

CHAPTER 15. Ultrastructure: the Sexual Reproductive System

The sexual apparatus in the water molds has not been accorded the same degree of subcellular examination that characterizes the fine structure studies on their asexual reproductive mechanism. Nevertheless, the work that has been done demonstrates that the sexual structures are certainly amenable to ultrastructural analysis. Transmission electron microscopy has greatly expanded that knowledge which limited cytological methodology had discovered about the morphology of the sex cells of the water molds, and has, as well, disclosed previously unsuspected events accompanying their development. However, much is yet to be learned of the structure, location, displacement, and function of organelles in the morphogenesis of the sexual system of water molds.

THE OOGONIAL AND ANTHERIDIAL CELL INITIALS

THE INCIPIENT OOGONIUM

From what little has been observed of the very early stages of oogonium development, the cytoplasm flowing into the expanding initial is furnished with the usual organelles characterizing the eukaryotic cell: dictyosome elements, nuclei, microbodies, vesicles of various electron densities and configurations, lipid bodies, storage vesicles, ribosomes, and the like. It has been reported by Gay and associates (1971) that in the earliest stage of formation the oogonial initials of at least one water mold, *Saprolegnia furcata*, lacks the central vacuole such as is characteristic of later phases in oogenesis. Investigators studying very young oogonia ultrastructurally (Gay *et al.*, 1971; W. L. Steffens, 1976, for example) agree that wall vesicles are prominent near the expanding oogonial wall.

According to Gay and his collaborators (1971) the nuclei in the developing oogonial initials of *Saprolegnia furcata* eventually occupy a peripheral position in the cytoplasm (Beakes, 1980d) and are spaced nearly equidistantly from one another. The centrioles associated with each nucleus are adjacent to the face of the nucleus nearest the periphery of the oogonium. Moreover, in this fungus, members of centriolar pairs are positioned at an 180° angle to one another, just as in somatic nuclei (Heath and Greenwood, 1970c).

Tontz (1969) detected rough and smooth endoplasmic reticulum in the ooplasm of *Achlya imperfecta*. The mitochondria in the peripheral cytoplasm of this sex cell occur along with vesicles of three types: granular (smooth membrane), electron transparent, and coated or "shaggy" ones. Prior to septum formation, mitochondria in the incipient oogonia of *Saprolegnia terrestris* are (Howard and Moore, 1970) typically elongate. After the cross wall at the base of the oogonium is complete, however, these organelles appear partially or entirely to encompass lipid droplets. Howard and Moore (1970) also

observed that mitochondria could be associated closely with storage vesicles (possibly dense-body vesicles) in this species.

Wall Formation: -- A very generous contribution to the knowledge of wall deposition in the oogonial initials of water molds is that of W. L. Steffens (1976). He observed that storage bodies, wall vesicles (Heath *et al.*, 1971), and lomasomes are associated with the developing wall in immature oogonia of *Achlya recurva*; the vesicles released from Golgi bodies are very similar to wall vesicles. By inference from microchemical tests, Steffens suggested that microbodies in the cytoplasm of the oogonial initial are of the glyoxosomal type, and he postulated that these could be involved in converting lipid in storage bodies into glucans which are then transported by vesicles to the site of wall synthesis. Both W. L. Steffens (1976) and Tontz (1969) found lomasomes in the walls of young oogonia of *A. recurva* and *A. imperfecta*[†], respectively. Heath and his collaborators (1971) noticed that vesicles were present in the cytoplasmic matrix in the vicinity of the wall of the oogonial initials in *Saprolegnia furcata* as these cells enlarged during oogenesis. Once the initial had attained a size beyond which it would no longer expand, vesicles were rare.

Pringsheim (1873-74), it will be recalled, reported that oogonia of some water molds produced fertilization papillae to which the antheridial cells became attached. Tontz (1969: figs. 16, 17) described what she termed "receptive papillae" between the oogonium wall and antheridial cell wall in *Achlya imperfecta*[†]: an osmiophilic flexible and compressible substance evidently secreted by the oogonial initial. This "fungal cement" (so named by Tontz) forms irregular or knob-like protrusions on the oogonium wall prior to contact by an antheridial cell. It seems that this so-called receptive material has not been sought for (or seen) in other species, and confirmatory evidence for it is needed. Howard and Moore (1970) reported that in the sexual apparatus of *Saprolegnia terrestris* a dense accumulation of vesicles took place in that region of the oogonial wall in contact with the boundary of the antheridial cell. These vesicles, they suggested, might be involved in the development (through enzymatic action) of wall pits.

The Basal Septum: -- Beakes (1976) studied septum formation in the delimitation of oogonia in several species of *Saprolegnia*. At the beginning of crosswall development, numerous electron-dense vesicles accumulate in a plate-like configuration at the septum site. These vesicles then become compacted, and wall material is deposited on both surfaces of this flattened disc of compressed vesicles.

THE ANTHERIDIAL CELL

At the time of septum formation, the antheridial cell in *Saprolegnia terrestris* is filled with multinucleate cytoplasm (Howard and Moore, 1970), and in *Achlya bisexualis* (Tontz, 1969) the point at which the wall is to form is rich in endoplasmic reticulum, dictyosomes, and vesicles. Tontz (1969) suggested that the antheridial cell septum is

composed of collapsed vesicles, a situation comparable to what is known of the structure of the septum in the oogonial stalk. Nuclei in the antheridial cells of *A. bisexualis* are accompanied by typical centrioles. In *S. furcata* (Heath *et al.*, 1971) the cytoplasm in that portion of the antheridial cell appressed to the oogonium wall is rich in wall vesicles prior to the appearance of the fertilization tube. Huizar (1978) has demonstrated that the antheridial cell in *A. recurva* may be attached directly to the distal end of an oogonium wall ornamentation (in this species, the wall projections are almost universally thin and truncate at the apex).

OOSPHERE CLEAVAGE

In gross morphological terms, oosphere cleavage in the developing oogonia of members of the Saprolegniaceae (except, perhaps, in species of *Aphanomyces*) coincides with extension of the tonoplast into the peripheral cytoplasm (Chapter 9). Subcellular structural changes during oosphere delimitation confirm that while this is, in fact, the basic pattern, the event is not simply the result of growth of the membrane of the central vacuole. A composite picture of oosphere cleavage emerges from an analysis of several accounts, those by Gay *et al.* (1971), Gay (1972), and Beckett *et al.* (1974) being most prominent.

STRUCTURAL ASPECTS

Oosphere delimitation presumably begins with the development of cleavage vesicles derived from dense-body units in the cytoplasm, but this remains to be proven. In any case, furrowing and separation of the cytoplasm into discrete aggregates (Fig. 40) occurs in such a manner as to suggest a two-stage process: vacuolation and organization of the peripheral protoplasm into roughly hemispherical units. Coalescence among cleavage vesicles, augmented by contributions from the vacuolar tonoplast (also believed -- Gay *et al.*, 1971 -- to derive from dense-body vesicles), may be responsible for cleavage. However, it also is possible that a redistribution of vacuoles rather than a synthesis of new ones entirely might at least supplement the process. It appears that the tonoplast ultimately fuses with the plasmalemma, and segments of the cytoplasm are then separated from one another. This being so, the oosphere initials when fully delimited are bounded in part by tonoplast and in part by plasmalemma (Gay, 1972: figs. 1-4), just as in spore cleavage. According to Gay and his associates (1971), no fragments of the plasmalemma remain after cytoplasmic separation has been effected. This has not been established with finality, and it is possible that some remnants of plasma membrane and tonoplast may persist.

Differentiation of the oogonial cytoplasm into oospheres has been investigated by Beakes (1976, 1980a) and Beakes and Gay (1978a). Calculations (formula for the volume of a prolate spheroid) show that in *Saprolegnia furcata* at least, the volume of the oosphere increases immediately after the tonoplast fuses to the plasmalemma.

Subsequently, the volume decreases, and as this reduction occurs (Beakes and Gay, 1978a), cytoplasmic protuberances appear at a few points on the periphery of some oospheres. In a few instances, fragments of cytoplasm detach from an oosphere, migrate across intersporal space, and then become incorporated into an adjacent oosphere. This migratory event in the developing oospheres of water molds had been seen by prior investigators using brightfield optics. The mechanism involved in movement of the cytoplasmic fragments is not known.

There is ultrastructural and cytochemical evidence, from some experimental work performed by Gay (1972) on the oogonia of *Saprolegnia furcata*, to show that dense-body vesicles involved in cytoplasmic cleavage contain phosphorus (possibly a phospho-glucan). His observations also suggest that there is a high ratio of phosphorus to sulfur in the cleavage vesicles and central vacuole during oosphere delimitation, and in the oogonial cavity following completion of this process. It has been noted (Gay *et al.*, 1971) that dense-body vesicles disappear or diminish in size as cleavage membranes "grow" and fuse, and granules identical to ones detected in these vesicles have been located as well in the intersporal fluid space in the oogonial cavity. There are very persuasive circumstantial grounds, then, for believing that some of the dense-body vesicles may have both a physical and chemical role in oosphere delimitation. Perhaps the organelle fragments and membrane segments which W. L. Steffens (1976) found in the central vacuole of the oogonia of *Achlya recurva* (and attributed to lysis) are dense vesicle remnants left behind after they had performed a function resulting in synthesis.

Tontz (1969) and W. L. Steffens (1976) noted that cytoplasmic cleavage in the oogonial initials of *Achlya bisexualis* and *A. recurva*, respectively, also is accompanied by evagination of the central vacuole. The latter investigator also found elongate vesicles in the area of oosphere delimitation. It has been reported by W. L. Steffens that linear structures appear in the oogonial initials of *A. recurva*. These likely were indicative of prophase since he noted that these elements later paired to become part of a synaptonemal complex (a structure characteristic of pachytene). Howard and Moore (1970) had earlier concluded that meiosis in *Saprolegnia terrestris* precedes furrowing of the oogonial cytoplasm (*See* section on meiosis).

CALCIUM AND ULTRASTRUCTURE OF THE OOGONIAL APPARATUS

It has been well established that calcium (Ca^{2+}) has a role in such biochemical processes in the physiology of water molds as amino acid transport and cytokinin activity (Chapters 22 and 23), and it is now evident from J. Fletcher's (1979a) work that certain aspects of ultrastructure also are influenced by this element. Fletcher explored qualitatively and quantitatively volume densities of various organelles in developing oogonia on mycelium in high calcium and calcium-deficient regimes. Noticeable subcellular changes accompanied the latter condition.

In the oogonia of *Saprolegnia diclina* in early stages of development on mycelium in a Ca-deficient medium, there is an increased volume density of mitochondria and nuclei. Increases also occur with respect to peripheral vacuoles and dense-body vesicles

in later stages of oogenesis. However, the mean diameter of the latter organelles is reduced when calcium is lacking in the medium. Calcium deficiency is associated as well with decreases in the volume densities of lipids, and with high levels of oogonium and oospore abortion as expressed by extreme disorganization of protoplasts. In unaborted immature oogonia produced on mycelium grown in a calcium-deficient medium the central vacuole contains crystalline inclusions, and some of these oogonia fail to develop secondary walls. If the release of wall vesicle content is retarded in a calcium-deficient environment wall formation would likewise be inhibited (J. Fletcher, 1979a).

During oogonial development in colonies of the watermold propagated in a medium containing calcium there is a decrease in the volume density of dense-body vesicles. This could be indicative of some contribution of these organelles to the developing central vacuole in the oogonial initial. With no calcium in the medium, the decrease in volume density of the dense-body vesicles during oogonial development is significantly curtailed, suggesting a failure of the normal functioning of dense-body vesicles in oogonial development. In general, in a calcium-containing medium, organelle volume densities (except for the central vacuoles and lipid bodies) decrease relative to total oogonium volume during oogenesis. As Fletcher noted, this change in volume densities is consistent with the view that supernumerary nuclei disintegrate as oogonial development proceeds.

OOSPHERE MATURATION -- THE CYTOPLASM

One of two major events following the delimitation of oospheres is a series of changes in their cytoplasm. It has been shown that a newly delimited oosphere is a membrane-bound cell lacking a wall, and that this cell persists in such condition (Beakes and Gay, 1978b) for 15-30 minutes following cleavage. Trow's claim (1895) that watermold oospheres are for a time without a wall has thus been salvaged from dispute.

The phases of oosphere development and oospore maturation as recognized by Beakes and Gay (1978a) have been mentioned in a prior chapter. These investigators also provided in their treatment substantive accounts of ultrastructural characteristics of these cells. Their observations were in part set down in diagrammatic form, a portion of which is redrawn here as Figure 41.

Shortly after an oosphere in the oogonial initial of *Saprolegnia furcata* is cleaved, it displays a mixture of membrane-bound dense-bodies and neutral lipid granules (Fig. 41 A-C). Endoplasmic reticulum, ribosomes, dictyosomes, mitochondria, and the nucleus also occupy the cytoplasm in the developing oosphere (Beakes and Gay, 1978a). As oosphere maturation proceeds, the dense-body vesicles migrate to the periphery of the cleaved cell and the neutral lipids move toward its center (Fig. 41 B). At first, small vacuoles are formed and coalesce (Fig. 41 D, E) presumably involving vesicles containing electron-dense material (variously termed dense-body, storage, reserve, or fingerprint vesicles). There evidently is no net gain in vacuoles through synthesis.

Subsequently, a central vacuole becomes fully delimited (Fig. 41 F) and as it does so, the other functional and storage bodies are displaced to the periphery of the maturing oospore (*sensu* Beakes and Gay, 1978a).

OOSPORE STRUCTURE

We have previously (Chapter 10) referred to oospore types in the Saprolegniaceae, and mentioned briefly their ultrastructural "morphology". The concept of oosphere maturation as it is viewed by Beakes and Gay (1978a; *see also*, Fig. 41) gives substance to a change in the definition and terminology applied to oospores.

It is the central vacuole surrounded by neutral lipid bodies that characterizes a centric oospore, as Beakes and Gay (1978a) have shown. Howard and Moore (1970) had earlier reached precisely the same conclusion, but they used the term ooplast to designate the central vacuole. If, in oosphere maturation, small vacuoles (derived from dense-body vesicles?) fused into a single large one that then became positioned eccentrically within the cytoplasm and invested by droplets of neutral lipids, a subcentric oospore would be the result. Howard and Moore (1970: fig. 60) show precisely this orientation of organelles for such an oospore.

At the time he analyzed saprolegniaceous oospore structure as a series of "phases", Dick (1969b: fig. 3) did not have the benefit of the knowledge of ultrastructural organization within these reproductive cells. With respect to a centric oospore, for example -- and quite in accord with prevailing thought -- he considered the central portion of the cell to be granular cytoplasm. The disposition of organelles in the oospore, however, shows this "Phase" or "Fraction I" (Dick's terminology) to be a vacuole (ooplast) and not cytoplasm.

OOSPHERE MATURATION -- THE WALL

The second major developmental event following oosphere delimitation is its investiture by a wall. Traditionally, the formation of a wall around the oosphere has been viewed as a signal that the oospore stage had been reached. Beakes and Gay (1978a) contended, however, that a cleaved reproductive unit in an oogonium is an oosphere even though equipped with an outer wall, and the delimited spheroid does not become an oospore until an inner wall is deposited prior to central vacuole development.

Immediately following completion of cleavage in the oogonia of *Saprolegnia furcata*, the resulting oospheres decrease in volume. The reason for this is elusive. Beakes and Gay (1978a) concluded that the volume change could not be traced to the loss of cytoplasm by blebs migrating from an oosphere. Moreover, the very young oospheres in this fungus have no contractile vacuoles to account for shrinkage. Beakes and Gay hypothesized that if the many peripheral vesicles of the oospheres were to be involved in wall synthesis the volume of the oosphere would decrease. In this context,

what is the subcellular nature of wall formation in oosporogenesis in the Saprolegniaceae?

Heath (1976) and W. L. Steffens (1976) reported that wall vesicles functioned in the elaboration of a wall about the oosphere of a watermold, an event not at all unlike that resulting in the maturation of spores and cysts. Steffens hypothesized that glyoxysomes (microbodies) are present in the oogonial cytoplasm in *Achlya recurva* and are involved in converting storage-body lipids into glucans (glyoxylate pathway). Subsequently, wall vesicles, he theorized, could operate to transport these glucans to sites of wall synthesis.

By far the most definitive account of oosphere and oospore wall development is that by Beakes and Gay (1978b) resulting from their study of *Saprolegnia furcata*. The oosphere in this species becomes globose, signaling that the first or outer wall (Fig. 42.2: W₁) has been elaborated. Development of this wall (the episporium, *sensu de Bary*, 1884) is associated with underlying vesicles (Fig. 42.2) that presumably contribute to elaboration of the plasmalemma (Fig. 42.1) and to deposition of the wall. Following fertilization, in *S. furcata*, an inner wall is added centripetally to the developing oosphere (Fig. 42.2, 42.5: W₂) over a period of several hours. Deposition of this investiture is not accompanied by peripheral wall vesicles and underlying dictyosomes, Beakes and Gay (1978b) reported, but cisternae of endoplasmic reticulum are aligned close to the developing layer. In his ultrastructural study of oosporogenesis in *Achlya recurva*, W. L. Steffens (1976) remarked that the plasmalemma had multiple foldings (at various points) which participated in the formation of lomasomes at the wall site.

After the inner wall (Fig. 42.5: W₂) has been deposited, there is a period of maturation involving several changes (Fig. 42). The outer wall becomes dense and fibrillar (Fig. 42.2, 42.3: W₁). A tripartite, dense layer is present between the outer and inner wall (Fig. 42.4) almost from the onset of maturation. The inner wall thickens (Fig. 42.5) and subsequently exhibits a three-layered partitioning (Fig. 42.6) -- an inner, electron-dense portion (Fig. 42.6: L₂), an outer layer (Fig. 42.6: L₁) and separating the two, a more electron-transparent fibrillar zone. There is cytochemical evidence that this central zone of the inner wall is a repository for polysaccharides. Beakes and Gay (1978b) postulated that the inner wall is a carbohydrate, lipid, and protein reserve, some fraction of which is mobilized at germination, much as Clausz (1968) had proposed. From observations with the light microscope, Dick (1969b) concluded that the endospore (in the de Bary sense, this being the secondary wall layer deposited during oospore maturation) of the zygotes of members of the Saprolegniaceae had at least two layers.

MEIOSIS

It seems reasonable to conclude that meiosis in the watermolds is gametic at least in those species carefully analyzed ultrastructurally. Reduction division in the antheridial cell (Ellzey, 1974, for example) occurs prior to entry of the fertilization tube into the oogonium, while in the oogonium the process takes place prior to oosphere

initial development after vacuolation (Bryant and Howard, 1969; Howard and Moore, 1970). Incomplete observations of the meiotic process¹ are available for a few members of the family: *Saprolegnia furcata* (Beakes and Gay, 1977), *Achlya ambisexualis* (Ellzey, 1974, Ellzey and Huizar, 1977; Ellzey *et al.*, 1976; Tontz, 1969), *Thraustotheca clavata* (Heath, 1976), *A. recurva* (W. L. Steffens, 1976), and *A. imperfecta*[†] (Tontz, 1969). Characterization of the subcellular events in meiosis in *S. terrestris* by Howard and Moore (1970) is not only the first record of details of this process in a watermold, it is also the most complete description (Fig. 43) at hand. Much of the following account is taken from their publication.

MEIOSIS IN THE OOGONIUM

Interphase nuclei in the oogonia of *Saprolegnia terrestris* and *Achlya radiosa* (Lie and Laane, 1979) are spherical prior to meiosis, and are accompanied by paired centrioles (Fig. 43 A) in a pocket in the nuclear envelope. These centrioles are identical in structure and position to those associated with somatic nuclei. Prior to late leptotene, the paired centrioles replicate and migrate (Fig. 43 B) to opposite “poles” of the nucleus. Some intranuclear tubules are associated with at least one pair of migrating centrioles. At this time the nucleus begins to increase in diameter to a size approximately twice that of a somatic one.

During prophase I, linear elements appear within the nucleus. Based on his study of prophase nuclear structure in higher organisms, Moses (1968) named such organelles axial elements, and Howard and Moore (1970: fig. 13) adopted his nomenclature for these structures in *Saprolegnia terrestris*. Also during prophase I each of the centriolar pairs becomes invested in an electron-dense sheath. Beakes and Gay (1977) saw a similar centriolar encasement in the meiotic figures of *S. furcata*, remarking that the presence of this sheath corresponded to the reports of prominent “aster bodies” by Trow (1904) and A. W. Ziegler (1953). In *S. furcata* and *S. ferax*, the centrioles also elongate at this time to nearly twice their normal interphase length. Subsequently, toward the end of prophase I, the centrioles reach their respective “poles”, and there become partially enveloped by endoplasmic reticulum (Heath, 1976) -- the “caps” described and illustrated by Howard and Moore (1970). Beakes and Gay (1977) showed that the cap of ER is discontinuous, hence microtubules can pass through this region. Howard and Moore contended that the centriolar caps persisted at least into metaphase II; Beakes and Gay (1977) reported that they were evident only through telophase I.

At metaphase I in meiotic nuclei of *Saprolegnia terrestris* and *Achlya radiosa* (Lie and Laane, 1979) the intranuclear spindle is evident, and there is an equatorial cluster of condensed chromatin material (Fig. 43 D). The micrographs provided by Howard and Moore (1970: figs. 17, 36) to illustrate metaphase I show the typical aspect of chromosomes fixed for TEM preparations. The polar centrioles at this stage are within

¹ See footnote, Chapter 24, for comment on terminology.

pockets in the nuclear envelope. Metaphase I and anaphase I (Fig. 43F) present essentially the same configurations as comparable stages in mitosis with some exceptions. Metaphase plates have not been seen in all instances of mitosis that have been reported for water molds, and there is no nucleolus associated with these stages of meiotic division as there is with the comparable phases of mitosis. Moreover, the staining properties of the nucleoplasm are noticeably different in meiosis and mitosis. Heath (1976) has pointed out that the physical association of microtubules with the nuclear envelope evidently is not (Howard and Moore, 1970) a characteristic of meiosis. The use of acrolein-glutaraldehyde fixation in some preparations made by Howard and Moore could have modified the specimens such that microtubular elements were not clearly delimited in all instances.

At metaphase II (following or accompanying constriction of the nuclear envelope) during oosporogenesis in *Saprolegnia terrestris*, the individuals of each centriolar pair separate without replication -- this occurs also in *S. furcata* (Beakes and Gay, 1977) -- and migrate to positions at right angles to the first division configuration (Fig. 43F). Subsequently (presumably following anaphase II and accompanying telophasic stages), the nucleus constricts a second time such that a cloverleaf results (Fig. 46), with each sinus partially enclosing a centriole (Figs. 43 G, 46). The resulting four gametic nuclei, each with a single centriole, are smaller than prophase I and interphase nuclei (Howard and Moore, 1970:332). It may be conjectured that continuing constriction of the membrane at the sinuses of the late telophase II nucleus eventually abstricts the gametic nuclei.

Phases of what were assumed to be meiotic divisions in nuclei of a few other water molds have been reported by a scattering of investigators since the pioneering work of Howard and Moore (1970). In 1976, W. L. Steffens reported that axial elements were associated with nuclei in oogonia of *Achlya recurva*; these structures eventually paired to form part of the synaptonemal complexes (see also meiosis in the antheridial cell). While Beakes and Gay (1977) did not detect a "cloverleaf" configuration (Fig. 46) in nuclear divisions in the oogonial initials of *Saprolegnia furcata*, they did observe division figures judged to be a blobbed condition of nuclei in prophase II. They also reported having located intranuclear axial elements, spindles, and cytoplasmic tubules, they noted that the spindle "poles" of metaphase II are associated with unpaired centrioles, and, as Howard and Moore found, that the spindle is oriented at right angles to the plane of metaphase I. It should be recorded that Barksdale (1968) -- using brightfield optics -- could not find any evidence of a spindle in the second division of meiosis in the antheridial cells of *A. ambisexualis*. Beakes and Gay (1977) saw no synaptonemal complexes in oogonia of *S. furcata* but the nuclear volume obviously had been decreased by the end of the division process. Although Win-Tin and Dick (1975) reported that chiasmata occurred at diplotene/diakinesis in *Achlya flagellata*, Beakes and Gay did not find any evidence for this event in their specimens (a reconstruction of chromosomes from thin sections would allow identification of chiasmata). Win-Tin and Dick also show clearly in their account of meiosis that there is a cruciform connection linking the presumed haploid nuclei in telophase II. Heath's (1976) observations on

meiosis in *Thraustotheca clavata* are consistent with those recorded in the more complete account by Howard and Moore (1970). Although Flanagan (1970) concluded that the nuclear envelope in the species of water molds he studied disappeared after prophase I, the weight of later evidence does not support this view.

An analysis by Lie and Laane (1979) of nuclear divisions in sex cell development in *Achlya radiosa* warrants particular attention. They used both a Feulgen-fluorescent methodology (Laane and Lie, 1975) and transmission electron microscopy to trace the morphology of the nuclear behavior pattern. Their study provides confirmatory evidence for meiosis occurring during gametogenesis, notably, prophase with pachytene configuration, and an increase in the number of chromatin units -- a ratio of 1:2:4 -- in, respectively, nuclei accompanying septum formation, and those in telophase I and II. During metaphase in nuclei in the sex cells of *A. radiosa* chromatin condenses symmetrically on both sides of an equatorial axis. The observations recorded by Lie and Laane agree with those described by Barksdale (1968), Bryant and Howard (1969), Ellzey (1974), and Sansome (1965), and also support the contention of Howard and Moore (1970) that the nuclear envelope persists (there is no karyokinesis intervening between meiosis I and II) until telophase II in the division process in the oogonium.

MEIOSIS IN THE ANTHERIDIAL CELL

In *Saprolegnia terrestris* antheridial meiosis precedes reduction division in the attendant oogonial initial according to Bryant and Howard (1969). Nonsynchronous companion division was confirmed by Howard and Moore in 1970, but their account of meiosis in the antheridial cells lacks detail. The most complete description of nuclear division in antheridial cells is that presented by Ellzey (1974) using preparations of induced branches in the male mating strain (E87) of *Achlya ambisexualis*.

The subcellular physical features of early meiotic stages in the male gametangium of *Achlya ambisexualis* are not unlike those of reduction division in the oogonial initial. Ellzey (1974) found axial elements (as had Howard and Moore) within the leptotene nucleus, and, in zygotene, these elements were associated with the chromatin that had condensed near the nuclear envelope. Spindle microtubules were also evident. In a later publication Ellzey and Huizar (1977) reported finding synaptonemal complexes attached to plate-like structures located at points adjacent to the inner periphery of the nuclear envelope. Bundles of microfilaments of an unknown function also have been observed in the antheridial cells of *A. ambisexualis* (Ellzey *et al.*, 1976) positioned either parallel or perpendicular to spindle microtubules. That synaptonemal complexes have been seen in some of the division figures in the sex cells of water molds provides additional evidence that meiosis is gametic.

FERTILIZATION

The subcellular events coincident with fertilization have not been explored extensively in members of the Saprolegniaceae. There is nevertheless sufficient

information at hand to conclude that the fertilization tube is a functional entity in some species. This contradicts those investigators who earlier had contended that the apparatus was nonfunctional (Chapter 9).

Howard and Moore (1970) made only brief mention of post-meiotic events in *Saprolegnia furcata*, stating that fertilization tubes occur in this species and illustrating generous vesiculation in the region of membranes separating juxtaposed gametic nuclei. The study by Beakes (1976) of *Saprolegnia* species provides visual proof that the fertilization tube develops during the period in oogenesis between the acquisition of the outer oosphere wall and the secondary or inner wall. Fusion also occurs, according to Beakes and Gay (1977), during this same period. Fertilization in some species is thus a process taking place after the initially naked oosphere has become encased in a wall.

The fertilization tube, which apparently penetrates the oogonial wall enzymatically (Heath, 1976) may contain mitochondria, one or more nuclei, plasmalemmasomes, and at its distal end, wall vesicles (Heath, 1976; Beakes and Gay, 1977). Vesicles and lomasomes also accumulate in the oosphere at the point where contact is to be made by the elongating fertilization tube. The oospore wall in this region of contact is thickened (Fig. 47), both in *Saprolegnia furcata* (Beakes and Gay, 1977) and *Thraustotheca clavata* (Heath, 1976).

Observations by Beakes and Gay (1977) on the interface region between the fertilization tube and the oosphere wall show (Fig. 45) that in *Saprolegnia furcata*, at least, the tube *per se* does not actually penetrate the oospore (*sensu* Beakes and Gay, 1978a). After contact between the antheridial tube and the oosphere is established, a thin-walled protrusion -- the penetration peg (not the same as the peg referred to by Heath, 1976: fig. 23.28) -- grows from the fertilization tube, ruptures the outer oospore wall, and pushes upon but does not at once sever and penetrate the plasmalemma (Fig. 47). It is assumed (Beakes and Gay, 1977) that the thin wall of the peg ultimately fractures at the point of contact with the plasmalemma, and the tube's content, including the gametic nucleus, is discharged into the oospore. As the thick inner wall of the oospore is deposited, the emptied fertilization peg is sequestered (Fig. 48) between the outer and inner wall where it may, in fact, take on the aspect of a lomasome.

Little is known of the physical nature of the fusion nucleus. Beakes and Gay (1977) reported in *Saprolegnia furcata* it is larger than the ordinary diploid vegetative nucleus (confirmed by Beakes, 1980d, from an emasculate *S. ferax*), and has an unusually dense fibrillar nucleoplasm.

THE SEXUAL APPARATUS IN *APHANOMYCES*

From the foregoing account, it is evident that there is a general pattern of subcellular changes in sex cell development common to species with multioosporous oogonia. Do species with unioosporous oogonia conform to this pattern? Traquair and McKeen (1980) have studied in detail the ultrastructure of the genesis of the sexual apparatus in an unidentified *Aphanomyces* (isolated from roots of *Medicago sativa* L.).

There is no peripheral positioning of dense-body vesicles in the oogonia of *Aphanomyces* sp. such as is found in *Saprolegnia furcata* (Beakes and Gay, 1978a) where these organelles are involved in multiple oospore cleavage. Fingerprint vesicles, seen for the first time in a representative of *Aphanomyces* and said to be derivatives of dense-body vesicles (vacuoles; Traquair and McKeen, 1980), fuse to form the membrane-bound reserve globules of the oospore. Lipid bodies in the developing oospore congregate toward the periphery of this cell. Traquair and McKeen did not detect any synaptonemal complexes in the developing gametangia, but did observe electron-dense, bar-like axial elements that have been interpreted as evidence of an early phase in meiosis. Thus, it may be postulated that reduction division in *Aphanomyces* sp., like that in other water molds, takes place within the developing gametangia. It is evident also that a functional fertilization tube is produced by *Aphanomyces* sp. As this tube develops it is seen to have a narrow channel in it, indicating that there is cytoplasmic continuity between the oogonium and the antheridial cell.

There is some cytological evidence (Traquair and McKeen, 1980) in *Aphanomyces* sp. that following fertilization, degeneration of unpaired, haploid nuclei occurs. As the oospore develops subsequent to fertilization, its wall becomes bipartite, but as maturation proceeds, the cytoplasm condenses, and both the oospore and oogonium wall shrink. As a result, the investing oogonium becomes noticeably wrinkled, and the oospore wall takes on a spiny appearance.

Aphanomyces sp. displays similarities in the subcellular nature of its sexual apparatus to both the Saprolegniales and Peronosporales (Traquair and McKenn, 1980). Unlike members of the latter order, however, the *Aphanomyces* does not produce periplasm in the oogonium.

GERMINATION

The refractory nature of oospores toward germination, and difficulties encountered in preparative manipulations of germinating zygotes, have hindered studies on the ultrastructural events accompanying this process. So far as we are aware, the first mention of the subcellular nature of a germinating oospore was that by Tontz (1969). She merely mentioned that there is a structural similarity between the apex of the oospore germ hypha in *Achlya imperfecta* and the filament apices in a species of *Pythium*. The ultrastructure of a germinating oospore of *A. recurva* was characterized briefly by Huizar (1978: fig. 23). The germinating zygote in this species contains a large, central vacuole, and peripheral cytoplasm in which are located the usual elements characteristic of eukaryotic cells. The pioneer studies on the subcellular features of zygote germination are attributable to Beakes (1980a-d; see Fig. 44). He investigated this process in an emasculate isolate of *Saprolegnia ferax*, finding that germination commenced after a brief dormant period of 12-240 hours.

The initial evidence (Beakes, 1980a) of oospore germination in *Saprolegnia ferax* is a thinning of the oospore wall and a decrease in the refractivity of the peripheral cytoplasm (indicative perhaps of hydration). The oospore increases in diameter, and

elaborate plasmalemma complexes appear (Beakes, 1980b). After the inner wall of the oospore is resorbed (Beakes, 1980b) a new germination wall layer is assembled. The electron-dense ooplast matrix gradually decreases in diameter, and then fragments into smaller units; dense-body vesicles increase in abundance in the early stages of germination prior to germ tube emergence. As the germ hypha develops and elongates, almost all of the organelle-containing cytoplasm in the oospore migrates into the hypha. During the continuing filament elongation the cytoplasm becomes vacuolate, and in some instances bar bodies (Gay and Greenwood, 1966) appear as terminal sporangia develop. The germling wall becomes thickened, an event accompanied by a substantial increase in the numbers of lomasomes. This is in agreement with the view of Heath and Greenwood (1970b) that lomasomes may result when vesicle discharge at the hyphal surface exceeds expansion of that surface.

Beakes (1980a) noted that there was no synchrony in germination of the oospores in a single oogonium. In most cases germ tube emergence from the oogonium takes place in the pit region, although some germ hyphae emerge through the secondarily thickened oogonial wall or through the basal septum.

In the gross aspects of oospore maturation, the pattern exhibited by the emasculate isolate of *Saprolegnia ferax* does not differ noticeably from the events recorded for *S. terrestris* (Howard and Moore, 1970) and *S. furcata* (Beakes and Gay, 1978a). The fact that germination occurred in an antheridium-deficient specimen suggests that the male apparatus has no direct influence on the subcellular changes taking place within the mature oogonium (Beakes, 1980a).

Wall morphogenesis in oospore differentiation in *Saprolegnia ferax* is very complex, and Beakes (1980b) has recognized at least 13 phases of differentiation during the formation, maturation, and germination processes: outer oosphere wall secretion, inner oospore wall deposition and subsequent digestion, and germination wall secretion, to name three. Most of the phases in wall morphogenesis are mediated by dictyosome-derived vesicles. In the emasculate isolate of *S. ferax* dictyosome abundance (and their association with ER and mitochondria) is correlated with detectable periods of vesicle-mediated wall accretion. The lowest numbers of these organelles occur in post-karyogamy oospheres and mature oospores, while the greatest numbers accompany germination stages. In the post-karyogamy oospheres, for example, Beakes found that 70% of the dictyosomes were unassociated, while in the germlings nearly the reverse was true with only 19% unassociated.

Beakes (1980b) consistently found that microbodies and vesicles accumulate in the germ hypha cytoplasm prior to its penetration through the oogonium wall. The role of these structures in the filament's exit is not known, but conceivably some enzymatic activity is at least in part involved. Since in this emasculate isolate of *Saprolegnia ferax* the oospore wall structure does not change during dormancy it may be expected that dormancy is controlled from some entity within the cytoplasm.

In a third paper in the series on oospores, