial diploids were prototrophic while the yellow-conidial diploids

required exogenous biotin, and some of them also had requirement for p-aminobenzoic acid.

Obviously, the yellow homozygous recombinants were products of crossing-over between y locus and the centromere. These are homozygous at **bi** locus. If the crossing-over occurred somewhere between **pyba** locus and the centromere, then they would be homozygous for the **paba** locus also. The markers **w** and **ad** remain heterozygous.

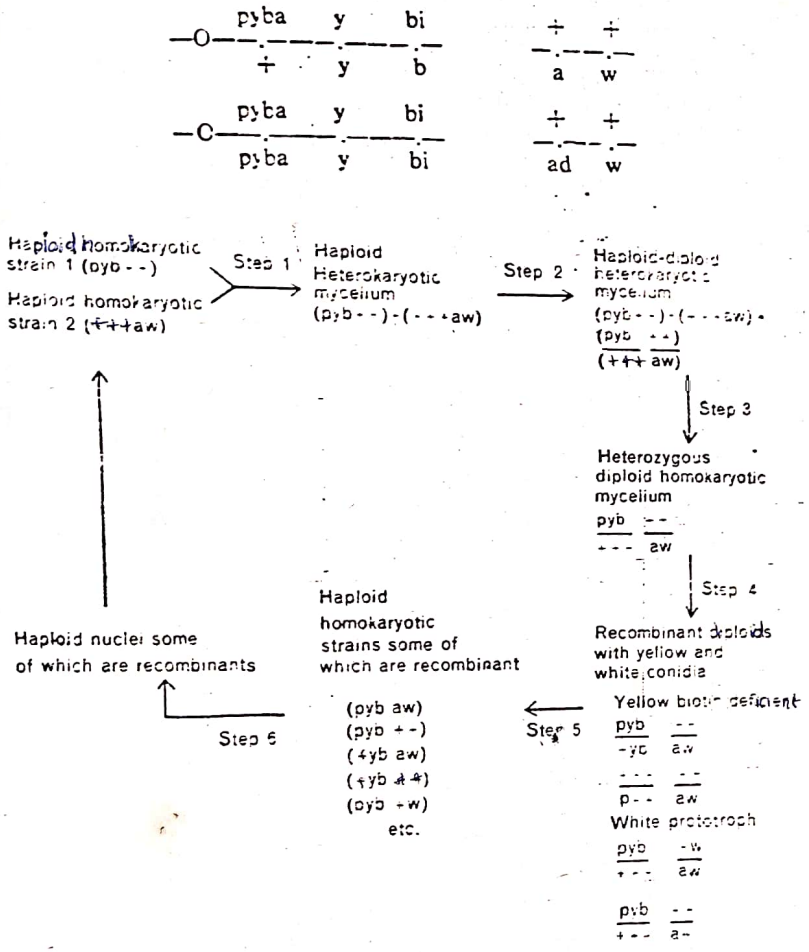


FIG. 37.4. Parasexual cycle in *Aspergillus nidulans*. Step 1, hyphal anastomosis; Step 2, 2×10^{-2} unlike nuclei fuse; Step 3, sorting out of heterozygous diploid nuclei through conidia; Step 4, mitotic crossing over; Step 5, haplodization in 10^{-3} diploid nuclei; step 6, sorting out of the haploid nuclei.

From the recombinant diploids were isolated aneuploid individuals with genotypes ranging from $(2n\text{-}1)$ to $(2n\text{-}n)$. The haploids (with

yellow and white conidia) were found to be of the following types ;

1. *The white haploids*, all reqiured adenine; approximately half of these had also requirement for p-aminobenzoic acid and biotin in addition to adenine. The latter haploids were new, recombinant haploids.

2. *The yellow haploids*, all required p-aminobenzoic acid and biotin but never adenine.

The various steps are shown in Fig. 37.4

Occurrence and Role of Parasexual Cycle in Nature

Parasexuality has been known only as a laboratory phenomenon. However, the results of Ingram (1968) strongly suggest that it may be widespread in nature. Though it has been demonstrated only in Asco-, Basidio- and Deuteromycotina, there are strong evidences of its occurrence in some coenocytic fungi, like *Phytophthora cactorum* (Buddenhagen, 1958). The phenomenon is linked with heterokaryotic condition which is very frequently encountered in natural populations. But every heterokaryotic species may not necessarily be parasexual. The more loaded question is whether the parasex has any significance in face of its being a rare, accidental phenomenon. The answer is that the role of parasexuality in the origin of new individuals remains to be assessed.

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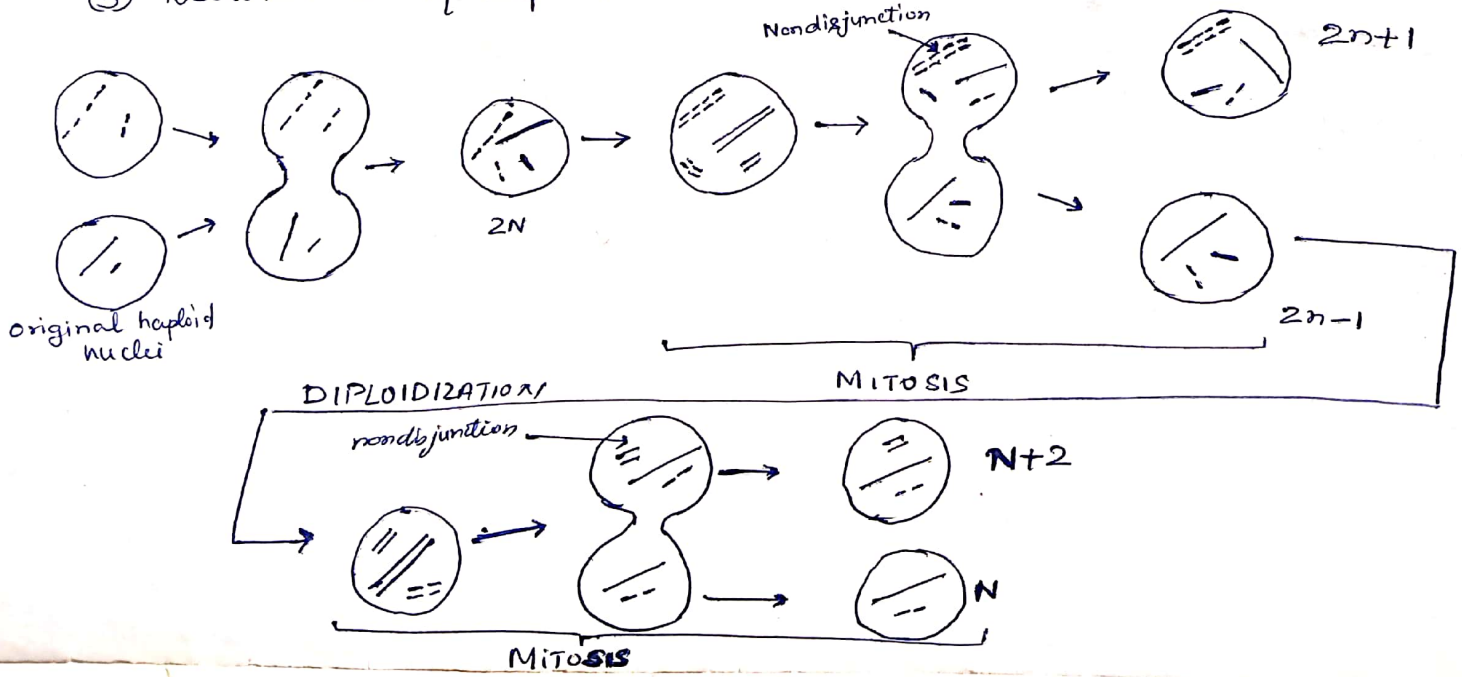
Parasexual Cycle :- Pontecarvo & Roper reported parasexual cycle in *Aspergillus nidulans*

In this process genetic recombination is achieved through "mitotic crossingover" & "haplodization". It is also called as "Somatic recombination"

The parasexual Cycle may take place within a heterokaryon.

The parasexual cycle is sequencing involving

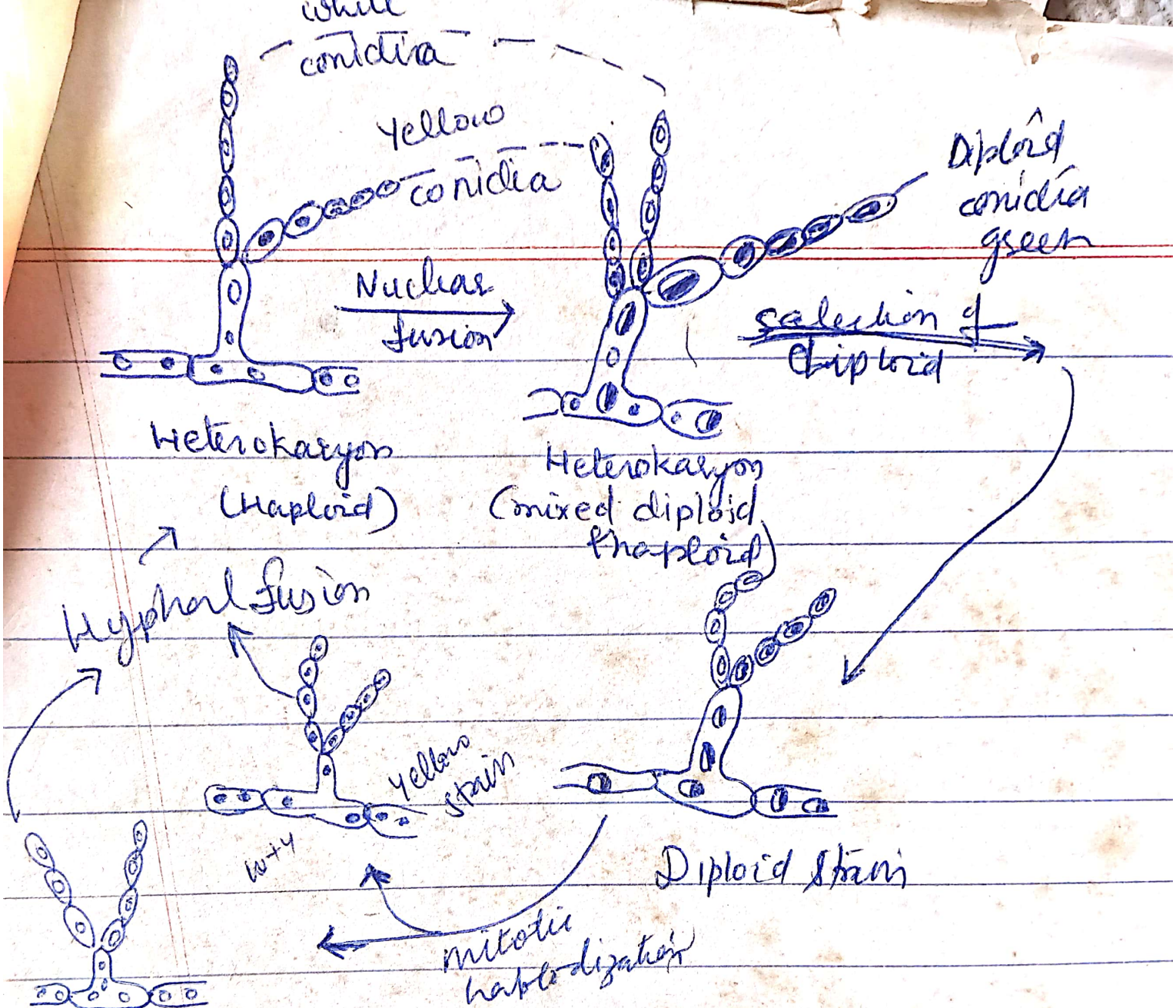
(1) Heterokaryon formation
(2) Diploidization of nuclei, and
(3) Restoration of diploid nuclei to their haploid state (Haploidization) fig (below)



Haploidization
beginning with genetically different haploid nuclei in the heterokaryon, & terminating with formation of a haploid nucleus that is genetically different from the original nuclei.

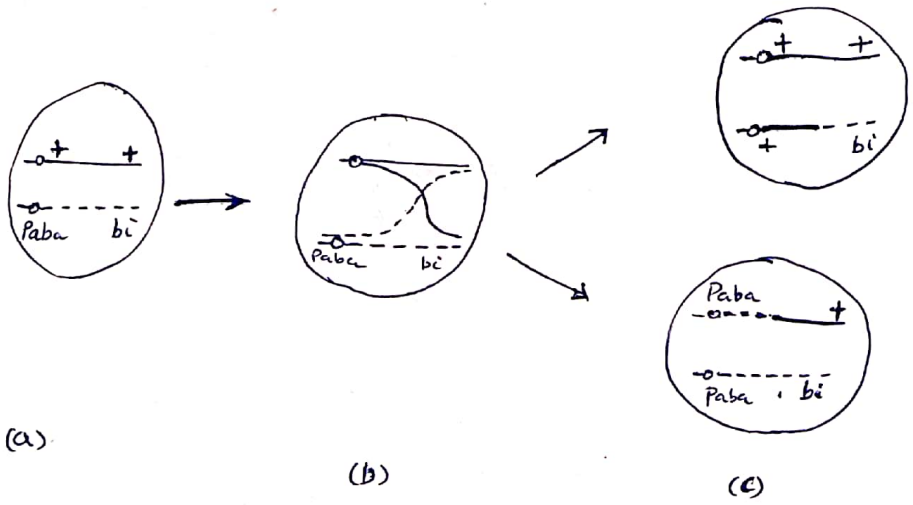
Haploidization involves a series of atypical and irregularly occuring mitotic divisions of the diploid nuclei. Frequently, daughter nuclei resulting from mitosis have unequal numbers of chromosomes because sister chromatids failed to separate (nondisjunction) during anaphase. This yields a daughter nucleus with one chromosome too many (2n+1), while the second daughter nucleus is lacking a chromosome (2N-1). Both of these Nuclei are now aneuploids, as they do not have even chromosome sets such as N or 2N. The deficient aneuploid nucleus (2N-1) may undergo additional loss of chromosomes in successive mitotic divisions of the same nature until it is reduced to the haploid condition. Pontecorvo (1958) estimated that only one out of every 1000 diploid nuclei in a diploid isolate of Aspergillus niger had undergone haploidization.

Mitotic Crossing-over may accompany the parasexual cycle. In mitotic crossing-over, segments of chromosomes are exchanged for exactly corresponding segments between homologous chromosomes, presumably occuring at the time when the chromosomes replicate. If dominant and recessive genes have been regrouped, subsequent haploidization may produce daughter nuclei that are genetically different from those that would be formed if this exchange had not taken place. For every 10 haploid nuclei



Parasexual cycle in Aspergillus, where diploid formation through heterokaryon & haploid formation without meiotic division are shown. Visible markers in the form of coloured conidia are used to demonstrate the parasexual

derived through the parasexual cycle, perhaps only one nucleus is a recombinant derived from mitotic crossing-over.



(a) (b) (c)

Mitotic crossing-over between a single pair of homologous chromosomes. this may occur at some point during haploidization.

(a) Diploid nucleus that is heterozygous for the the genes paba (p-amino benzoic acid-requiring) and bi (biotin-requiring).

(b) Crossing-over at chromosome replication (four-strand stage);

(c) genetically different nuclei formed after chromosome segregation.

The parasexual cycle mimics sexual reproduction. Unlike hyphae may fuse (plasmogamy) & their (gametic) nuclei occupy the same mycelium. These nuclei may fuse (karyogamy) to form a diploid (zygotic) nucleus. Genetic recombination may occur, and the diploid nucleus is reduced (but without meiosis) to the haploid condition.

The recombinant, haploid nuclei are segregated into so-called nonsexual spores, which differ genetically from the parent mycelium.

Chapter 35

HOMOTHALLISM AND HETEROTHALLISM

A.F. Blakeslee, an American geneticist in the year 1904 observed that while some species of *Rhizopus* formed zygospores freely, others e.g. *R. nigricans* (=*R. stolonifer*), formed these only rarely. He examined zygospore formation in several mucorales and discovered the phenomenon of heterothallism in fungi. When two isolates of *R. nigricans* or *Mucor hiemalis* were grown together in a Petri dish (Fig. 35.1), zygospores appeared at the point of contact between

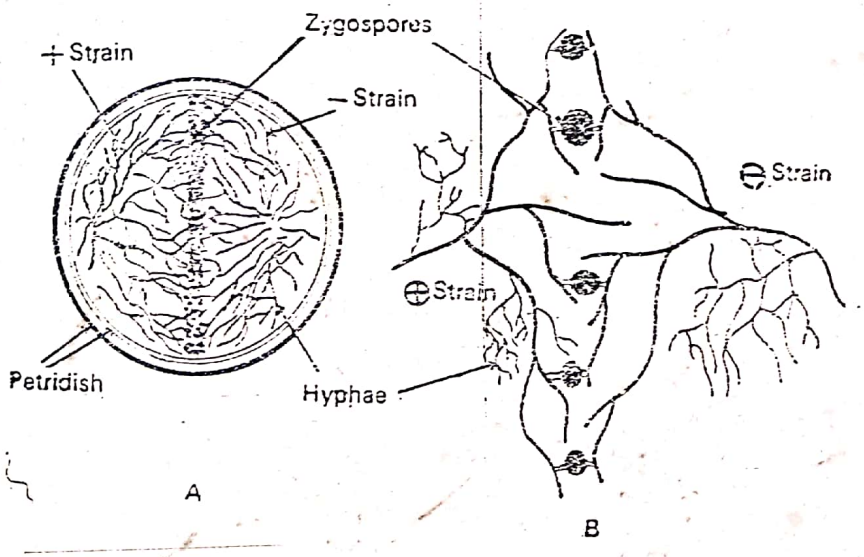


FIG. 35.1. Heterothallism in *Mucor hiemalis*. A, Petri dish culture showing zygospores along the zone of contact between the two mycelia of opposite mating types; B, magnified view of the zygote formation.

hyphae of the two isolates. Blakeslee called these fungi, which required another isolate for zygospore formation, as **heterothallic** and the phenomenon as **heterothallism.** He designated the two isolates, or mating types, the (+) and (−) strains, as their gametangia were morphologically similar and indistinguishable into male and female individuals. The opposite condition in which an individual, originating from a single asexual spore, was capable of forming zygospore independently, was called **homothallism.**

In heterothallism the two mating partners are derived from two genetically-unlike spores, designated as (+) and (−) spores. Subsequently, several common species of Mucorales were examined and described as homo- or heterothallic. The heterothallic species always consisted of two types of individuals, the (+) and (−) mating types, Blakeslee assumed that the (+) strain represented the female and the (−) the male.

To test this hypothesis Satina and Blakeslee (1928, 1929) undertook extensive tests between homothallic and heterothallic species of Mucorales. *Zygorhyncus moelleri*, *Absidia glauca* are homothallic, forming female macro-, and male microgametangia on the same thallus. In mating experiments, the (+) mating type gametangia of heterothallic species fused with the male and (−) mating type gametangia with the female, suggesting that the (+) mating type was female and the (−) male. But the opposite reaction occurred with the gametangia of *Zygorhyncus heterogamous* and, thus, the femaleness of (+) strain and maleness of (−) strain remained inconclusive.

No physiological differences could be found between the gametangia of (+) and (−) strains, and therefore, we do not disignate the (+) and (−) as male and female sexes but as **mating types.**

Since Blakeslee's discovery, heterothallism has been reported from all major groups of fungi, though in varied forms. Several patterns of intermycelial reactions are recognized in heterothallic fungi, but all share one characteristic feature viz. the inter mycelial contact. In some heterothallic fungi, the sexual differences are distinct and sex organs are formed on different thalli. In others e.g. in hermaphrodite heterothallic fungi, the reasons for dependence on another individual are physiological and more subtle.

Morphological and Physiological Heterothallism

Whitehouse (1949) distinguished two major types of heterothallism: **morphological** and **physiological**. When the two interacting thalli produce morphologically distinct male and female sex organs (or gametes), the heterothallism is called morphological heterothallism. In physiological heterothallism, the interacting thalli differ in mating type or incompatibility, irrespective of the presence or absence of the sex organs or gametes. Homothallism was accepted by Whitehouse in the original sense i.e. sexual fusion between elements of the same thallus; or in unicellular organisms, between cells of the same clone. In addition, he added a new term, the **secondary homothallism**, which is explained like this. In some heterothallic fungi e.g. *Exobasidium japonicum*, it so happens that two genetically-different nuclei, formed during meiosis and which ought to have gone to different spores, are regularly contained in a single spore. Such spores on germination give rise to a mycelium which contains nuclei of the opposite strains and the fungus behaves as if homothallic.

Genetic Determinants of Heterothallism

We have seen above that heterothallism may be due to: (1) lack of both male and female sex organs on the same thallus, or (2) incompatibility between the sex organs formed on the same thallus with regard to karyogamy. The two conditions are genetically controlled by factors called, respectively, the sexual and incompatibility factors. **Sexual factors** are responsible for the formation of sex organs and gametes. The maleness or femaleness is determined by the direction of nuclear migration during plasmogamy. The male serves as the donor of the nucleus and the female as the recipient. **Incompatibility factors** determine the mating capacity in addition to, or in absence of, sexual factors. In presence of sexual factors, and consequently of sex organs, the incompatibility factors determine the possibility of mating between the male and female sex organs or gametes. In absence of sex factors, when no sex organs or gametes are formed, as happens in most higher Asco- and Basidiomycotina, the incompatibility factors are the sole determinants of sexual fusions.

The segregation of these factors during meiosis determines the sexual status (i.e. whether sex organs or gametes are formed) and the mating behaviour (i.e. whether homothallic or heterothallic). Three types of segregations may occur during meiosis: (1) segregation of sexual factors (2) segregation of incompatibility factors and (3) no segregation of either factors. The segregation pattern is exhibited by the thalli that develop from the spores containing the meiotic nuclei. The spores of type (1) and (2) give rise to thalli that are self-sterile i.e. heterothallic. The spores of type (3), on the other hand give rise to homothallic thalli.

HOMOTHALLIC FUNGI

Homothallic species are more common than heterothallic in all groups of fungi, except in Basidiomycotina. The sexual or incompatibility factors do not segregate during meiosis and differentiation of compatible sexual elements is intra-mycelial. All individuals are alike and morphologically and/or functionally hermaphrodite. Is not the sexual fusion between genetically similar nuclei, in homothallic fungi, in terms of genetic recombination, a biological absurdity? No! Nature would not encourage biological absurdities; they are swiftly eliminated. The widespread occurrence of homothallic fungi in nature is eloquent testimony to this. During the extensive vegetative phase, the nuclei undergo genetic changes due to mutations, and become heterokaryotic (see later).

HETEROTHALLIC FUNGI

Raper (1966) distinguished six types of heterothallic fungi. The segregation of the sexual and incompatibility factors of these various

heterothallic fungi fall into two categories: (1) two alleles at one locus, and (2) multiple alleles at one or two loci.

Two Allele—or Bipolar Heterothallism

Fungi in this category have two mating types; each containing genetically different nuclei. The sexual compatibility is controlled by a pair of genetic factors **A** and **a** (which may be sexual or incompatibility factors) located at a single locus on different chromosomes. The use of (+) and (−) designations seems preferable because **A/a** implies dominance of **A** over **a**, which has never been demonstrated for these alleles. This is, therefore, also called as "two-allele heterothallism". During meiosis, the two chromosomes, containing the (+) and (−) alleles are separated in the haploid spores (viz. germ spores, ascospores or basidiospores). The spores give rise to two types of thalli which must fuse in order to bring the two compatible (+) and (−) nuclei together. The two mating types are designated as (+) and (−) strains.

When the two alleles represent sex factors, the two types of thalli formed are unisexual, producing male or female sex organs or gametes. Relatively few groups of fungi show sexual dimorphism. Examples are found in unifiagellate aquatic fungi which develop into morphologically distinguishable male and female gametangia.

The heterothallic hermaphrodite (=self-sterile) individuals also come under two-allele heterothallism. The sexual factors, as in homothallic species, are not segregated while the incompatibility factors segregate. This results in the production of self-sterile, hermaphrodite individuals. Examples of this type of heterothallism are found in majority of Ascomycotina (e.g. *Neurospora*) and the rust fungi. Esser (1963) has explained the genetic basis of heterothallism of the hermaphrodite fungi as shown in Fig. 35.2.

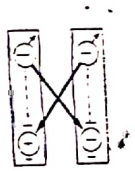


FIG. 35.2. Bipolar, 2 allele heterothallism (After Esser 1963). For explanation see text.

The rectangles represent the two mating types (+) and (−); the differentiated or undifferentiated sex organs contain nuclei which are incompatible. This is indicated in the diagram by broken lines which are blocked. Sexual fusion requires the presence of individuals of different mating types (represented by heavy arrows) and reciprocal crosses occur.

MULTIPLE ALLELES AT ONE LOCUS

(Bipolar Multiple allele Heterothallism)

This type of heterothallism is controlled by multiple alleles at a

single locus, instead of a pair of alleles. If we name the locus as A, the multiple alleles can be designated as A_1, A_2 A_3 . . . . A_n. The meiotic products give rise to thalli, which are of several mating types (equal to the number of the alleles). The thallus containing the allele A_1 can mate with a thallus of any mating types except A_1 (Fig. 35.3) Like the two-allele heterothallism, here also the genetic factors may involve sexual or incompatibility factors. Incompatibility factors are more commonly involved. It is a characteristic of the Basidiomycotina (excluding rusts and smuts). The mating is reciprocal i.e. each cell of the thallus is capable of donating or accepting the nucleus of the compatible mating type.

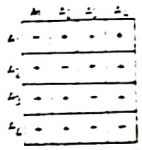


FIG. 35.3. Bipolar, multiple-allele heterothallism. A_1-A_4 represent two multiple alleles at a single locus (+, compatible cross; −, incompatible cross).

When the alleles involve sexual factors, several types of strains are formed, each typically self sterile but capable of mating with the other types. This type of heterothallism has been found in all the studied members of Saprolegniales,. Leptomitales and Peronosporales. The sexual strains constitute a linear series in which only those strains which lie at the two ends are strictly male or female; the rest have **relative maleness** or **femaleness**. Raper (1947) collected 10 such sexual strains of *Achlya ambisexualis* and arranged them in a linear series on the basis of their sexual (male or female) potential

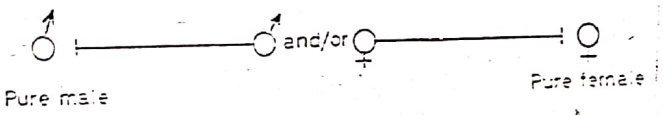


FIG. 35.4. The sexual strains of *Achlya ambisexualis* linearly arranged according to their male and female sexual potentialities. Each strain can react as male, female or both, depending on juxtaposition (♂, male; ♀, female, after Raper 1966).

(Fig. 35.4). Except the terminal pure male or female isolates, the rest of the isolates behaved as female with those on their left and as male to those on the right. To quote Raper (1959) these show "sexual ambivalence in which maleness or femaleness is determined in each mating type by common consent of the mated".

MULTIPLE ALLELES AT TWO LOCI

(Tetrapolar Multiple-allele Heterothallism)

This type of heterothallism is found in Basidiomycotina, excepting the rusts. It involves only incompatibility factors and therefore, sex organs are not formed.

The incompatibility factors are present at two loci, say **A** and **B** on different chromosomes. Each locus comprises of multiple alleles, as many as 100 or even more. In *Schizophyllum commune* 122 alleles of **A** and 61 of **B** factor have been identified in laboratory. In natural populations the number of alleles may be even more. According to one estimate (Raper *et al*, 1958 b, 1960) locus **A** may have as many as 350-450 alleles, while **B** about 65. As a rule, the multiple factors of the two loci are unequal; locus **A** always has more alleles than **B**. The **A** and **B** allelic incompatibility factors segregate independently at meiosis and give rise to thalli of numerous mating types. Mating occurs (Fig. 35.5) between thalli which have nuclei carrying different alleles at both loci (Kneip 1920). The diploid nucleus comes to have the alleles A_1, B_1, A_2, B_2.

	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	−	FL	B	+
A_1B_2	FL	−	+	B
A_2B_1	B	+	−	FL
A_2B_2	+	B	FL	−

FIG. 35.5. Mating reactions between the four possible isolates of a tetrapolar fungus. (+, compatible cross; — incompatible cross: FL, flat reaction; B, barrage reaction).

In this type of heterothallism the out-breeding is complete, (100%) against 25% in bipolar. This is due to enormous increase in the number of the possible mating type of the thalli.

Flat and Barrage Reactions

When two thalli with a common A or B alleles are mated, though plasmogamy takes place and heterokaryons are formed, fruiting never occurs. If the heterokaryons have common A alleles (and dissimilar B alleles), the mating is termed *flat reaction* (see Fig. 35.5). Such heterokaryons show poor growth: hyphae are curly and irregularly branched. Parag (1965) reported that the presence of common A alleles prevent formation of clamp connections; disrupt nuclear distribution and also cause morphological and metabolic abnormalities. The mating between thalli containing similar B alleles (but different A alleles), show what is called a **barrage reaction**. The hyphae at the point of contact show cessation of growth, resulting in a "barrage" or 'zone of no growth' between the colonies of the two thalli. Common B factors, according to Parag (*loc. cit.*), prevent karyogamy and meiosis.

ESSER'S TERMINOLOGY FOR HOMO- AND HETEROTHALLISM

There are fungi which do not fit into the concept of homo-, versus heterothallism, as described above. There have been proposals to abandon these terms by reputed mycologists like Korf

(1952). Hartman (1956), Burnett (1956) and Esser (1959, 1963). The terminology and concepts but forward by Esser deserve attention. Esser introduced the terms *monoecious* and *dioecious* analogues to higher plants disregarding the absence of sexual differentiation in most fungi. He used the concept of monoecism and dioecism in the broader sense, emphasizing its physiological rather than morphological aspects. An individual which can function both as donor and recipient of nucleus is monoecious. Individuals which act only as recipient or donor of the nucleus are dioecious. Monoecious fungi may possess both male and female sex organs on the same thallus or may have none, but, nevertheless, can receive or donate nuclei. A monoecious species may be compatible (self-fertile) e.g. *Glomerella* or incompatible (self-sterile) e.g. *Neurospora.*

The dioecious species may be *morphologically* or *physiologically* dioecious. Morphologically-dioecious species bear either of the male and female sex organs, which are morphologically dissimilar. The physiologically-dioecious species either bear sex organs (which can not be identified as male or female) or lack them.

Esser's proposed terminology roughly paralleles homo- and heterothallism.

Since the terms homothallism and heterothallism have been widely used and have attained a historical importance, their abondonment may cause unnecessary confusion. Hence, the *status quo* is being maintained in spite of several accepted shortcomings.

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Chapter 38

SEX HORMONES IN FUNGI

The participation of hormones in the sexual reproduction of fungi [which was proposed by de Bary (1881) for *Achlya*] is now an established fact. Studies on sex hormones of fungi have come of age. With the recent success in their isolation in and chemical characterization, the 'gaps' in knowledge may soon become non-existent.

DEFINITION AND TERMINOLOGY

Van den Ende (1976) has defined sexual hormone as a *diffusible substance playing a specific role in the sexual reproduction of the organism that produces it.*

Machlis (1972) coined the terms, **erotactins, erotropins** and **erogens**, imaginatively derived from the word *Eros* (the name of the god of love and son of aphrodite). A sexual hormone which attracts motile gametes is **erotactin;** it is **erotropin** when it induces chemotropic growth of sexual structures; and an **erogen** when the sex hormone controls the induction and differentiation of sexual structures. These terms only refer to the phenomenon and not the mechanism of response, which vary with the different organisms. Van den Ende (1976) doubts the usefulness of such clear-cut segregation of the hormones. Where to put those which show more than one type of action? For example in the fungus *Achlya*, the hormone eliciting the formation of sexual structures also influences their direction of growth.

THE HORMONES

There are good evidences of the involvement of sex hormones in almost all groups of fungi. We shall study only those which have been chemically characterized and extensively investigated. These are: **sirenin, antheridiol, trisporic acid** and **yeast α factor.** These hormones do not show any marked similarity either in chemical structure or biological activity. Sirenin (from *Allomyces*) is a sperm-attractant i e. (an erotactin *sensu* Machlis). Antheridiol and trisporic acid induce differentiation of sex organs and fall in the category of erogen. Antheridiol also functions as a chemotactic agent i.e. erotropin. Table 38.1 lists the better-known sex hormones, the organisms that produce them, the site of their synthesis, the morphogenetic actions they induce, and the emperical formula, where known.

TABLE 38.1

Fungal Sex Hormones and Their Properties

Sex hormone	*Produced by*	*Emperical formula (mol. wt.)*	*Site and control of synthesis*	*Morphogenetic action*
Sirenin	*Allomyces* sp.	$C_{15}H_{24}O_2$ (236)	Female gametes	Chemotaxis of male gametes
Antheridiol	*Achlya* sp.	Sterol $C_{29}H_{42}O_5$ (470)	Female cells	Antheridia formed by male hyphae
Hormone—B	,, ,,	Sterol (Ca 500)	Female cells in response to antheridiol	Oogonia formed by female cells
Trisporic acid	Several mucorales	$C_{18}H_{26}O_4$ (306)	(+) and (—) cells in collaboration	Zygophore formed by (+) and (—) hyphae
Yeast x factor	*Saccharomyces* sp.	Peptide (Ca 1400)	α yeast cells	Elongation of cells

SIRENIN

This is a sperm-attractant (Fig. 38.1) produced by the water mold *Allomyces*. The species *A. macrogynous*, and *A. arbuscula* which have

FIG. 38.1. Sirenin.

been employed in the study of sex hormones, belong to the sub-genus *Eullomyces*, characterized by isomorphic alternation of generations. (see page 68 for life cycle).

The gametophyte thalli produce male and female gametangia in pairs. The male gametangia are orange-coloured and, depending on the species, lie above or below the colourless female gametangia. When mature, the motile male and female gametes are released into the water from the gametangia. The smaller, orange-coloured male gametes are attracted by the female gametes which are twice as big as the male gametes, and sluggish in movement.

The female gametes release sirenin while still inside the gametangium. The hormone diffuses into the surrounding water and male gametes, under the influence of sirenin, start collecting around the female gametangia. The female gametes are faced with numerous male gametes as soon as released. The purpose of sirenin production is achieved. The female gametes by anisogametic copulation give rise to diploid motile zygotes.

The synthesis of sirenin by female gametes and their attractive action for the male gametes was demonstrated by Machlis (1958). The fact that sirenin is not extractable from the male gametes after its up take, suggests that the hormone is metabolized and converted into inactive form.

Bioassay of Sirenin

The test solution is taken in a small glass tube with cellophane base and immersed in a vessel containing water and male gametes. The clustering of male gametes around the cellophane indicates the presence of sirenin in the test solution. The number of male gametes collected is proportional to the concentration of sirenin.

Isolation and Chemical Characterization

For isolation, interspecific female hybrids of *A. macrogynous* and

A. orbuscula, which produce exclusively (96-99%) female gametangia were used. The methods of collection and purification of the hormone are highly technical, and we can be spared of great deal of pain by simply referring it as a specialists' job. The chemical structure of sirenin was reported in 1968 by Machlis *et al*. It is an oxygenated sesquiterpene and may be species specific. Two forms (D and L) have been isolated but only the L-form is biologically effective. The L-sirenin has also been synthesized.

Two Kinds of Chemotaxis in Allomyces

Five types of motile cells are formed in the life cycle of *Allomyces* (viz. haploid and diploid zoospores, male and female gametes, and the zygotes) but only the male gametes respond to sirenin. The two kinds of zoospores and the zygote show no response; instead, they are attracted towards amino acids. Immediately after fertilization the male gametes cease to respond to sirenin and the resulting zygote shows positive chemotactic response towards amino acids.

ANTHERIDIOL AND HORMONE-B(=OOGONIAL)

J.R. Raper in his classical studies (1939-1959) demonstrated a multihormonal regulatory system in *Achlya ambisexualis* and *A. bisexualis*. He envisaged four hormones, named A, B, C and D; but now it has been conclusively proved that only two hormones viz. antheridiol (erstwhile hormones A and C) and hormone-B(=oogonial) are involved. To have a better understanding, we shall examine it in a historical perspective.

The Hormonal System in *Achlya* sensu Raper

Raper (1952) worked with two isolates of *Achlya ambisexualis* (T_5, a typical female, and E_{57}, a typical male strain). The two isolates, which did not form any sex organs when grown independently formed them readily when grown in juxtaposition. The development of the sex organs was found to be dependent on sequential production of hormones by both the individuals. Raper divided the sexual reactions in the following four steps (Fig. 38.2.).

Step 1. *Development of Antheridial Hyphae*. According to Raper, this step is regulated by four hormones (A A_1 A_2 A_3), collectively called A-complex. The hormones A and A_2 are produced by the female isolate which induce formation of antheridial hyphae in the male isolate. The male isolate produces hormones A_1 and A_3; while A_1 augments the action of A and A_2, A_3 retards it.

Step 2. *Development of Oogonial Hyphae*. This step is induced by hormone B secreted by the antheridial hyphae.

Step 3. *Chemotropic Growth of Antheridial Hyphae towards Oogonial Hyphae*. This step is induced by hormone **C** secreted by oogonial hyphae. In addition, the hormone also induces delimitation of antheridia after the antheridial hyphae have come in contact with the oogonial hyphae.

Step 4. *Delimitation of Oogonia and Oospheres*. This is controlled by hormone **D**, secreted by the antheridia. The hormone also leads to fertilization.

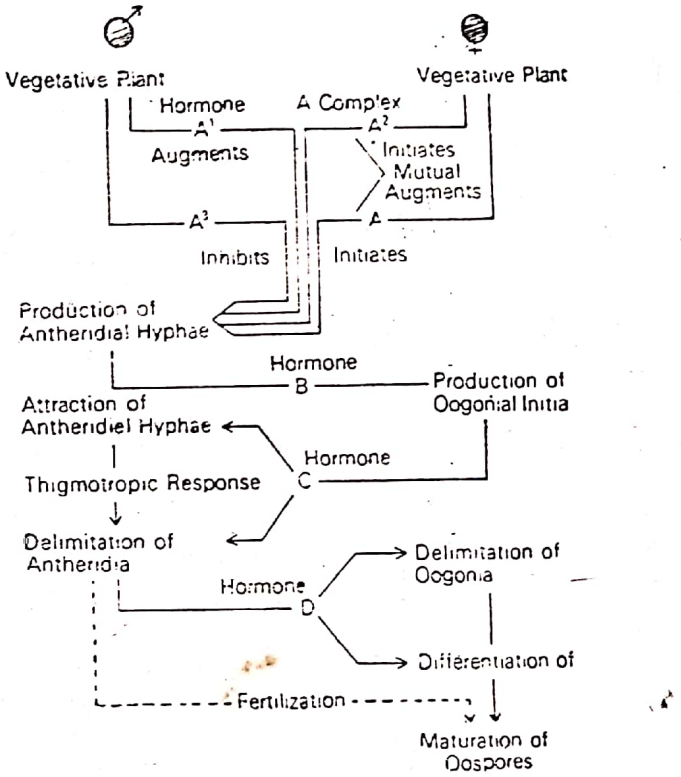

FIG. 38.2. Showing multihormonal regulation of sexual reproduction in *Achlya ambisexualis*, sensu Raper (1952).

Antheridiol (Erstwhile Hormones A and C)

Barksdale (1963), working with a purified sample of sex-hormone A observed that the hormone, in addition to the initiation of antheridial hyphae, caused responses earlier attributed to hormone **C** (chemotropic growth of the antheridial hyphae and delimitation of the antheridia). She found that the purified hormone **A** could be absorbed on particles of plastics, which then mimicked oogonia in certain respects. These particles induced formation of antheridial hyphae in the male isolate. The antheridial hyphae grew towards the "plastic oogonia" and antheridia were delimited. She concluded that hormone **C** was misconceived and did not exist. To summarise, antheridiol

elicits the following responses: (1) induction of antheridial hyphae, (2) chemotropic stimulation of the antheridial hyphae, (3) stimulation of male hyphae to produce hormone **B**, and (4) delimitation of antheridia.

Chemical nature and structure. Mc Morris and Barksdale (1967) isolated the hormone in a crystalline form and named it antheridiol. It's structure (Fig. 38.3) was proposed by Arsenault *et al* (1968) and

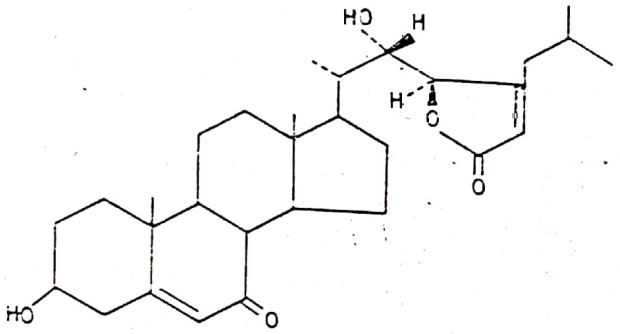

FIG. 38.3. Antheridiol.

Edwards *et al* (1969). It is a steroid (the only steroid-hormone known outside animal kingdom). It is active at concentrations down to 10^{-10} M and elicits distinct responses.

Bioassay of antheridiol. The test sample should induce formation of antheridial hyphae in pure male *Allomyces ambisexualis* in absence of the female isolate. The number of antheridial branches produced is directly related to the concentration of the hormone, and this forms the basis of estimating the amount of the hormone. The fate of the antheridiol in the male thallus is not known.

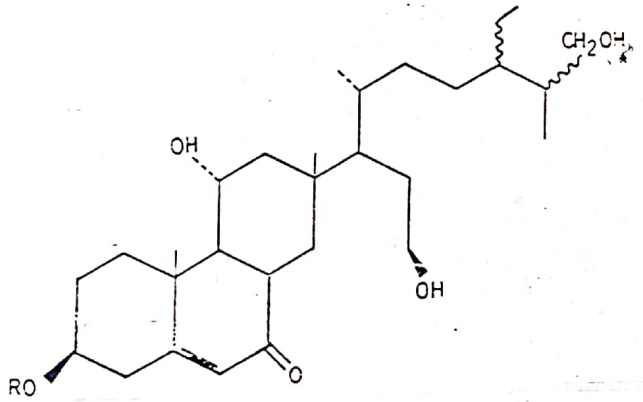

FIG. 38.4. Oogonial. (Oogonial–1, R = $(CH_3)_2$ CHC=O; Oogonial–2, R = CH_3CH_2C=O).

Hormone B (Oogonial)

It is produced by the male isolates only in presence of antheridiol.

Barksdale *et al* (1974) reported that oogonial is produced by some hermaphrodite strains without the stimulus of antheridiol. Recently, Mc Morris and his co workers (1975) have isolated two crystalline compounds from the culture filtrates of *Achlya heterosexualis*, which possess hormone B activity. The following structures have been proposed to these compounds which have been named oogoniol-1 and oogonial-2 (Fig. 38.4).

Hormone D

There is no direct evidence for the existence of this hormone, which was postulated by Raper to induce delimination of oogonia and the events leading to fertilization. It's involvement was suspected because of the fact that it needs 2-4 hours of contact between antheridial and oogonial initials before oogonia are formed. This constant time-lapse suggested the formation of some substance by the antheridium during this period. So, this hormone too has gone into the oblivion.

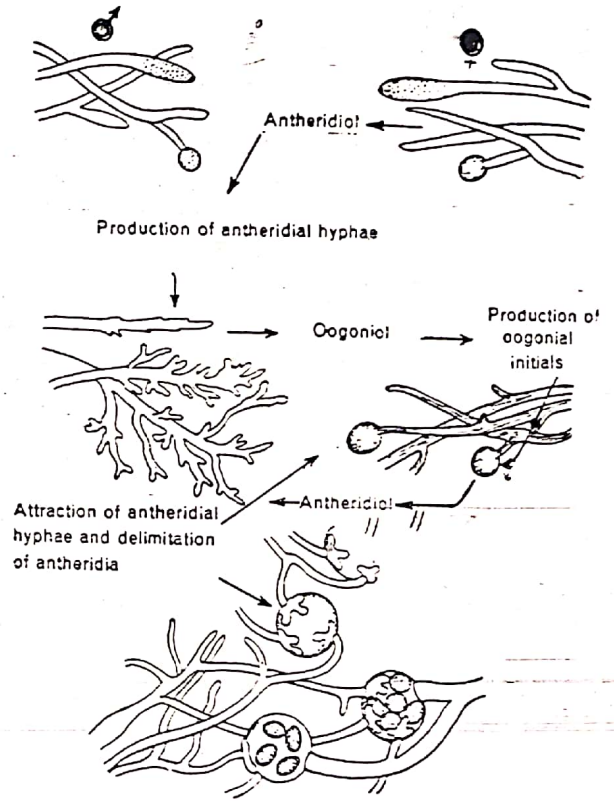

FIG. 38.5. Present concept of hormonal regulation of sexual reproduction in *A. ambisexualis* involving only 2 hormones antheridiol and oogonial (after van den Ende, 1976).

The hormonal regulation of sexual reproduction in the light of recent discoveries, involves only two hormones, antheridiol and oogonial or hormone-B (Fig. 38.5).

TRISPORIC ACID

Trisporic acid, has been found to be active in the sexual reproduction of several mucorales e.g. *Mucor mucedo*, *Blakeslea trispora* and *Phycomyces blakesleeanus*. Sexual interactions involve mutual stimulation for zygospore formation. The hormone is synthesized only in presence of the compatible partner, otherwise, only asexual reproduction takes place.

Discovery

Though the hormonal control of zygospore production was demonstrated by Burgeff in 1924, it was only after 30 years, (1956) that he isolated a cell-free extract showing hormonal activity. The realization that trisporic acid was a sex hormone came as a surprise. The acid was known for a long time as a metabolite associated with carotenogenesis in mated cultures of *Blakeslea trispora*. It could induce carotenogenesis in unmated cultures as well. When the sex hormones of *B. trispora* and *M. mucedo* were isolated and their structures ellucidated (Fig. 38.6) it was found that the hormones

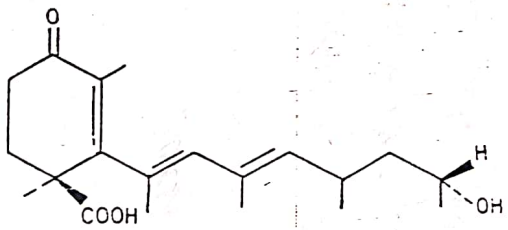

FIG. 38.6. Trisporic acid.

were nothing but trisporic acid (Van den Ende, 1967; Gooday, 1968). Austin *et al* (1969) directly showed that the hormone extracted from *Mucor* and the authentic trisporic acid were one and the same thing. There are three kinds of trisporic acids, trisporic acid A, B and C. Trisporic acid C has the major (80%) hormone activity; trisporic acid B has 15%, while trisporic acid A shows only 1-2% of the activity. Perhaps trisporic acid A lacks the functional group in the side chain.

Relationship with Carotenogenesis

β carotene, the major carotenoid present in Mucorales, is the precursor of trisporic acid synthesis. This is supported by: (1) co-inhibition of carotene and trisporic biosynthesis by diphenylamine, and (2) impairment of sexuality in carotene-deficient mutants of

P. blakesleeanus. Trisporic acid itself stimulates β-carotene synthesis in the zygosphores. So, the system is self-amplifying. β-carotene, in addition to being the precursor of trisporic acid, forms **sporopollenin**, the resistant protective material of the zygospore wall. Sporopollenin is found also in the pollen grains of angiosperms. It is an oxidatively-polymerized product of β-carotene.

Production of Trisporic Acid by Mucorales

When two compatible mating types grow together, they institute a "collaborative" biosynthesis (Fig. 38.7) of trisporic acid; they

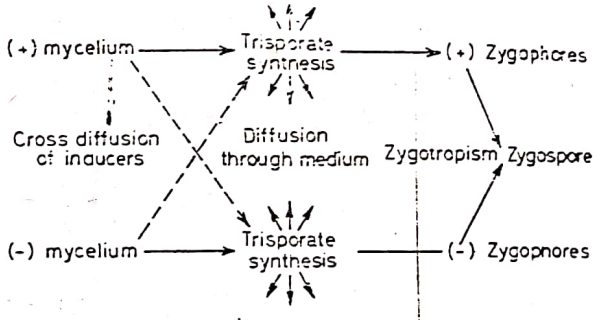

FIG. 38.7. Collaborative synthesis of trisporic acid by the compatible mating types of Mucorales.

mutually 'switch on' the biosynthesis of each other through inducers, which, however, have not been identified. The trisporic acid, formed in both mating partners, diffuses into the medium and induces zygosphore formation. Cell contact is not necessary. Both partners contribute to the final trisporic acid yield.

The hormone also stimulates greater β-carotene production, to insure its own, as well as, sporopollenin synthesis.

Zygotropism

But what controls zygotropism (the growth of the (+) and (−) zygophores to towards each other)? The answer is not known. That some volatile substance may be involved was suggested by Banbury (1954) and Plempel (1960-1963). Zygophores are also attracted towards the vegetative hyphae of the compatible strain (Mesland *et al*, 1974), suggesting that the volatile substance is produced in the vegetative hyphae. Gooday (1973) suggested that the inducer of trisporic acid synthesis could be the volatile chemical which causes zygotropism. This has found strong support from Mesland *et al* (1974) who found that the volatile substance obtained from (+) and (−) cultures could induce trisporic acid synthesis and also attract the resultant zygophores in the opposite mating type.

Note. There are strong indications suggesting hormonal control of sex in homothallic species similar to that in heterothallic mucorales. Trisporic acid and its precursors induce zygophore formation in homothallic species.

YEAST α FACTOR

There are evidences suggesting the involvement of some hormone in the sexual reproduction of *Saccharomyces cerevisiae*. The haploid cells are of two mating types (*a* and α), which conjugate and give rise to diploid cells. Levi (1956) showed that the α cells produce a diffusible chemical which induces the formation of copulatory processes by the compatible *a* cells. As yet no *a* factor has been demonstrated. Physical contact is not necessary for the production of copulatory processes. The *a* cells, under the influence of the α factor, stop growth and budding. They, instead, swell and turn into giant cells of various shapes; occasionally 10 or more times bigger than the normal cells. The giant cells show a dry weight, 30 or more times greater than that of the haploid vegatative cells. The α-factor is specific; it acts only on *a* cells and has no effect on the α cells. It inhibits DNA replication in the *a* cells.

Chemical Structure

Duntze *et al* (1970-1973) purified and partly characterized the α-factor; it is a peptide with a molecular weight of 1400. The peptide is complexed with copper ions which do not dissociate even during purification.

Bioassay

The test sample should induce the formation of copulatory processes specifically in the *a* cells, and transforms into giant cells.

Note. In addition to the yeast α-factor, two other hormones called α-, and *a* hormones, have been implicated in the mating reactions between α and *a* cells. These are steroids (Yeast α-factor is a peptide) possibly related to ergosterol. The hormones are active on the opposite mating types (without need for physical contact) and cause swelling of the cells, which is a prerequisite for mating. However, such swellings are induced by several other chemicals also, like *testosterone*, *estradiol* and *auxins*. Thus, the effect of the α and *a* hormones is less specific than the yeast α-factor.

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