

CHAPTER 12. Cytology: The Reproductive System

There is no generally acceptable starting point from which to launch a historical account of studies on the cytological features of the reproductive elements in the Saprolegniaceae. Indeed, we have already touched generously upon aspects of morphology that quite properly can be thought of as classical cytology -- spore cleavage and septum delimitation, to name two examples. Ignoring any dictates of what is and what is not cytology, we turn principally to those events that surround the behavior of the nuclei in the reproductive cells of the water molds.

It will be at once apparent that most of the traditional cytological work on members of the family has been on the sexual apparatus. This emphasis may be traced directly to the mycologist's preoccupation with unraveling the nature of the fertilization process in water mold species, an activity that persisted until ultrastructural methods were applied to these fungi and their asexual (and somatic) stages once again commanded due concern.

THE SPORANGIUM AND SPORES

The most prominent of the pioneer investigators of sporangium morphogenesis in the water molds -- de Bary, Trow, Hartog, and Humphrey -- were absorbed largely with tracing the structural changes accompanying spore cleavage. At best, they gave passing attention to nuclei in the sporangia, and others as well also viewed nuclear behavior of these fungi in a cursory manner. Istvánffi (1895), for example, simply stated that each spore in a water mold sporangium obtained a nucleus, and in this Murdia (1939a) concurred for *Achlya dubia*. Cooper (1929b), working with *Brevilegnia diclina*, reported that spores could have as many as 7-15 nuclei. Cytological observations on an unidentified *Aphanomyces* led P.-A. Dangeard (1890-91) to state that some spores had a single nucleus while others possessed as many as six (such multinucleate spores were alleged to be viable and capable of germination). Most of the early investigators believed that there were sufficient nuclei in the incipient sporangium so that each spore obtained at least one at cleavage. Trow (1899), B. M. Davis (1903a), and F. E. V. Smith (1923) concluded that no nuclear divisions took place within the sporangium, but there is convincing ultrastructural evidence from the work of Heath and Greenwood (1970c) quite to the contrary.

It is necessary to turn to Cotner's (1930b) study on *Saprolegnia monoica* var. *glomerata*[†], *Isoachlya paradoxa*[†], *Achlya conspicua*[†], and *Aphanomyces euteiches* for the first reasonably detailed account of the cytology of water mold spores. Some of her interpretations were faulty, later work was to show, but historically her publication is a prominent one. The principal findings in Cotner's report follow.

The protoplast of each spore examined in the various species was divided into two parts, a very dense portion surrounding the nucleus, and a peripheral zone containing small vacuoles. In the center of each nucleus there was a dense body connected to peripheral chromatin by thin strands. The flagella of the primary spores

were inserted into basal granules lying within the plasma membrane. As a spore emerged from its cyst, the nucleus elongated, became lunate, and the ends touched the membrane. At each of these two points, a pseudopod emerged. In *Achlya conspicua*[†], the curved, fusiform nucleus (with chromatin condensed at the equator) was provided with granules, some of which migrated to each end of that lunate nucleus, and it was from these granules that the flagella arose. When the spore subsequently encysted, the granules migrated back into the central portion of the nucleus. Each flagellum was attached to a granular blepharoplast (having its origin "at" the nucleus), and this organelle in turn was in contact with the nucleus by means of a rhizoplast (*see*, for contrast, Chapter 14, describing the ultrastructure of water mold spores at ex- and encystment).

The events in spore nuclear behavior as Cotner interpreted them occurred in all the species she studied. For *Aphanomyces euteiches*, however, she also described a peculiar configuration of "pseudopodia" developing as the secondary spores excysted. These pseudopodia waved about in the medium, and in some instances attached the spore to some bit of microscopic debris, or to become enmeshed among the tips of the flagella. This remarkable production of pseudopods in addition to the two flagella on each spore evidently has not since been seen in any species of Saprolegniaceae.

The configuration of nuclei in spores of *Leptolegnia caudata* was first described in detail in 1932 by A. C. Matthews. In the spore initial (prior to discharge), the nucleus contained a spherical body which, Matthews reported, put out a beak-like process bearing a centrosome at its summit. Just before release of the spore from the sporangium, the flagella appeared to be continuous from this centrosome to the outside of the cell. When the spore emerged from the sporangium and became pyriform, its nucleus was seen to be in a position near the point of flagellar insertion. The bases of the two flagella appeared to lie within the spore body but were connected to the nucleus at a "chromatic" body; this structure Matthews believed to be the centrosome. As later ultrastructural work was to show, the basic subcellular features ascribed by A. C. Matthews to the primary planont in this species were essentially correct.

Astral rays were reported by Setzer (1941) to be present occasionally in the sporangia of *Isoachlya anisospora*[†]. The illustrations accompanying the account depicted the rays as very faint lines connecting the spore initial nucleus with the cytoplasmic membrane. At the homogeneous stage (Chapter 7) of sporogenesis, these astral rays disappeared. The pattern of microtubules in sporogenesis (Chapter 14) is not far removed from what Setzer described from traditional cytological preparations.

Trow (1895) described nuclear division in the spore of *Saprolegnia ferax*. He maintained that the process was typically mitotic consisting of the elongation of a central chromosome and the formation of a transverse wall at the median constriction that developed between the two daughter chromosomes. It is quite apparent that Trow recognized the persisting nature of the nuclear envelope, and thus as he described the division it was in part mitotic and in part amitotic! It is pertinent to interject at this point P.-A. Dangeard's (1931) contention that the two "chromosomes" which Trow referred to were only the two nucleoli within a single nucleus.

Cytological features other than the nuclei of the spores and sporangia of the water molds were of course seen and reported by the classical cytologists. The cleaving spores of *Aphanomyces laevis*, P.-A. Dangeard (1890-91) said, were not separated by granular plates (Körnerplatten) as some observers had maintained. Guilliermond (1941) described the "chondriosomes" (see Chapter 11) in the sporangia of species of *Saprolegnia* and *Achlya* as elements having the form of mitochondria and assembling themselves around each nucleus to thus delimit its outline. At the time of spore germination these mitochondria were alleged to be transformed into "chondriocents" (Chapter 11). The observations on *Thraustotheca clavata* by R. K. Saksena and Bhargava (1946) differ little from Guilliermond's with respect to the mitochondria. In the first-formed, polygonal spore initials (see Chapter 7) of *T. clavata* the mitochondria were scattered in the cytoplasm, but in the homogeneous stage, they aggregated around the nuclei, and remained so as the second (and final) cleavage took place (Fig. 12). The granular mitochondria could elongate and thus become rod-like or filamentous structures.

Guilliermond's (1941) method of staining water mold mycelium with neutral red (primarily) led him to conclude that the single large vacuole appearing in a spore at the time it germinated arose by the fusion of small, granular vacuoles already present in the cell's cytoplasm. Not finding neutral red in the very first spore initials to cleave in sporangia of *Thraustotheca clavata* bathed in this vital stain, R. K. Saksena and Bhargava (1946) decided that vacuoles were absent. Each of the spores to cleave from the sporangial cytoplasm after the homogeneous stage, however, took up the dye. By way of explanation, these two investigators suggested that the stain gave visibility to a vacuole that remained in the spore during its release, and persisted into germination. In addition, they reported, vacuoles arose *de novo* in the apex of the germ hypha.

THE SEXUAL APPARATUS

Published accounts -- some convincing, some not so -- that deal with nuclear behavior and fertilization in the sexual apparatus of water molds are many, and only an occasional review paper has appeared. Two of the earliest summary statements of the nature of sexuality (as it was known at the time) in saprolegnians are those in Wager's paper of 1899, and Gallaud's account published in 1907. A relatively early synthesis published after the emotion of the Trow and Hartog controversy had passed was that by Ramsbottom (1912). A review published in 1973 by Dick and Win-Tin provides considerably more coverage than that by Ramsbottom.

NUCLEAR STRUCTURE AND DIVISION

Although de Bary had seen and commented on nuclei in the water molds he described, these structures seem not to have commanded much of his attention. An early student of the cytology of water molds, P.-A. Dangeard (1890, 1890-91, 1892a, b) believed that the oospores of saprolegniaceous fungi were multinucleate, that this

condition persisted even into germination) and that some observers (Hartog, notably) had confused vacuoles with nuclei. In fact, Dangeard forthrightly disagreed with de Bary's contention that the single staining body in each oospore was a nucleus and in an analogous fashion Trow (1896) considered that the latter's "Kernfleck" was but a lipid droplet.

Much discord is found among the various accounts treating the nature of any nuclear divisions within the sexual apparatus of members of the family. Inconsistencies in fixation and staining procedures, and, as well, the "age" of the specimens when prepared for observations surely contributed to discrepant presentations. Typical mitotic figures for nuclear divisions in the oogonia of *Saprolegnia mixta*[†] were illustrated by B. M. Davis (1903a): characteristic metaphase and anaphase configurations, and intact spindles. He noted that the nuclear membrane persisted during division, and illustrated the nucleolus as a structure outside of the spindle (ultrastructural studies much later were to corroborate these observations). Davis maintained that the nucleolus simply disappeared as division proceeded. A most singular aspect of Davis' cytological study of water molds was his discovery of the "coenocentrum," a transitory structure recognizable as a system of radiating fibrils appearing after oogenesis had begun. He regarded the coenocentrum as the focal point of oosphere origin, and contended that the nucleus positioned nearest these fibrils was destined to continue to function while any others disintegrated. If two or three nuclei were equidistant from the coenocentrum, Davis pointed out, they would be uniformly favored, as it were, and would enlarge but not fuse. The essence of Davis' belief was that the coenocentrum induced a chemotactic response from nuclei in its vicinity, and also promoted nuclear disintegration. It is now evident that he had observed the configurations of astral rays, but had not recognized them as such. Subsequently, B. M. Davis (1905) blundered into a misconception of Trow's "ovicentrum," demonstrating little understanding of the revelations of cytological preparations.

Of course B. M. Davis (1903a) was not alone in seeing typical mitotic figures of nuclei in the oogonia of water molds. The illustrations of nuclear division in *Achlya polyandra*[†] provided by Trow (1899) are explicitly of equational divisions -- spindle, metaphase, and anaphase configurations. Astral rays, centrosomes, and division figures were described for this same species by Mücke (1908). Höhnk (1935b) reported characteristic mitotic stages in the nuclei of *Saprolegnia ferax* as in Cooper (1929b) did *Brevilegnia diclina*. According to the Moreaus (1935b) simultaneous equational divisions occurred in the oogonia and antheridia of *A. conspicua*[†] and *A. flagellata*[†] and they claimed that successively smaller nuclei resulted. The Moreaus (1938) also illustrated typical astral rays -- a single grouping for each nucleus -- in these same two *Achlyas* and in *A. colorata* and *S. ferax* as well. Most of the accompanying illustrations (Moreau and Moreau, 1938) showed the rays directed toward the oogonium wall rather than toward the center of the oogonium. This being so, the figures provided visual evidence of B. M. Davis' (1903a) prediction that these rays functioned in oosphere cleavage and delimitation. The Moreaus also showed spindles and one centrosome for each nucleus. Quite to the contrary, Bhargava (1946b) evidently did not find astral rays associated

with the nuclei of *Isoachlya anisospora* var. *indica*^t but he did observe spindles in the dividing nuclei. Shanor (1937) detected astral rays in the cytoplasm of the oogonia of *Thraustotheca clavata*, and reported that they extended out to the periphery of the oosphere cytoplasm as well as into cytoplasmic "bridges" between adjacent oospheres. He did not see an intact nuclear membrane associated with each division figure, and thought that such instances were evidence of an early stage in nuclear degeneration.

It was P. M. Patterson's (1927) contention that nuclei in the oogonia of *Achlya colorata* divided indirectly although the nuclear membrane persisted, and in *A. flagellata*^t Wolf (1938) likewise found that the division spindle was intranuclear. Typical mitotic figures -- spindle fibers connecting centrosomes at each pole -- occurred in *A. bisexualis* J. R. Raper (1936) reported. Stained preparations of the oogonial initials of *Achlya recurva* enabled A. W. Ziegler (1941) to describe nuclear division in some detail. Each nucleus had a deeply staining centrosome (*see* spindle pole bodies in ultrastructural account of mitosis). At the onset of division, the chromatin condensed into chromosomes, a spindle appeared (Ziegler thought the nucleolus probably contributed to its formation), and at its opposing ends, astral rays developed. Ziegler's preparations evidently did not show a nuclear membrane at metaphase, but apparently did demonstrate movement of the "chromosomes" to the opposite poles.

Slifkin (1967a, b) used colchicine treatment in her ultrastructural and light microscope study of nuclei in the oogonia of *Saprolegnia diclina*, finding that division could be halted at metaphase by this compound. The illustrations of dividing nuclei in Slifkin's (1967b) account are typical of mitosis (amitosis took place in the nuclei of spores and mycelium, she noted, as had Bakerspigel in 1960) with clearly defined spindles and astral bodies. The latter were directed toward the oosphere periphery, just as the Moreaus (1938) had discovered. In sum, mitosis in the classical sense has been reported to occur in the sexual development of several species of water molds among them *S. ferax*, *S. diclina.*, *Achlya debaryana*, *A. bisexualis*, *A. recurva*, *Brevilegnia diclina*, and *Thraustotheca clavata*.

The size of nuclei in the sexual apparatus of species of Saprolegniaceae has been almost entirely ignored. As has been pointed out, the Moreaus (1935b) thought that the nuclei decreased in diameter through successive divisions, but they offered no supporting evidence. Deducing from preparations of *Achlya hypogyna*, Cooper (1929a) concluded that the antheridial nuclei were slightly smaller than the ones in the oogonia (neither had nucleoli). The nuclei in the two members of the sexual apparatus in *Thraustotheca clavata*, however, were identical in size according to Shanor (1937).

THE NUCLEI IN MORPHOGENESIS OF THE SEXUAL CELLS

During oogenesis and the formation of the antheridial apparatus, more than one nucleus is incorporated into the developing cells. In the case of the oogonia, Humphrey (1893) thought there were not less than 100 nuclei in these structures; Shanor (1937) stated that about 35-100 nuclei flowed into the enlarging "female" cell of *Thraustotheca clavata*, and Burton (1940) reported 40-60 in oogonia of *Pythiopsis intermedia*. What is the

fate of these numerous nuclei? According to B. M. Davis (1903a:323) oogenesis in the Saprolegniales had as its outcome the “ ... sacrifice rather than the preservation of nuclei...” That the nuclei in the incipient oogonia and antheridial cells of water molds decrease in number is not seriously contested in the literature; how this reduction occurs has proven to be debatable. Two schools of thought prevail.

Strasburger (1880) held that the number of nuclei in the oogonia of saprolegnians (no particular species are identified in his account) was reduced by fusions, and Hartog (1895, 1899) agreed. Having already called Trow to task for his views on fertilization, Hartog (1896) at once claimed that what Trow saw as disintegrating nuclei were really cases of fusion. Humphrey (1893) spoke in favor of reduction of sex apparatus nuclei through fusion, and although he did admit that some disintegration possibly also occurred, he was satisfied that nuclear divisions did not take place in the oogonial initials. Evidence cited by Hartog (1895) to support his claim was that some oospheres were provided with two or more nuclei. As was to be demonstrated later by others, the presence of two nuclei in one oosphere could equally well be viewed as a precursor to fertilization.

Alternatively, the decrease in the number of nuclei in the oogonial initial was explained as a manifestation of disintegration, and most investigators obviously favored this view (Bhargava, 1946b; Burton, 1940; M. C. Carlson, 1929; Claussen, 1908; Cooper, 1929a, b; P.-A. Dangeard, 1894-95; B. M. Davis, 1903a; Dayal, 1961b; Höhnk, 1935b; Moreau and Moreau, 1935b; P. M. Patterson, 1927; Schrader, 1938; Trow, 1895; Wolf, 1938). There was not unanimous agreement, however, on the point at which disintegration occurred. Claussen (1908), Höhnk (1935b), Bhargava (1946b), Wolf (1938), and Trow (1904) believed that all nuclei in the oogonial initial underwent mitosis before disintegration occurred. At least in *Brevilegnia diclina*, according to Cooper (1929b), many nuclei in the oogonium initials disintegrate, the remaining ones undergoing a single mitotic division succeeded by a second disintegration of all but one functional nucleus in the single oosphere cleaved out in each oogonium. From observations on the oogonia of *Achlya aplanes* var. *indica*[†], Dayal (1961b) also concluded that some nuclear disintegration occurred before a mitotic division of the remaining ones took place. The results of a cytological study by J. N. Couch (1932) on *Leptolegnia caudata* suggested that mitosis in this species occurs in both members of the sexual apparatus followed by disintegration of all but two products, one nucleus each in the oogonial initial and the incipient antheridial cell.

Discord in published opinion surrounds the behavior of the antheridial nuclei in members of the family. The reported number of these organelles is quite varied, and it can be surmised that one reason is attributable to the age of the thallus at the time it was examined. The antheridial cells in *Saprolegnia thuretii*[†], P.-A. Dangeard (1890 - 91) remarked, each had 2-4 chromatin masses, and 3-6 nuclei occurred in these cells in *Aphanomyces laevis*. Several nuclei appear in the antheridial cells in *Achlya colorata* according to P. M. Patterson (1927), but eventually all but 1-6 disintegrate. Burton (1940) counted 8-10 nuclei in the antheridial cells of *Pythiopsis cymosa*, but after a mitotic division by each, all but one broke up.

Humphrey (1893) reported that there were no nuclear divisions in the antheridia (cell) of watermolds, but Hartog (1895) was of a contrary opinion. There was a division of nuclei in the antheridial cell, Trow (1895, 1904) discovered, and this was followed by disintegration. Among those who also decided that a division occurred in antheridial cells with a subsequent breakdown of some of the resultant products were P. M. Patterson (1927), Wolf (1938), Burton (1940), and Bhargava (1946b). A single nucleus remained in each antheridial cell of *Achlya flagellata*[†] (Wolf, 1938), and *Isoachlya anisospora* var. *indica*[†] (Bhargava, 1946b). Dayal (1961b) did not see any nuclear divisions taking place in the antheridial cells of *A. aplanes* var. *indica*[†] but assumed that this process nevertheless occurred.

The cytological study by J. Fletcher (1979b) of *Saprolegnia diclina* and *S. terrestris* relates particularly to oospore abortion, but contains observations as well on nuclear behavior in the gametangia. Some nuclei in the oogonia of *S. terrestris* complete two successive divisions, but all but a few of these gametic products disintegrate prior to oosphere formation. The antheridial nuclei either divide to form gametic nuclei, Fletcher reported, or take on the form of large, torus-shaped organelles (reported also by Bryant and Howard, 1969). A similar situation occurs in the antheridial cells of *S. diclina* except that no gametic nuclei are evident. Furthermore, Fletcher saw no oogonial nuclei of *S. diclina* in division figures beyond prophase (the implication of these cytological events in these two species is discussed in the following section).

FERTILIZATION

Subsequent to Pringsheim's reference in 1855 to appendages coming from side branches and penetrating the oogonia (he called them sporangia at this time), dissent ensued over whether fertilization tubes actually functioned. He repeated his views in 1858 and 1859, but admitted that parthenogenesis likely occurred also (Pringsheim, 1873-74). Some watermolds lack fertilization tubes yet the oospheres still develop into oospores, and it may be surmised that this behavior was in part instrumental in de Bary's (1881) decision to consider all species in the family apogamous. He had expressed this stand in 1866(a, b), stating that it was impossible to prove whether or not fertilization actually occurred in these fungi. De Bary's conclusion was accepted by H. M. Ward (1883), and De-La-Rue (1873) promoted, curiously, the idea of apogamy for those species whose oogonia were without pits in the wall.

Undoubtedly, the beliefs extant on fertilization in the Saprolegniaceae were either weakened or strengthened by Pringsheim's (1860; 1882a, b) reports of motile male cells or "spermamoebae," emanating from the antheridia of *Achlya dioica*[†], *A. colorata*, and *A. polyandra*[†]. These uniflagellate cells, he maintained, swam into the oogonia, and there brought about fertilization. Zopf (1882, 1883) and de Bary (1883) were quick to point out that the alleged fertilizing cells seen by Pringsheim were in fact spores of some parasitic organism. Zopf's twenty-one theses on the matter were particularly penetrating. Pringsheim responded feebly in 1883(b) and 1884, but even cursory examination of the illustrations he provided along the way (1860-1882) proves that he

was confusing infection with fertilization. In some cultures of water molds Hartog (1890) found monadine parasites. He decided that they were similar to what Zopf had found (and had referred to in his criticism of Pringsheim's work), but were not what Pringsheim had described as fertilizing sperm cells. Nevertheless, Hartog (1890, 1895) rejected Pringsheim's discovery, and, in fact, suggested (1895) that bits of cytoplasm set free in the oogonia during oosporogenesis might well have been what the latter thought were spermamoebae.

The motile sperm concept of fertilization in water molds did not perish easily. Hine (1878) believed that motile spermatozooids were formed in the "bulbous antheridia" (the antheridial cells), moved down the fertilization tube, penetrated the oogonium, there to contact the "goniospheres" (oospheres). The fungus in which this startling fertilization pattern occurred was identified by Hine as a new species, *Monoblepharis lateralis*; the organism was, in fact, an infected, saprolegniaceous form. Reinke (1869) reported seeing motile sperm cells slip into the "fertilization spheres" (oospheres) of *Saprolegnia monoica*[†] and C. Müller (1883), who stated he had seen some of the material on which Zopf refuted Pringsheim's view, also accepted the motile sperm concept. A new taxon, *Archilegnia latoica*[†] (and a new subfamily to accept it), was proposed by Apinis (1933a, b) to accommodate a water mold with fertilization by uniflagellate male gametes. Apinis' specimens, too, were parasitized.

De Bary (1881) rejected the idea of gametic nucleus transfer through the medium of a fertilization tube for two principal reasons. First, he argued, the internal pressure in an oogonium would, until the oospheres rounded up and turgor was gone, prevent the fertilization tube from penetrating the oogonial cavity. Second, even in instances where penetration occurred, the end of the fertilization tube never breached an oosphere. Hartog (1889b, 1895) and Lechmere (1911a) accepted de Bary's view, with the former taking the stronger stand. According to Hartog (1892:25), the antheridia of water molds neither opened nor emitted any material, and thus their nuclei went to "... absolute waste without fulfilling any functions whatever..." He announced that the "rejuvenescent" nucleus in the oospore was a product of the fusion of many "closely related" nuclei, and not the union of nuclei from two different (sexual) origins.

As a result of very careful observations, Trow demonstrated to his satisfaction (1895) that the fertilization tube was functional in *Saprolegnia dioica*[†] and occasionally in *S. mixta*[†]. In 1899, he proved fertilization in *Achlya americana* var. *cambricat*[†], and in *A. polyandra*[†] Hildebrand and *A. debaryana* in 1904. Trow (1895) also believed that Humphrey's (1893) account of *A. americana* demonstrated fertilization in this species as well. This conclusion was contrary to Humphrey's view since he had stated flatly that the fertilization tubes in oogonia of *A. americana* were closed. To Trow, the presence of two nuclei in oospheres was evidence that antheridia and fertilization tubes functioned to transfer the male gametic nucleus. Hartog (1895, 1899) took exception to Trow's observations, criticizing him bitterly in print, alleging that Trow could not possibly see fusion occur in living specimens. Hartog's view was shared by B. M. Davis (1903a, 1905) who thought Trow's views were improbable and his illustrations incorrect.

Trow (1895, 1905) did not respond in kind to either Hartog or Davis, and

accepted the former's "accurate and reliable" observations, stating only that he interpreted events differently (1895) than did Hartog. In fact, Hartog (1895:701) argued that the nucleus of the oosphere contained many more chromosomes than was the "norm", a condition disproving the assumption that chromosome number was an indicator of fertilization.

Later cytological studies on various species of saprolegniaceae were to prove Trow correct in at least three very critical decisions, two of which may be cited at this point. In the first place, he concluded that fertilization took place in those species where functional antheridia (actually producing gamete nuclei) occurred. That Trow was correct has since been shown in studies by Beakes (1976: *Saprolegnia furcata*), Bhargava (1944, 1946b: *Isoachlya anisospora* var. *indica*†), Burton (1940: *Pythiopsis intermedia*†), M. C. Carlson (1929: *Achlya racemosa*), Claussen (1908: *S. monoica*†), Coker (1909: *Leptolegnia*), Cooper (1929a: *A. hypogyna*; 1929b: *Brevilegnia diclina*), Dayal (1961b: *A. aplanes* var. *indica*†) Ksanowsky (1911: *Aphanomyces laevis*), Moreau and Moreau (1935b, 1938: *A. conspicua*†, *A. colorata*), Mücke (1908: *A. polyandra* de Bary), P. M. Patterson (1927: *A. colorata*), Schrader (1938: *Thraustotheca clavata*), S. L. Singh and Pavgi (1977a: *Aphanomyces brassicae*), Shanor (1937: *T. clavata*), Valkanov (1931b, 1932: *Aphanomyces hydatinae*; incompletely known), Wolf (1938: *A. flagellata*†), A. W. Ziegler (1953: *A. megasperma*, *A. recurva*, *A. orion*, *S. litoralis*, and *T. primoachlya*†). Other investigators were not certain that fertilization occurred in some of the species they examined, although they thought that it possibly did so: P.-A. Dangeard (1890-91; 1892a, b; 1894-95; 1931), Häyren (1954: *S. dioica*†), Moreau and Moreau (1935b, 1938: *A. flagellata*†), J. R. Raper (1936: *A. bisexualis*), Wager (1899), A. W. Ziegler (1941: *A. recurva*). In still other species of water molds, fertilization tubes have been observed but not seen to function. This is true, for example, for a number of species in which but a single oospore is produced -- *Aphanomyces euteiches* (F. R. Jones and Drechsler, 1925), and *A. cochlioides* and *A. raphani* (Drechsler, 1929). The earliest report of a fertilization tube in a species of *Aphanomyces*, *A. laevis*, seems to be that of P.-A. Dangeard (1890-91). He saw canal-like structures emanating from the antheridial cell and growing into the oogonium to touch the oosphere. There were chromatin granules in these canals, Dangeard reported, but he evidently did not see nuclei.

The second point on which Trow (1895, 1899, 1904) has been upheld was his belief that the oospheres in the water molds he studied were devoid of a wall at least for a short time in their development. Later (1927), P. M. Patterson was to state that the oospheres in *Achlya colorata* were provided from the outset with a thin wall. Trow's observation has since been corroborated by the results of ultrastructural methodology Beakes and Gay, 1978b).

It is well to interject at this point certain observations published by J. Fletcher (1979b). Cytological preparations of the sexual apparatus in *Saprolegnia diclina* failed to show any nuclei that completed a division sequence, hence there were no gametic nuclei. Accordingly, Fletcher thought it was improbable that fertilization could take place in this species, and he suggested that the nuclei in the oospheres were ones incorporated into these cells as they were delimited from the oogonial cytoplasm.

