

CHAPTER 9. Morphogenesis: The Sexual Apparatus

In the Saprolegniaceae the morphological accouterments of sexuality are oogonia and antheridia (the latter cells may be absent). Specifically, sexual reproduction is accomplished by a nonmotile oosphere, the bearer of the so-called female gamete (gametic nucleus) and, save where parthenogenesis prevails, an aplanetic male gamete nucleus conveyed to the vicinity of the female gametic nucleus by a tube or pore. The result of sexual fusion is the zygote, almost universally referred to as the oospore when it is mature. Oospore germination completes the sexual part of the development (life cycle) of the fungus. It was C. E. Bessey's (1903:53) contention that the antheridia and oogonia of members of the Saprolegniaceae were "... so modified on account of their parasitic habit, as to result in suppression of the antherozoids..." so that transfer of the contents of the male cell to the female one had to take place only after direct contact had been established.

The majority of species of Saprolegniaceae are functionally hermaphroditic, but in a few representatives multiple alternate patterns (J. R. Raper, 1966) of sexuality operate. Where the hermaphroditic or parthenogenetic mode prevails, the terms usually used to express this condition at the species level are homothallic or monoecious. The contrasting terms, heterothallic or dioecious, lose precise meaning when applied to some of the water molds in which multiple alternate patterns operate. Gynandromixis (J. R. Raper, 1939b) may well be the best word to designate some of the diverse patterns in these fungi.

Barbier's (1971a) observations on *Achlya flagellata*[†] have a direct bearing on the general concept of sexuality in the water molds. He propagated continually for ten years a single spore isolate on PEHyPH (an extract medium containing hydroquinone; Barbier, 1969) periodically examining the specimen for the types of antheridial branches produced (androgynous, monoclinal, diclinal). At the end of ten years, the fungus no longer produced androgynous antheridial branches (during the first two years, 20-50% of the branches were of this type), and the number of diclinal ones far exceeded the monoclinal ones. Since the culture conditions were constant over the decade, Barbier concluded that the change in predominant branch type was possibly mutational involving some genetic mechanism of exclusion. He suggested that some extrachromosomal factor transmitted by vegetative multiplication modified the expression of sexual reproduction such that there occurred "male and female localization" in a hypha (Barbier, 1971a; see also R. E. Wheeler and McGahen, 1952, and the origin of heterothallic isolates from homothallic ones in *Glomerella*). Certainly Barbier's results do not bear out Petersen's (1909a) contention that there was a trend in the Saprolegniaceae toward elimination of "fructification."

While it is not unreasonable to assume that the biologists of the early 1800's who looked at water molds on dead fish and flies saw oogonia as well as sporangia, a description of the former structure did not appear in the literature until 1842. At that time, Schleiden reported that there were two kinds of spores in *Achlya prolifera* (in actuality a *Saprolegnia*), and the largest of these occurred in spherical sporangia. Without a doubt Schleiden saw oogonia containing oospores.

In 1850 Thuret published his observations on Kützing's *Saprolegnia ferax*. Some "sporangia", Thuret noticed, were filled with dark cytoplasm, and others with spherical bodies that never escaped from the surrounding cell. On some of these "sporangia" he saw small protuberances; possibly these were pits through which wall substance protruded, a not uncommon occurrence in this *Saprolegnia*. A year later, A. Braun's (1851) paper on rejuvenescence in plants appeared. His description of large resting spores in globular or expanded cases and in cylindrical tubes makes it very likely that he, too, saw oogonia and oospores.

Understandably, neither Schleiden, Thuret, nor Braun recognized the significance of the large, globose structures associated with their fungi. Leidy (1850) -- he held that at some point in the life of every plant a "sexual admixture" occurred -- reported what he thought was a sexual process in *Achlya proliferata* (probably *Saprolegnia ferax*), but offered nothing to substantiate his claim. Soon after, Pringsheim (1851) unmistakably described oogonia, oogenesis, centric oospores, and oospore germination in a watermold that he identified as *Achlya proliferata* (see *S. ferax*). He believed the oogonial wall pits were pores, and in 1855¹, referred to them as the micropyle. Subsequently, Pringsheim (1858) admitted that the thin places in the wall perhaps were not openings. De-La-Rue's (1873) thinking on the matter of wall pits is at best curious. He contended that the *Saprolegnia* sp. he had collected was a monoecious species because the oogonia lacked pits. Nevertheless, he stated that the oogonial wall occasionally showed small globules on it; wall pitting, of course, shows precisely this configuration when seen in refracted light. Hine (1878) too, thought that only dioecious species of watermolds had pitted oogonia, but he gave to the concept of dioecious a far different meaning than it now commands. It seems likely that as early as 1852 (pl. 7, figs. 26, 27) de Bary saw oogonia of a watermold. In any case, he clearly illustrated a pitted wall of a "spherical sporangium."

The first description of antheridial branches in a saprolegnian appears in Pringsheim's 1851 paper, but he did not comprehend the significance of these "closely appressing side branches" as he designated them. In 1858, he published a detailed account of these structures, and recognized by the "appendages" which they put into the oogonial cavity, that they were involved in the sexual process. He reported that these side branches and their appendages "drained" into the oogonium where the content was distributed among the "goniospheres" (oospheres). Thus, contrary to accepted dogma, Cornu (1872) was not the first to see fertilization tubes; Pringsheim saw them in 1858, and de Bary did likewise in 1866(a, b). By 1858, then, the morphological necessities of sexuality in the Saprolegniaceae had been discovered.

¹ Pringsheim himself, in 1858, cited his 1855 paper incorrectly. His 1855 publication appeared in the "Bericht über die zur Bekanntmachung" of the Königlich Preussischen Akademie der Wissenschaften zu Berlin, and not in the "Monatsberichte" series of this same journal. It was a prior account of his 1858 paper that appeared in an 1857 issue of the latter series.

OOGENESIS AND ANTHERIDIAL DEVELOPMENT

As in the case of the formation of the asexual apparatus, morphogenesis in the sex cells of water molds is a recognizable pattern of changes leading to maturation, and there is surprising agreement in the literature on the sequence of events. Differing opinions have arisen chiefly over two major questions: does fertilization occur, and is there nuclear division or nuclear degeneration in the gametangia? The assemblage of evidence -- it is largely cytological -- that allegedly answers these questions appears in Chapter 12.

The subsequent account of morphogenesis is taken from several major sources, as well as from our own observations: de Bary (1866a, b; 1881; 1884), Bhargava (1946b: *Isoachlya anisospora* var. *indica*†), Burton (1940: *Pythiopsis intermedia*†), M. C. Carlson (1929: *Achlya racemosa*), Claussen (1908: *S. monoica*†), Coker and Hyman (1912: *Thraustotheca clavata*), B. M. Davis (1903a: *S. mixta*†), Dayal (1961b: *Achlya aplanes* var. *indica*†), Dick (1973), Hartog (1895, 1899), Häyrén (1954: *S. dioica*†), Humphrey (1893), Krause (1960), Lechmere (1911a: *S. thureti*†), Mäkel (1928: *S. thureti*†, *S. mixta*†), Moreau and Moreau (1938: several species), Mücke [1908: *A. polyandra* de Bary (see *debaryana*)], P. M. Patterson (1927: *A. colorata*), Schrader (1938: *T. clavata*), Shanor (1937: *T. clavata*), W. L. Steffens (1976: *A. recurva*), Strasburger (1880), Trow (1895: *S. dioica*†, *S. mixta*†, *S. thureti*†); 1899: *A. americana* var. *cambrica*†, and 1904: *A. polyandra*, *A. debaryana*), H. M. Ward (1883: *A. polyandra*), Weston (1918: *T. clavata*), A. W. Ziegler (1941: *A. recurva*).

The process of sex cell development (Fig. 14) can be divided into any number or groups of events, but the five stages recognized by Krause (1960) are convenient reference points.

The first stage in sex cell genesis, the induction phase, is simply the outgrowth from the hyphae of lateral branches destined to become oogonia (or antheridia), or the accumulation of cytoplasm in a hyphal tip if a terminal oogonium is to develop. It is an established fact that oogonium induction in dioecious species is triggered by hormonal mechanisms (Barksdale, 1963b; Horgen, 1977a; J. R. Raper, 1951a, b; 1952; 1966), but the triggering mechanism in monoecious species has not been discovered. In any case, lateral branching to form the rudiments of the sexual apparatus involves cellulase production (Thomas and Mullins, 1969; Mullins, 1973). As the oogonial branch (stalk) elongates, its central vacuole is usually continuous with that of the parent hypha.

The second phase (Krause, 1960) in oogenesis is a period of growth in which the lateral branch enlarges at the tip. As this occurs, multinucleate cytoplasm streams into the burgeoning apex. If the branch is to become an antheridium, cytoplasm also collects in its apex, but to a much less obvious degree than occurs in the developing oogonial branch.

Dick (1969b:753) proposed that there are three possible routes by which a lateral branch expands into an incipient oogonium. In the first of these the "... plastic dome..." of the branch enlarges progressively "... until the cessation of volume increase and elongation." A second pattern of expansion, Dick envisioned, might involve apical dilation (from internal pressure) of the branch apex because the rate of solidification in the plastic wall was itself retarded. In the third mechanism of inflation, previously

solidified wall material at the end of the oogonial branch could become softened through lysis, and the turgor of the protoplasm cause the apex to bulge outward to become the enlarged oogonial initial.

Several species in the family have ornamented oogonia, but only Dick (1969b) seems to have given much attention to the origin of these protrusions. He recognized three types of papillae. When a sudden and total loss of plasticity at localized points in the oogonial wall occurs bullate papillae result (the papilla wall is the same thickness as that of the oogonium). In cases where there is a slow reduction in localized wall plasticity, coupled with a decrease in the width of the plastic papilla apex, the resulting ornamentation, Dick suggested, is mammillate (mammiform). The third type -- truncate -- is noticeable only after wall deposition has commenced. The apex of such an ornamentation appears as a thin apical membrane stretched over an otherwise thickened wall extension.

We have not found a consistent pattern of synchrony in the appearance of the two parts of the sexual apparatus in all the monoecious species of water molds we have examined, and most accounts in the literature strengthen this impression (but see following paragraph). Trow for example, noted that the antheridial branches contacted the oogonial initials when these latter bodies were "full size" but before the oogonial septum appeared. Antheridial branches in *Achlya polyandra*, Mücke (1908) reported, developed at the time of oosphere cleavage, hence formed considerably later than Trow had observed for his species. In *Thraustotheca clavata* the antheridial elements are present before the oogonial initials are evident, according to Weston (1918). Indeed, he found (as had Horn, 1904, working with another species) that if an oogonial initial was not in contact with an antheridial branch, it did not form oospheres, but either proliferated or reverted functionally to a sporangium. Shanor (1937) also studied oogenesis in *T. clavata*, and reported that antheridial branches were usually present in contact with the oogonium initial by the time the basal septum was formed. In *A. racemosa*, the antheridia attach to the oogonial initial before the basal septum is in place (M. C. Carlson, 1929), but in *A. recurva* (A. W. Ziegler, 1941), the reverse is true even though the "male" branches arise synchronously with the "female" ones.

While Trow (1899) had touched briefly upon stages in sex apparatus development with respect to time of occurrence, the most comprehensive effort in this regard is attributable to Fletcher (1978). He repeatedly timed the appearance of morphogenetic events in the formation of the oogonia, antheridia, and oospores of *Saprolegnia diclina*. The antheridial cells of this species were always delimited before the oogonia were cut off from their respective stalks. Fletcher also found that there was a very consistent time interval (varying no more than $\pm 5\%$) between the formation of the oogonial septum and the appearance of discrete oospheres. On the other hand, the timing of stages in oospore maturation was demonstrably variable not only within individual oogonia but also among oogonia on the same mycelium.

In the incipient oogonium the cytoplasm is at first dense, with many small oil bodies and vacuoles. As differentiation proceeds, a central vacuole appears and enlarges concomitantly with the expanding initial (Horton, 1921, reported that there was no such vacuole in the young oogonia of *Aplanes*^t sp., but she did state that the

cytoplasm was peripheral). Precisely how the central vacuole develops and remains contiguous with that of the oogonial stalk is not known (but see ultrastructure). Pringsheim (1858) thought that the "clear spots" visible on immature oogonia marked the site of pits, but de Bary (1881) explained that these hyaline areas were parietal vacuoles. According to Humphrey (1893), such peripheral vacuoles emptied their "cell sap" into a central vacuole which then enlarged at their expense. In any case, once the central vacuole is prominently displayed in the oogonial initial the third stage in oogenesis, the isolation phase (Krause, 1960) begins.

Delimitation of the oogonium from the subtending hypha is by centripetal development of a septum. A hyaline area (Häyrén, 1954) first appears in the region in which the septum is to form -- just as occurs in delimitation of the sporangium -- but the precise physico-chemistry of septum formation is unknown. Dick (1969b) reported that secondary wall material is deposited on the cross wall after it is complete. In some species of Saprolegniaceae, the septum protrudes noticeably into the oogonial cavity in various configurations. Trelease (1882:107) called any such invagination a "pseudo-pollinodium"; Dogma (1966) referred to it as a "pseudocolumella." Beakes (1976) traces the ultrastructural events in septum formation (*see* Chapter 15).

The fourth stage in oogenesis Krause (1960) referred to as differentiation, that is, the centrifugal (Dick, 1969b) cleavage of oospheres. This delimitation process has been variously described. Strasburger (1880) maintained that the oospheres in the Saprolegniaceae were formed at the wall of the oogonium (just as were the spores in a sporangium) around "concentration points" made up of several nuclei. The cytoplasm in the oogonium segmented into "goniospheres" (oospheres), Pringsheim (1858) reported, and these then arranged themselves peripherally. At the point where the oosphere touched the oogonial wall, material from that wall was absorbed to form the openings where, Pringsheim said, the "appendages" (fertilization tubes) of the antheridia were to penetrate. To H. M. Ward (1883) and P. M. Patterson (1927) the cytoplasm of the immature oogonium simply condensed in some unexplained fashion into clumps along the inner periphery of the oogonial wall. Trow also wrote (1895), as did Hartog (1895) that the parietal protoplasm aggregated into clumps, but the latter additionally stated that each aggregation presumably took place around a nucleus. A curious interpretation of oosphere cleavage appears in Berthold's (1886) paper. He described rounded protrusions of parietal protoplasm increasing in size and pushing into the central vacuole. Subsequently, these large fragments of protoplasm separated from the oogonial wall, and became even more spherical. It may be recalled in connection with Berthold's description of oosphere cleavage that he championed the theory of free cell formation.

In a paper published in 1912, Coker and Hyman reported that oosphere cleavage began with the development of a central clear space in the oogonium, and from this region radial fractures appeared and enlarged to divide the cytoplasm into pyramidal units. This account was a forerunner of what is now recognized as the method of cleavage, namely, the evagination of the central tonoplast into the parietal cytoplasm to separate it into units (W. A. Becker, 1934; Bhargava, 1946b; M. C. Carlson, 1929; Cooper, 1929a; Dayal, 1961b; Schrader, 1938; Shanor, 1937). As Dick (1969b) noted -- and Beakes

and Gay (1978a) have confirmed by observations of subcellular structure -- the tonoplast continues to push outward, and fuses with the plasmalemma to define the oospheres (see also Howard and Moore, 1970). The segregation of cytoplasm into oospheres, then, is a series of events identical to that which delimits the primary spores in the sporangia. Humphrey (1893) contended that the basal wall of the oogonium (the septum) was concave (toward the stalk) prior to the appearance of the oospheres, but bent upward toward the oogonial cavity afterwards. This spatial change in the crosswall, he concluded, was indication of a loss in turgidity in the oogonium just as occurred in the sporangia during their formation. The sequence of events in oosphere delimitation (in *Saprolegnia diclina*) depicted by J. Fletcher (1978) does not bear out Humphrey's view.

THE ANTHERIDIA

The antheridial filaments [named "antherocysts" by Lechmere (1911a), and "pollinides" by Rioux and Achard (1956)] of water molds grow as hyphal branches each of which, when functional, subsequently delimits one or more terminal, multinucleate antheridial cells. The sequence of antheridium appearance in relation to the development and maturation of the oogonium has already been treated.

Studies on the antheridial apparatus in the water molds have emphasized principally the fate of nuclei within the antheridial cell and the development of the fertilization tube (Chapter 12), but other aspects also have been explored. Pringsheim (1873-74, 1882a) directed attention to the attachment of the antheridium to the oogonial wall. Quite possibly in conformity with the prevailing view that successful sexual reproduction required both a female and a male apparatus, he stated that in *Achlya racemosa* and *A. polyandra* the oogonia produced female copulation papillae. These protrusions (the accompanying figures in Pringsheim's paper suggest that they were only ordinary wall ornamentations) were allegedly the points at which the antheridium attached to the oogonium and into which the fertilization tubes penetrated. Pringsheim also (1882a) described what is essentially percurrent antheridial cell proliferation, but confirming evidence for such a feature is lacking. Zopf (1893) was impressed by the profuse antheridial branch development in *Dictyuchus carpophorus*[†] and likened it to the multicellular ascocarp of species in the genus *Podosphaera*.

Antheridial branch types are defined according to the point of origin of the branch itself or by the position of the antheridial cell alone (when the branch is absent). Coker (1923) recognized two types of antheridial filaments androgynous and diclinous, qualifying the former as to whether they were of near or distant origin (with respect to the oogonium to which they were attached). According to T. W. Johnson (1956b) five antheridial types were distinguishable in members of *Achlya*, and Dick (1969b) recognized eight types of branch origin within the Oomycetes (not all are applicable to the Saprolegniaceae).

For convenience, the conventional antheridial filament types (T. W. Johnson, 1956b; Seymour, 1970) are defined in the following paragraphs, together with reference to Dick's (1969b) terminology where there is a divergence in meaning.

Epigynous: an antheridial branch originating as a protrusion from the oogonial wall, and being continuous with the lumen of the oogonium.

Androgynous (Fig. 15): the antheridial hypha grows from the stalk of the oogonium to which it then becomes attached. This type in part represents Dick's (1969b) "closely monogynous" antheridium.

Monoclinous (Fig. 15): the antheridial branch arises from the same hypha as the oogonium to which it is attached, but not from the stalk of that oogonium. The two branch types recognized by Dick (1969b) -- closely monogynous and distantly monoclinous -- we include in a single designation.

Diclinous (Fig. 15): the antheridial branch originates on a different hypha from that bearing the oogonium which it attends. Dick proposed for homothallic species that this type of origin be labeled "diclinous and homothallic," while those antheridial hyphae coming from a separate and sexually distinct mycelium be designated "diclinous and heterothallic." If a term is needed to refer to antheridial origin in these dioecious or heterothallic fungi, "interthallic" seems adequate.

Hypogynous (Fig. 15): an antheridial cell only, delimited in the oogonial stalk immediately below the oogonium, and allegedly producing a fertilization tube that penetrates the basal septum of that oogonium.

Hemihypogynous (Fig. 15): the distal portion of a hypogynous cell evaginates to form a very short, lateral protrusion that contacts the oogonium wall apically or laterally. This term -- as the etymologically incorrect semihypogynous -- was introduced in 1974 by Nolan and Lewis to describe antheridial origin in *Pythiopsis cymosa*.

FERTILIZATION

Krause (1960) defined the fifth stage of oogenesis in water molds as the maturation phase, that is, the transition of oospheres into oospores. Fertilization (if it occurs at all) is, of course, a key event in this maturation process; but contrary to the situation in sporogenesis, there is no homogeneous stage during generation of the oospheres.

Either by accident or design, investigators studying the fertilization process in the water molds have generally done so based on prevailing cytological methodology. As would be expected, this approach -- particularly in the formative years of histochemical work -- gave conflicting results such that fertilization was both championed and denied. Whether fertilization is a process better viewed as a morphological rather than a cytological event could be debated endlessly. We are following the precedent established by the first mycologists to explore fertilization in the Saprolegniaceae, and treating the process in a subsequent chapter (12). Suffice it to say at this point that fertilization has been proven beyond doubt for some species, but has not even been traced in many.

OOGENESIS IN SPECIES WITH SINGLE OOSPORES

Most of the work on development of the sexual apparatus in the water molds has been with multiovulate forms. Representatives with a single oospore in each oogonium digress somewhat from the pattern of events found in other taxa. However, only a few of these uniovulate species have been examined to any appreciable degree.

APHANOMYCES SPECIES

The most painstaking treatments of the morphology of species of *Aphanomyces* are those of F. R. Jones and Drechsler (1925), and Drechsler (1929). For such detailed observations as these authors recorded, it is surprising that they really do not treat adequately the sequential morphogenesis of reproductive structures. Much earlier P.-A. Dangeard (1890-91) recorded some observations on oogenesis in *A. laevis*, but the details of the process are unclear from his account. According to Dangeard the cytoplasm in the oogonial initial condensed parietally, became progressively thinner, and then bulged centripetally to form a central swelling. Subsequently the parietal remnants of the cytoplasm folded into the central mass, and the oosphere was thus developed. It is necessary to turn to accounts by Kasanowsky (1911) and Scott (1961a), notably to find much information of a morphogenetic nature regarding sex apparatus development in members of *Aphanomyces*.

The oogonial and antheridial rudiments of *Aphanomyces laevis* are very similar structurally to those of species in other genera. The initials are multinucleate (10-15, according to P.-A. Dangeard, 1890-91), and at first conspicuously vacuolate. Shortly after its appearance, however, the cytoplasm in the oogonial initial becomes increasingly dense and opaque, and there are no traces of vacuoles (Scott, 1961a). If this pattern of vacuole disappearance during morphogenesis holds for other species of *Aphanomyces*, it is unique among the water molds. Kasanowsky's (1911) account suggests that the central vacuole persists, which we have seen in *A. laevis* also.

According to Kasanowsky (1911) there are simultaneous mitotic divisions in the oogonia and antheridia of *Aphanomyces laevis* followed by disintegration of all but one nucleus in each. No such division is mentioned by Scott (1961a) or others. In any case, when the oogonium reaches maximum size, a septum -- formed like that in other water molds -- delimits it from the subtending stalk. Antheridial branches appear at about the time that the oogonial initials are developing (P.-A. Dangeard, 1890-91; Scott, 1961a) or shortly thereafter. A septum cuts off a terminal cell on each antheridial branch, but details of this process are unknown. About 3-6 nuclei are enclosed in the antheridial cell, P.-A. Dangeard (1890-91) reported, but this observation has never been confirmed.

Unlike the situation in species with multiple oospheres (where the cells are cleaved out at least in part by furrowing action of the tonoplast of the central vacuole), the single oosphere in each oogonium of *Aphanomyces laevis* originates through contraction of the cytoplasm from the oogonial wall. This evidently happens in all species of *Aphanomyces* for we have not seen any indication in other species of the genus that cleavage furrows develop. Only Kasanowsky (1911) interpreted oosphere formation differently. He stated that the cytoplasm shrunk from the oogonial wall, but

concomitantly the central vacuole fragmented. Neither Drechsler (1929) nor F. R. Jones and Drechsler (1925) treated this aspect of oogenesis. After fertilization allegedly has occurred, the oospore wall thickens and the numerous small, refractive droplets coalesce into a single, large globule.

PLECTOSPIRA SPECIES

Drechsler (1927, 1929) published exceptionally detailed observations on the structure of *Plectospira* species, but the structural changes in sex cell morphogenesis of the various forms are still unknown. His statements suggest that oogenesis and sporogenesis are saprolegniaceous, but specimens have not been studied developmentally and cytologically. *Plectospira myriandra*, Drechsler thought (1927), might produce fertilization tubes, but the evidence for this is not wholly convincing.

LEPTOLEGNIA SPECIES

If a feature of sex apparatus development in representatives of *Leptolegnia* can be said to be unique, it is that the oosphere is not always organized into a discrete body visibly separable from the oogonial wall prior to fertilization. In *L. subterranea*, for example, the oosphere fills all the projections in the oogonium (J. V. Harvey, 1925a), and is alleged to mature into a symmetrical oospore. A paper by J. N. Couch (1932) on sex cell morphogenesis in *L. caudata* constitutes the only complete account of the events in the reproduction of a leptolegnoid watermold. Although more than one antheridial branch may contact a single oogonium in *L. caudata* (J. N. Couch, 1932), only one becomes functional. Wherever an antheridial cell adheres to the oogonial wall, a protrusion -- the receptive papilla -- grows out from the oogonium.

After the receptive papillae have formed, the wall of the oogonium and that of the adjacent antheridial cell (the functional one?) thicken perceptibly. Subsequently, the wall common to both cells bulges into the oogonium (the oosphere is appressed to the oogonial wall) and ruptures. At this point the gametic nucleus and some cytoplasm from the antheridial cell flow into the oosphere. After karyogamy the oospore wall thickens. Dick (1969b) considered that species of *Leptolegnia* have centripetal oosporogenesis, and thus digress in this respect from the family Saprolegniaceae as a whole.

REVERSION AND REGENERATION

In 1892, A. Fischer made mention of sporangia becoming gemmae, an instance of reversion of function and morphology. The first experimental work on the change in developmental sequence of various structures of watermolds appears to be that by Trow, in 1899. He excised hyphae of *Achlya americana* var. *cambrica*[†] bearing oogonial initials. If antheridial initials were also present on such hyphae these "male" elements continued to develop, but grew away from the incipient oogonia, and would not contact them. If an oogonial initial on the hyphal segment had not been delimited from its stalk

by a septum when the hypha was excised that initial would continue to mature and develop oospores apogamously. Trow concluded that the oogonium exerted some influence on the nature and direction of growth of the antheridial branches -- and, like de Bary, in a sense presaged J. R. Raper's discovery of a hormonal mechanism.

Götze (1918) experimented with the effect of temperature and various solutions on reversion in three species of water molds. He demonstrated that a sporangium could be induced to assume a vegetative function even though the basal septum was formed or spore cleavage had commenced. The oogonia responded in a similar fashion to exogenous irritants except that they would not revert to a vegetative stage once oosporogenesis had begun.

Like Trow, Schlösser (1929) experimented with the reversion phenomenon in several species of Saprolegniaceae but was far more thorough, testing for what he thought was the distribution of sex potential within individual isolates. If an oogonium in which oosporogenesis had begun was excised and placed on agar, the incipient oospheres dedifferentiated, and the oogonium produced hyphae. Other excised oogonia containing oospheres but having antheridial cells attached to them subsequently formed spores -- as did the attached antheridia themselves! In still other instances, Schlösser observed, the oospheres in excised oogonia continued to develop and mature, suggesting, of course, that at some point in maturation reversion could not occur. Schlösser also experimented with monoclinal and diclinal antheridial branches. For instance, he excised a hypha bearing a single incipient oogonium and two developing monoclinal antheridial branches. This hyphal section then was placed near an intact vegetative filament having on it oogonial initials and young (but unattached) antheridial branches. The antheridial filaments from the intact hypha grew toward and subsequently attached to the oogonium on the excised hypha, while the two monoclinal branches on that same hypha failed to develop further. In the normal development of properly nourished mycelium of water molds, Schlösser concluded, three sets of influencing factors -- "currents" was his word -- operated successively: one for control of vegetative growth followed by a second to "operate" male sex differentiation (antheridia), and a third to control the female or oogonial differentiation. He further interpreted the results of his excision and mating experiments as evidence that immature oogonia induce a tendency toward maleness in hyphae (that then function as antheridia).

Three remarkable cases of functional and morphological change in the sexual apparatus of water molds have been published. In a culture of *Achlya racemosa* var. *stelligera*[†] the Moreaus (1935c) saw several regenerational reversions in sex cell development. Among these were the following changes. In one culture an antheridial branch in part developed into a new oogonium, but still retained an antheridial function for adjacent oogonia. An oogonial initial produced a short hypha bearing a second initial, and concomitantly developed antheridial branches that attached to the new initial. In some instances an androgynous antheridial branch converted prior to its attaching to the attendant oogonium into a terminal oogonial initial which in turn formed a normal antheridial branch that attached to the original oogonium. An even more bizarre case of functional reversion was reported by T. W. Johnson (1973a) for a

related species, *A. racemosa*. In the two isolates of *A. racemosa* that Johnson cultured the rudimentary suboogonial branches sometimes matured into functional antheridial branches, as is characteristic of *A. racemosa*, but in other instances on the same hypha converted into uniovulate, apogamous oogonia (Fig. 66 H). In the latter cases, as many as 48 satellite oogonia were produced by suboogonial androgynous "antheridia" on a single oogonial stalk. Depending upon incubation temperature and the source of culture water used, 7-27 small oogonia usually clustered about the base of a typical oogonium. Unlike the changes occurring in *A. colorata* (Moreau and Moreau, 1935c), the satellite oogonia of *A. racemosa* did not dedifferentiate into antheridial branches or even proliferate such filaments. Newby (1948b:267) reported " ... anomalous structures ... " in *Saprolegnia paradoxa*[†] that recall Johnson's specimens of *A. racemosa*. Oospore-bearing outgrowths from the wall, neck (above the basal septum), or stalk of the oogonium itself were produced by Newby's isolate. The protuberances from the stalk often attached to the terminal oogonium just as a functional androgynous antheridial branch would do. In *S. paradoxa*[†] these "miniature" oogonia were sometimes pitted (Newby, 1948b). The genetic and hormonal control (or lack thereof) in these three fungi with such high levels of functional change is yet to be explored.

As Barbier (1975) so adequately demonstrated by perfusion experiments on *Achlya flagellata*[†] it is possible to induce reversion and regeneration artificially. When a hypha bearing a terminal oogonium was perfused with distilled water, the oogonium was stimulated to proliferate and in a monopodial fashion continue the hypha. Since septation to delimit a terminal oogonium interrupts apical dominance, a lateral branch develops. Repeated formation of septa, each instance being followed by lateral branching, resulted in monopodial growth.

SELF-PARASITISM

In the discussion of *Pythium graminicolum* Subramanian, V. D. Matthews (1931: 59, pl. 14, figs. 4, 7) reported instances in her specimens where antheridial branches penetrated into the oogonia and " ... used up the oogonial contents as food." She stated that J. N. Couch also had seen a similar phenomenon in a species of *Achlya* (as is confirmed by his unpublished notes; communication to authors). While the figures provided by Matthews may be the first illustrations of this unusual phenomenon of "self-parasitism," hers is by no means the first mention of its occurrence.

Klebs (1899) apparently saw self-parasitism in *Saprolegnia mixta*[†]. He reported that there was a correlation between profuse antheridial branch development, their penetration into the oogonial cavity, and degeneration of oospheres. Kauffman (1908), too, thought such a coincidence existed, and we have observed the same effect in some isolates of *Achlya prolifera* but without attributing any significance to it.

It is Nolan (1975a) chiefly, who has devoted more than passing attention to self-parasitism in the water molds. In an isolate of *Saprolegnia megasperma* he noticed that oogonia with mature oospores sometimes lacked attendant antheridial branches. In 10-100% of the cases where antheridial hyphae were attached to oogonia, the branches (none bore antheridial cells) grew through wall pits into the oogonia, destroyed the

oospheres, and subsequently might emerge extramatrically from the oogonial cavity. Nolan saw for these instances of self-parasitism some taxonomic and evolutionary implications. If a fungus had evolved to the extent that its own antheridial branches functioned parasitically -- and thus destructively to sexual reproduction -- the most propitious mutations for survival would be those in which antheridial branches were eliminated in individuals of the successive generations.