

CHAPTER 24: Genetics

Information on the genetics of the Saprolegniaceae is in short supply, even though these fungi are properly equipped with the essentials of sexual reproduction -- karyogamy, meiosis, and a structural mechanism to provide for the union of the sexual elements. Why have genetic studies lagged so far behind those of the other biological aspects of the water molds? Several factors mitigate against these fungi as suitable subjects for experimental hybridization and genetic analysis. For instance, there is among the water molds a preponderance of monoecism (homothallism) and self-fertility, all have nonmotile gametes, and apomixis evidently is common. Moreover, a certain means of germinating the zygotes (oospores) has proven to be elusive, hence little genetic analysis can be derived from sexual manipulation of isolates. While the water molds, unlike certain Ascomycetes, do not display an orderly arrangement of the products of sexual reproduction, there is sure to be the incorporation of random meiotic products or even mitotic recombinations of markers. One task is to discover these products. Since the water molds seem not to develop hyphal fusions (at least *in vitro*), even parasexual analysis is not possible.

The members of the Saprolegniaceae have long been considered haploid organisms -- the diploid phase being reduced to a single nuclear generation -- but the evidence from the analysis of DNA and the interpretation of ultrastructure points to quite the opposite view (see Chapter 12). The inheritance of characteristics in these fungi must therefore be read in this context.

For the most part, the progress in studies on the genetics of members of the family has centered on determining (rather incompletely, it must be admitted) from a diverse array of patterns which "sexes" emerge from interstrain matings in dioecious species. Even where matings between monoecious and dioecious species have been accomplished, the phenotypic analysis ends with identifying the mating potential of the offspring. The underlying genetic basis for the various sexual strains of the water molds remains a mystery. It seems certain that the patterns of inheritance in these fungi are dictated by gene control: how to read and evaluate these patterns as expressions of genetic recombinations and segregation is the great task for future research. The information at hand suggests that the inheritance of characteristics in the water molds is not a simple, one-locus, two-allele system. This conclusion is adequately supported by the analysis by Lasure and Griffin (1975) of the results of a series of parental crosses, repeated backcrosses, and sib matings within isolates of *Achlya bisexualis*. A complex pattern of sexual inheritance appeared in the data as evidenced, for example, by the fact that first backcrosses of progeny from matings gave a high frequency of antheridial strains, while second and third backcrosses yielded very high frequencies of oogonial strains.

Terminology with respect to sexuality in the Saprolegniaceae is treacherous, and we make no pretense of relieving the attendant difficulties. A brief word on usage, however, is necessary at this point.

When first devised and used, the terms “homothallism” and “heterothallism” conveyed precise intentions, but some later applications of these terms were to be less rigorous. While the derivation of the terms is explicit the implications are not necessarily so exact. Thus “heterothallism” can be used to imply morphological differences in mating participants, or can be limited to mean solely a dissimilarity in sexuality that is not expressed visually until the time of mating (if then). In a noble attempt to put meaning back into the term, Whitehouse (1949) proposed that two sorts of heterothallic behavior could be recognized. Morphological heterothallism is applicable when one member of a mating pair can be recognized as male, and the other as female. Whitehouse equated such a condition with “haplo-dioecism”, and cited *Achlya bisexualis*, *A. ambisexualis*, *A. regularis*, and *Dictyuchus monosporus*, as examples of this pattern of sexuality. Fungi that expressed differences in mating type unaccompanied by some constant morphological feature displayed physiological heterothallism. Whitehouse (1940) admitted that the water molds he cited were not necessarily strictly haplo-dioecious because of the “degrees” of maleness and femaleness exhibited by some strains. Further, he remarked that J. N. Couch’s (1926b) use of “heterothallic” as applied to *Dictyuchus* isolates was equivalent to haplo-dioecism.

It is possible to muster two objections to Whitehouse’s terms and examples. First, the various compatible mating strains of *Dictyuchus monosporus* (to name one instance) cannot be distinguished from one another on structural grounds -- until sexual reproduction occurs. We interpret “morphological heterothallism”, *sensu* Whitehouse, to mean that the sexes are indeed different morphologically prior to any sex-accompanying physical contact. Second, if it should be proven that *D. monosporus* is in fact predominantly diploid (gametic meiosis¹ as in *Achlya ambisexualis*; Tontz, 1969), then haplo-dioecism in these instances is not equivalent to heterothallism (Whitehouse, 1949). To be sure, the terms heterothallic and dioecious have been used interchangeably -- and, indeed, we obviously prefer the latter term -- but, as Whitehouse notes, “dioecious” is in general use for describing diploid plants with the two sexes separated on different individuals. This seems to be precisely the condition in the water molds -- they are evidently diploid -- and this being so, dioecious becomes a perfectly serviceable term that does not convey (unless one wishes it to do so) any impression of morphologically differentiated sex. Emerson (1950) considered most of the Saprolegniaceae to be self-fertile hermaphrodites, that is, haplo-monoecious or homothallic organisms. Of course the former designation is not appropriate in view of the current concept of gametic meiosis. Whitehouse (1951) attempted to show that an

¹This is a confusing adjectival form, but as it is used here (and is understood by mycologists generally) the term refers to the point in sexual reproduction at which meiosis occurs. In the water molds, reduction division appears to take place within the gametangia (production of gametic nuclei) rather than being one of the events associated with germination of the zygote (zygotic meiosis).

evolutionary sequence could be traced from isogamous homothallism to haplodioecism -- representatives may be heterogamous or oogamous (Whitehouse's terms) -- via primary heterothallism (isogamous, as in representatives of *Blastocladiella*). Increased interbreeding, he suggested, could lead to monoecism (homothallism) with the species being either heterogamous or oogamous (his terms). Whitehouse's scheme implies to us a claim that the unisexual water molds are the link between heterothallic and homothallic expressions. In a review account of heterothallism in fungi, published in 1958, Olive also suggests that two kinds of heterothallism might exist. Unbalanced heterothallism, he proposed, was exhibited in crosses between non-allelic, self-sterile individuals the progeny of which either were self-sterile or self-fertile. If, when two self-sterile thalli were crossed, none of the recombinant progeny was self-fertile, and balanced heterothallism was achieved.

Terminology is *still* treacherous. While we prefer to interpret the water molds as being either monoecious or dioecious (but also recognizing that some display gynandromixis), in the account to follow we will use the terms chosen by the authors of the particular papers cited.

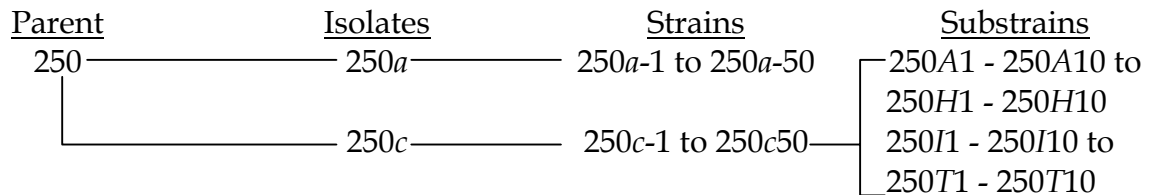
SEXUAL MATINGS IN DIOECIOUS SPECIES

Genetic data in the Saprolegniaceae have come largely from matings performed with species of *Achlya* and *Dictyuchus* (some biochemical aspects of genetics in water molds are treated in Chapter 23). The first work of this nature was that by J. N. Couch (1926b) using isolates of the latter genus. He demonstrated that heterothallic strains of *Dictyuchus* "species" existed, but the evidence for sexual segregation and recombination was scanty. Oospores developed from successful matings (crosses) among isolates of *Dictyuchus* were germinated and individual spores from the resulting germ sporangia assayed for mating type. Colonies propagated from some spores responded as males (antheridium-producing), others were females (forming oogonia), and still others were neutral as to sex. It should be recognized that in conformity with prevailing thought Couch considered the mating strains to be haploid (derived from zygotic meiosis).

It was to be some four decades later that sexuality in the genus *Dictyuchus* was again to be explored experimentally, but in the meantime, Hartmann's (1931) theory of relative sexuality was published. According to this postulate the sex potential of the zygote or diploid generation of an organism was equally male and female. During meiosis, however, one potential developed at the "expense" of another such that maleness or femaleness was expressed in that nuclear generation. In Hartmann's view, sexuality in isolates of *Dictyuchus* ultimately was determined at the time of meiosis, that is, these fungi were (according to his terminology) haplogenotypic. From the results of later work on representatives of this genus, it is unlikely that the complex sexuality of water molds can be explained solely by Hartmann's theory. Nevertheless, potentially productive research might well be found through a reconsideration of Hartmann's arguments in light of the predominantly diploid nature of the mycelium of water molds.

In 1969, W. A. Sherwood published an account -- from his thesis of 1966(b) -- of the results of matings between clonal subcultures from two parental strains of *Dictyuchus monosporus* that responded strictly as heterothallic (dioecious) isolates. The numerous matings demonstrated that among the cloned individuals there were inherited homothallic as well as heterothallic tendencies. Moreover, Sherwood found that the ability of a strain to react in a particular sexual fashion could decrease with repeated subculturing of isolates. In essence, his culture work signaled three sexual reactions; these were analyzed in detail and the results published by him in 1971.

From an initial set of three collections of *Dictyuchus monosporus* W. A. Sherwood (1971) isolated six compatible mating strains, three "female" (oogonial) and three "male" (antheridial). From these he derived 680 single spore isolates having a definite and traceable pedigree, one example of which follows:



Some matings among the cultures (W.A. Sherwood, 1971) resulted in a sexual behavior pattern in which a strain formed antheridial branches when paired with an oogonium-producing individual. Such pairings, Sherwood stated, were indicative of heterothallism. A second type of behavior (expressed by other cultures from the parent *Dictyuchus*) was a heterothallic response preceded by homothallism in one of the individuals in the region of contact with an opposing compatible strain. In such cases, the colony exhibiting a homothallic tendency acted as a male to the opposing one, and the nonhomothallic one functioned as the female of the mating pair. Sherwood described a third reaction type as homo-heterothallism. Both individuals of the mated pair were homothallic in the areas of direct contact, yet each was at the same time involved in reciprocal cross-reactions. Research to uncover the genotypic basis for such a varied phenotypic expression in *D. monosporus* has not been undertaken.

Species of *Achlya* also display a variety of sexual behavior patterns, J. R. Raper (1936, 1940b) discovered; these patterns have since been explored further and attempts made to analyze them. The percentages of sterility, Raper reported, were very high in the matings among isolates of *Achlya bisexualis* and other nonsexual strains. Combinations of the sexual strains in *A. ambisexualis* signified that all intrastrain matings were sterile, and all interstrain ones were fertile (J. R. Raper, 1940b). The various morphological responses in matings indicate that there are six sexual strains in *A. ambisexualis*, expressed by four sexual factors: F (dominant ♀), M (dominant ♂) f (latent ♀), and m (latent ♂). Raper deduced that the genotypes of the strains were:

FM = hermaphroditic and self-fertile

Ff = pure ♀

Fm = predominantly ♀ with ♂ tendencies

Mf = predominantly ♂ with ♀ tendencies

Mm = pure ♂

fm = sexually sterile

It is important to recognize that this genetic analysis was based on the concept -- held and so stated by J. R. Raper (1940b) -- that the production of fusion nuclei by this species was not immediately preceded by meiosis. Therefore, he regarded the nuclei in the vegetative hyphae of strains of *A. ambisexualis* to have the same genetic potentiality as the gamete nuclei.

Barksdale (1962b) analyzed data from matings, among 34 isolates of *Achlya bisexualis* that formed spherical gemmae. Oospores were produced in 208 of the matings, but the percentages of germinable zygotes was as low in self-conjugating as in cross-conjugating strains. Fifteen of the 34 strains behaved as pure females, an equal number were pure males, and four proved to be predominantly male in their reaction with certain female strains.

The results of some experimental crossings of strains in *Achlya ambisexualis* and *Dictyuchus monosporus* led Mullins and Raper (1965) to conclude that the genetic control of mating competence in these water molds could not be explained simply by paired alleles at a single locus (which is, of course, an assumption in Hartmann's theory of relative sexuality). Mullins and Raper analyzed the sex potential of colonies from 56 germinated oospores of a mating between *A. ambisexualis* ♂ × ♀, and from 36 oospores of a cross between an antheridium- and an oogonium-producing isolate of *D. monosporus*. In the former, 39 colonies were male-reacting, 11 were predominantly male, and six had both male and female potentiality. Eighteen of the 36 germinated oospores from isolates of *D. monosporus* produced male colonies, 12 were female, four were predominantly male, and two were predominantly female. Although J. N. Couch (1926b) reported the occurrence of strains mixed -- as to sexual reaction -- from single germinated oospores of *Dictyuchus* isolates, Mullins and Raper (1965) found no instances in which both "male" and "female" isolates could be derived from the products of a single germinated oospore, Barksdale (1966) determined the mating potential of 103 oospores germinated from products of a male × female mating in *Achlya bisexualis*. When the colonies from the germinating oospores were back-crossed to both parents, 99 were found to be female, two were males and two were neutral. Lasure and Griffin (1975) working with the same species had found first backcrosses to yield predominantly antheridial (male) progeny.

INTERSPECIFIC MATINGS

In 1960, Barksdale published the results of an extensive series of mating experiments among isolates of *Achlya* representing both heterothallic and homothallic species. She discovered that interthallic sexual reactions took place between heterothallic *Achlyas* and some homothallic ones, but the process of sexual reproduction was not always completed with matings. *Achlya ambisexualis*, for example, acted as a female strain when mated with *A. flagellata* (= *debaryana*), but the

sexual cycle ended with the production of oogonial initials. The same was true for matings between *A. ambisexualis* and *A. caroliniana*. When *A. ambisexualis* was paired with *A. americana*, *A. conspicua* (= *debaryana*) or *A. klebsiana* (= *debaryana*), on the other hand, oospores were produced, indicating further progression of the reproductive cycle. Barksdale's data leave little doubt that there is a tendency toward heterothallism in reputed homothallic species of water molds.

INTERGENERIC MATINGS

The first attempts at intergeneric crosses with members of the Saprolegniaceae were included in the experimental work by J. N. Couch, reported in 1926(b). *Thraustotheca clavata* and *T. primoachlya* (= *Achlya primoachlya*) were propagated in two-member cultures with strains of *Dictyuchus* that had proven to be heterothallic. Even though antheridial branches from the mycelium of the *Thraustothecas* contacted the oogonial initials of the *Dictyuchus* isolates, there was no evidence of fertilization, and oospores were never developed. Some matings attempted between specimens of *Dictyuchus* and those of *T. primoachlya*, exhibited a neutral stimulation: the former produced oogonial initials, and the latter developed very profuse antheridial branches. Salvin's (1942c) experiments on intergeneric matings involved an analysis of 51 combinations of attempted matings among *Achlya flagellata*, *A. dubia*, *A. oblongata*, *Brevilegnia* sp., (his isolate C-2; Salvin, 1942a), *Dictyuchus* sp., *D. achlyoides* (= *Achlya achlyoides*), *Aphanomyces laevis*, *Thraustotheca clavata*, *T. primoachlya*, and two nonoogonial isolates of *Saprolegnia*. Several methods designed to determine if mating in the various combinations of cultures had taken place proved to be intractable, hence Salvin could only judge the success of a "cross" by tracing the origin of antheridial branches that had contacted oogonia. In a two-member culture of *A. flagellata* and *T. clavata*, antheridia from the latter were followed to points of contact on oogonia of the former. Salvin reported that fertilization tubes were produced and oospores were developed as a result of the cross, but he could not germinate the zygotes. His work on intergeneric matings, accordingly, is inconclusive. Liles (1969) reported a positive sexual reaction between a nonoogonial *Dictyuchus* and *A. ambisexualis*, as well as between *D. sterile* (discarded name, see systematic account) and the same *Achlya* (male strain).

MEIOSIS

In 1892(a) P.-A. Dangeard commented on sexuality in the Oomycetes, without benefit, of course, of the information to be derived from genetic analysis. It was his view that of the very large number of nuclei produced by these fungi, many assumed a vegetative role while others were reduced to a single function, that of being either a "male" or a "female" nucleus. In each oospore these two kinds of nuclei fused into one, he thought, and at germination of this zygote, the single nucleus was the progenitor of the numerous ones that were to be present in the developing thallus and spores. He

recognized the basic pattern of nuclear behavior in sexuality, even though he apparently had no concept of the role of meiosis in sexual reproduction.

Evidence from the behavior of some subcellular organelles (*see* Chapter 15) has shown that reduction division in some Saprolegniaceae -- if not all -- is gametic (*see* footnote, p. 383) and therefore the vegetative thallus is diploid. Does genetic evidence yield an identical conclusion? Olive (1953) concluded that there was insufficient cytological and genetic evidence at that time to support the case for zygotic meiosis, but in 1965 stated that the evidence for gametic meiosis was also not convincing.

In the crosses attempted by Barksdale (1966) using progeny from matings of self-conjugating strains of *Achlya bisexualis*, the production of male strains was interpreted to mean that the original isolates were heterozygous for "sex". This, Barksdale (1966) thought -- and Dick and Win-Tin (1973) concurred -- was evidence for meiosis taking place in the gametangia. Sansome (1963a) reviewing J. N. Couch's (1926b) work, reasoned that the data he presented were in accord with what would be expected of heterozygous diploids, and therefore were supportive of gametic meiosis. The investigation by Mullins and Raper (1965) also supported this view. Employing cycloheximide resistance as the phenotypic marker, Lasure and Griffin (1974) analyzed the results of matings (oospore germination) between cycloheximide-resistant induced mutants and "wild" types of *A. bisexualis*. The progeny of F₂ crosses, they reported, exhibited a 3:1 ratio of resistant to sensitive individuals, as would be expected from heterozygous parental types. All F₁ progeny were cycloheximide-resistant, and the matings were thus consistent with gametic meiosis.

Dick and Win-Tin (1973), summarizing in part a cytogenetic study by Win-Tin, stated that evidence for reduction division taking place in the gametangia of water molds was confirmed (although complete sequences were not necessarily traced in the oogonial initials and antheridial cells) for the following species: *Achlya ambisexualis*, *A. americana*, *A. apiculata*, *A. benekei* (= *Protoachlya benekei*), *A. caroliniana*, *A. colorata*, *A. flagellata*, *A. hypogyna* (= *Protoachlya hypogyna*), *A. inflata*, *A. racemosa*, *A. radiosa*, *A. recurva*, *A. sparrowii* (= *racemosa*), *A. treleaseana* (= *androgyna*), *Aplanopsis terrestris*, *Pythiopsis cymosa*, *Saprolegnia ferax*, and *S. furcata*.

CHROMOSOME NUMBERS

Although chromosome counts have been made (chiefly from cytological preparations) on some water molds, the spectrum of species used is rather narrow. The counts are not in all instances precise, and certainly do not necessarily reflect accepted speciation. The numbers in Table 42, compiled from the literature, should be considered tentative since without exception the data have not been confirmed by repetitive investigation. We have included in the table reference to the structure in which counts were made or (where applicable) to the indirect methods of estimating chromosome numbers.

Colchicine, colcemid, and camphor appear to be effective in inducing stable tetraploid nuclei in *Saprolegnia* sp. without preventing formation of "normal" spindles.

Heath (1977) has proposed that these antimitotic drugs reduce the velocity of spindle fiber polymerization relative to the rate at which the chromosomes replicate.

If Heath's (1980c) observations on mithramycin-treated hyphae of *Saprolegnia ferax* are confirmed (the treatment is found not to induce artifacts) he will have thrown considerable doubt on the validity of prior chromosome counts in the water molds. Chromatin in nuclei in the hyphal tips of an isolate of *S. ferax* did not appear to undergo condensation at any stage in mitosis, Heath reported. Presumptive meiotic nuclei in the antheridial cells, however, did contain condensed chromatin. Heath believes it likely that artifacts resulting from the pretreatment of hyphae may have led those who have reported chromosome numbers (brightfield optics) to erroneous conclusions.

THE SEXUAL CYCLE IN THE SAPROLEGNIAEAE

From time to time, review papers have labored strenuously to give meaningful, broad interpretations of the sexual life practiced by the Saprolegniaceae (Emerson, 1950; J. R. Raper, 1959, 1966; Olive, 1958; Madge and Blackwell, 1942). These reviews deal largely with interpretation of heterothallism (dioecism).

Within the fungi, J. R. Raper (1954, 1966) recognized seven basic types of life cycles founded upon the two absolute events in sexuality, meiosis and karyogamy. These types ranged, he proposed, from a haploid cycle through, for example, a haploid-diploid one to a cycle that was characteristically diploid (except for the immediate meiotic products). The latter type may well be characteristic of all members of the Saprolegniaceae. In addition, J. R. Raper (1954, 1966) proposed that the fungi, *in toto*, embraced seven fundamental patterns of sexuality; one homothallic, and six heterothallic. The Saprolegniaceae appear to represent two of the seven patterns formulated by Raper: Type 0 and Type II. All individuals having the Type 0 (homothallic) pattern are alike, and are functionally hermaphroditic. The Type II pattern (heterothallic) is portrayed by those species in which several sexual strains exist, each being typically self-sterile but cross-fertile with all others (J. R. Raper, 1966). This, of course, is the pattern of the dioecious *Achlyas*.

Dick (1972) has discussed the theoretically possible genetic systems that may operate in the Oomycetes, and how those systems could influence species concepts in this class. Two main features of the account emerge. In the first place, Dick suggests (1972:1155, 1156) that there are five possible pathways of sexual reproduction for homothallic species; apospory, homokaryotic and heterokaryotic diplospory, parthenogamy, and automictic sexual reproduction. These terms are discussed and defined, particularly as to situations (theoretical, in most instances, in our view) in which any one of the pathways might be expected to occur. Secondly, Dick (1972) has attempted to relate sexual behavior patterns to species concepts in the family. Homothallism (with a functional union between an antheridium and an oogonium) he noted (Dick, 1972:1157) would result in a species concept (his term) that would "... be relatively narrow, with well-marked species boundaries ..." Obligate heterothallism would "... be expected to provide the stable... species concept expected of

outbreeding." Diplospory, on the other hand (Dick, 1972:1158) "... would result in segregate species within an aggregate." Parthenogamy is conceived by Dick as yielding narrow concepts of taxa not unlike those derived from species whose behavior (Dick, communication, states that diplospory is nonsexual) followed a pattern of diplospory. If automictic sexual reproduction were the behavior pattern, relative "... stability of the genotypes would be expected but a broad range of genotypes could evolve if [this type of] sexual reproduction coexisted with homothallism." These subtly defined theoretical species precepts are too tenuously linked to genetic principles to be accepted without substantial and precise documentation.