Trow's (1895) statement that the fertilization tube in this species (identified as de Bary's *S. dioica*) is functional appears to be disproven, and other reports that this process takes place in water molds with a high degree of oospore abortion need confirmation in view of Fletcher's study.

Some additional structural aspects of the fertilization process have been touched upon by various investigators. In some water molds -- at the point where the antheridial tube contacts the oosphere wall (Beakes, 1976, reported that fertilization takes place after the oosphere has been invested with a wall) -- a clear area appears in the cytoplasm (J. N. Couch, 1932; Burton, 1940; Shanor, 1937). The nature and function of this "fertilization spot" (Shanor, 1937) is not known. In *Brevilegnia diclina* (Cooper, 1929b), *Leptolegnia caudata* (J. N. Couch, 1932), *Thraustotheca clavata* (Shanor, 1937), and *Pythiopsis intermedia*[†] (Burton, 1940) the route taken by the male gametic nucleus through the cytoplasm of the oosphere persists as a visible furrow that has been called the penetration path (Cooper, 1929b). Whether such a region is formed consistently by the water molds remains to be determined; Cooper (1929a) did not find such a path in *A. hypogyna*. Kauffman's (1908) remarks on this latter species are of interest. He inferred that his observations supported Maurizio's (1894: 149) contention that the evagination of a portion of the oogonial septum into the oogonial cavity was not a fertilization tube, but instead represented a tendency toward internal proliferation as in the sporangia of *Saprolegnia* species. The hypogynous antheridial cell, Kauffman thought, was not an antheridium but an aborted or "latent" cell that had either lost its sexual function or had not developed one.

The study of nuclei in members of the Saprolegniaceae can be promoted by the application of a modified Feulgen staining method developed by Laane and Lie (1975) and Lie (1977). They treated *Achlya radiosa* with Feulgen reagents and the fluorochrome pararosaniline, and viewed the fungal preparations by incident green light excitation. The fluorescing nuclei could be followed during fertilization and meiosis.

MEIOSIS

Seen in retrospect, the concept of haploidy in the somatic nuclei of the water molds was an eminently logical outgrowth of prevailing thought in the early 1900's. The study of life cycles had become firmly entrenched as a research effort quite some time before knowledge of nuclear events began to unfold. For the most part, patterns of nuclear behavior proved to fit quite well the facts of life cycles except with respect to the water molds. A central figure in the study of water mold reproduction was A. H. Trow, who was drawn into a controversy over fertilization in these fungi. To his misfortune, his choice of water molds as subjects for nuclear behavior studies was to call further into question his reasoning ability.

The prevailing belief during the formative years of study of nuclear behavior in the Saprolegniaceae was that a single mitotic division took place in the gametangia. Hence, when meiosis occurred, it did so in zygote germination. In 1895, Trow published some observations on nuclear behavior and development in what at that time

were recognized as three species, *Saprolegnia mixta*[†], *S. dioica*[†], and *S. thuretii*[†]. Two critical points emerged. First, he recognized that nuclear degeneration took place in the cytoplasm of the oogonial initials. Second, Trow (1895:628) noted that some nuclei underwent a division which was "... exactly of the nature of a reducing division. The whole chromosomes are converted into half-chromosomes which do not ... become whole chromosomes by a process of growth." Later, in a discussion of life-cycle analogies among *Achlya* species and members of *Fucus*, Trow (1899) most certainly implied that he regarded the vegetative nuclei of these fungi to be diploid. In part, at least, Trow's early belief in gametic (*see* footnote in Chapter 24 for comment) meiosis was indirect, that is, he simply could not find acceptable evidence for zygotic reduction division. He had reported in 1895 that the number of nuclei in the *Saprolegnia* species he studied was reduced by abortion, and, when the oospore of *S. ferax* germinated, there was a single direct (amitotic) division of its one nucleus. More than five decades later, A. W. Ziegler (1953) was to state that following the division of the oospore nucleus there was no disintegration or abortion of residual nuclei. If watermolds conformed in their nuclear behavior to some other organisms, meiosis should produce four nuclei, three of which would then disintegrate.

To be sure, nuclear disintegration (following a division in the gametangia) was observed by most of those who applied cytological methodology to the study of watermolds (Burton, 1940; M. C. Carlson, 1929; Höhnk, 1935b; P. M. Patterson, 1927; Wolf, 1938, for example), and even Trow reported that nuclear disruption did in fact take place. In 1904, Trow provided acceptable evidence of two nuclear divisions in the gametangia of Hildebrand's *A. polyandra* and Humphrey's *A. debaryana*, and the disintegration of some nuclei following the second division. Hartog (1895, 1896, 1899), who had objected harshly to Trow's claims that fertilization took place in the watermolds, now also (1899) disputed Trow's belief in gametic meiosis. He argued that Trow had misinterpreted incomplete stages in nuclear fusion as representing reductional divisions. It was Hartog's contention, of course, that the reduction in the number of gametangial nuclei in watermold sex cells was the result of a multitude of nuclear fusions.

Cytological studies on sexuality in the watermolds for several decades following the Hartog/Trow disagreement did little, it must be said, to advance knowledge of the nature of nuclear behavior in these fungi. However, such investigations assuredly solidified the belief that members of the family were haploid organisms, with meiosis taking place in the oospore or zygote. From a study of *Saprolegnia monoica*[†] Claussen (1908) concluded that meiosis was zygotic. Kasanowsky (1911) saw that nuclear divisions in the oogonia of *Aphanomyces laevis* were followed by disintegration of all but one nucleus in each cell, but attached no significance to this event. Without presenting any evidence to substantiate his view, Coker (1923) simply stated that Trow erred in believing in gametic reduction division. Observations on six species of Saprolegniaceae (but with emphasis on *S. thuretii*[†]) led Mäckel (1928) to conclude that there was a single synchronous division in the oogonial initials followed directly by abortion of some division products. Mäckel also decided that some oospores in *S. mixta*[†] and *S. thuretii*[†]

were unfertilized, and never had more than a single nucleus. These, he maintained, were indicative of "haploid parthenogenesis". Although she observed disintegration of some nuclei (following division) in the sex cells of *Achlya racemosa*, M. C. Carlson (1929) was uncertain where meiosis took place, and left open both alternatives (gametic or zygotic) as possible routes. It remains to be explained how Apinis (1933a, b) could conclude that meiosis in *Archilegnia latvica*[†] occurred in the gametangia, for his specimens were infected by some parasitic organism. In 1937, Shanor stated that some division figures in the gametangia of *Thraustotheca clavata* were of nuclei without a membrane and he speculated that these were degenerating. Abortion of nuclei in *Achlya bisexualis* was reported by J. R. Raper (1936) to take place before the division process was complete, but this would be an unlikely event in normal mitosis. Shrader (1938) concluded that meiosis in *T. clavata* was zygotic, in part because only a single division occurred in the gametangia. Prior to division, the oospore nuclei (one per spore) were 4-4.5 μm in diameter (2n), Shrader noted, and following division they were 3.2-3.7 μm in diameter (n).

The most broadly based cytological work on meiosis in the water molds was that published by A. W. Ziegler, in 1953, describing prophase, metaphase, and anaphase configurations in the germinating oospores of *Achlya colorata*, *A. megasperma*, *A. orion*, *A. recurva*, *Isoachlya intermedia*[†], *S. litoralis*, and *Thraustotheca primoachlya*[†]. He concluded that all information he had assembled -- such factors as chromosome count, pachytene and leptotene configurations, anaphase and metaphase figures, diads, and two and four nuclei in germinating oospores -- confirmed that meiosis was zygotic. As further evidence he cited measurements for the so-called male and female nuclei, and the diploid nucleus; the former averaged 3.2 μm in diameter, while the latter were 6.2-6.6 μm . Some of the figures provided in Ziegler's paper might admit to more than one interpretation. Metaphase "chromosomes" in the gametangial nuclei of *A. recurva*, for instance, are illustrated as being considerably larger than those alleged to be of metaphase I in the oospore.

At least up to and including Ziegler's published account, evidence assembled for and against zygotic meiosis was largely circumstantial. Events such as synchronous divisions, nuclear abortion, nuclear and metaphase chromosome size changes, and differences in prophase configurations and chromosome counts were used to sanction one conviction over another. Two reviews of reproduction in the fungi serve appropriately to reflect the general views of meiosis in the Saprolegniaceae about 1950. Emerson and Wilson (1949) perceptively pointed out that evidence favoring the hypothesis of zygotic meiosis was essentially negative: meiosis simply had not been demonstrated to occur preceding gametogenesis. Olive (1953) accepted provisionally the idea of meiosis in the zygote, but cautioned against careless acceptance of chromosome counts as supporting evidence. In his view, there was insufficient proof that reduction division took place in the germinating oospore of species of Saprolegniaceae.

Something over a decade after A. W. Ziegler's paper (1953) appeared, the generally accepted belief of a haploid vegetative thallus in the water molds was called

openly into question. Mullins and Raper (1965) germinated oospores produced by matings in *Achlya ambisexualis* and *Dictyuchus monosporus*, and while the resulting data were consistent with the theory of zygotic meiosis, they could be interpreted as suggestive of gametic meiosis. Slifkin (1967a, b) demonstrated that colchicine did not affect division in the somatic hyphae, but did so when applied to oogonia. This would suggest, as Dick and Win-Tin (1973) remarked, that the nuclear divisions in the vegetative and reproductive phases of the fungus *Saprolegnia delicata* were different. By inference from the result of colchicine sensitivity tests divisions in the oogonial initials were meiotic. Lie (1977) concluded that his very detailed study of *A. radiosa* gave evidence for the occurrence of meiosis during gametangial development.

Sansome and Harris (1962), and Sansome (1965) attacked the problem of meiosis in *Achlya colorata* and *Achlya* sp. by the usual cytological staining methods alone and after treating the fungi with camphor (to cause the nuclei to enlarge). Some nuclei in the antheridial cells and oogonia of the specimens had a ring configuration postulated as consisting of four bivalents. Sansome (1965) also found quadrivalents in the sex cells of *Achlya* sp. treated with camphor. Such structures, she stated, could not be interpreted as mitotic. In earlier papers, Sansome (1963a, b) predicted that the Oomycetes as a class were diploid organisms, not haploid. She cited J. N. Couch's (1926b) work with mating strains in *Dictyuchus* as supporting this view, and (Sansome, 1964) was of the opinion that the results of crossings reported by Barksdale (1960) and J. R. Raper (1960) could be most readily explained on the basis of the fungi in question having a diploid thallus. In *Dictyuchus anomalus*[†] which Couch identified as "parthenogenetic strain N", not all the products of mating were alike -- a situation that could be explained if the parthenogenetic line was a heterozygous diploid. Among the lines of evidence put forth (Sansome, 1965) to support the view that meiosis was gametic were these: (1) two successive divisions in the gametangia accompanied by a nucleus size reduction; (2) about four times more nuclei in the gametangia following the second division than the first; (3) multiple associations of chromosomes in gametangia, and (4) the occurrence of typical metaphase configurations only in the gametangia.

Shortly after Sansome's paper appeared a few additional reports favorable to the hypothesis of gametic reduction division were published. On the basis of genetic evidence (Chapter 24), Barksdale (1966) concluded that meiosis in *Achlya bisexualis* was gametic. Later work by her (1968) of a cytological nature on the antheridia of *A. ambisexualis* E87 (♂), demonstrated that two successive divisions occurred, followed by disintegration of some nuclear products. She detected diakinesis at the first of the two divisions, three bivalents at pachytene, and of three chromosomes visible in the preparation, one was accompanied by a terminal satellite. Barksdale could not see a metaphase I organization of chromosomes on a spindle apparatus. At the second division, three dyads moved at the onset of anaphase II, and monads subsequently appeared. To be sure, the metaphase chromosomes illustrated by Barksdale are much larger than those reported by Sansome for the material she studied. In any case, the configuration of the second division stages seen in the antheridial cells were different from those encountered in the first of the two successive divisions. Barksdale suggested

that this would be expected of a reductional division. The nuclear sizes which Barksdale (1968) reported also are supportive evidence of meiotic division: at mid-prophase I the nuclei were 4.6-4.8 x 4.8-5.2 μm , but those in the interphase between the two divisions were about half this size -- 1.9-2.1 x 2.0-2.4 μm .

By cytochemical means and microspectrophotometry, Bryant and Howard (1969) sought evidence for meiosis in *Saprolegnia terrestris*. They analyzed randomly selected somatic nuclei, and ones in early divisional stages in the gametangia and late divisional phases in the oospheres. The amounts of DNA were measured and reported, as Swift (1950) had done, as a quantity relative to that of the haploid condition (for instance, a 2 C value for a particular nucleus meant that it had twice the DNA of a haploid one). The predivisional nuclei in the oogonia and antheridial cells of *S. terrestris* had a value of 4 C, but this level was reduced to C following a series of divisions. The somatic hyphae had nuclei with a 2 C value, that is, were diploid. In one oogonium and antheridial cell of *S. terrestris* the DNA value for nuclei in prophase and early metaphase was 8 C, suggesting polyploidy (Bryant and Howard, 1969).

In *Achlya klebsiana*[†] and *Saprolegnia ferax*, Flanagan (1970) saw two nuclear divisions in the oogonial initials and antheridial cells, and at the onset of germination, a single division of the oospore nucleus; and the latter in every way resembling somatic nuclear division. Cytological preparations of *A. klebsiana*[†] showed evidence of diakinesis and bivalents, and 24 chromatin bodies in the somatic nuclei at metaphase. Nuclei in the first division in the oogonial initial and attached antheridial cell, however, had but 12 such bodies. For *S. ferax* comparable observational analysis gave counts of ca. 20 and 10, respectively. These numbers seem quite precise -- in view of the very small size of "chromosomes" in the water molds -- but Flanagan maintained that nuclei in these fungi could be studied meaningfully if properly fixed and stained. The divisions (meiotic according to Flanagan) in the antheridial cells and oogonia were synchronous. Flanagan's remarks on the fusion of gametic nuclei are unusual. He reported that when fertilization took place an oospore was formed around the diploid nucleus to the exclusion of any other diploids or gamete nuclei not involved in fusion. This condition was interpreted as meaning that the two species did not form oospheres.

Lastly, two publications from Michael Dick's laboratory have further cast support to the concept of gametic meiosis. Dick and Win-Tin (1973) concluded that observational data from existing literature favored this view. Later, Win-Tin and Dick (1975) found multiple chromosome associations at first telophase or second metaphase in *Achlya flagellata*[†], *A. hypogyna*, *A. inflata*, *A. racemosa*, and *Aplanopsis terrestris*. For evidence of gametic meiosis in their preparations they relied upon chromosome numbers (Chapter 24) as well as other cytological indicators: (1) a two-stage reduction in nuclear volume during division in the gametangia; (2) abortion of interphase supernumerary nuclei; (3) tetrads of gametic nuclei in both the antheridial cells and oogonial initials; (4) condensation and pairing of homologous chromosomes; (5) chromosome size differences in the metaphase stage of the two successive divisions; (6) bridges or bridge fragments at anaphase I, and (7) chiasmata at diplotene/diakinesis. Curiously, in *Aplanopsis terrestris*, meiosis was congenitally aberrant,

involving chromatin lag during late anaphase and early telophase such that the division figure showed only a linear series of three discrete chromatinic zones. Perhaps the reason for the absence of sporangia in this species resides in its aberrant meiotic pattern.

The visible evidence -- from light microscopy -- needed to establish nuclear divisions as reductional seems well summed up by Dennett and Stanghellini (1977). Reliable evidence of meiosis, they stated, was at hand if diplotene/ diakinesis stages were found, but four other expressions could be sought. These are: multiple associations of chromosomes, a metaphase plate configuration unique to meiosis (Sansome, 1965), anaphasic bridges or fragments, and reduction in nuclear diameters as divisions proceed. Subsequently (Chapter 15), we record the ultrastructural evidence for meiosis (Beakes, 1976; Beakes and Gay, 1977; Lie, 1977; Lie and Laane, 1979).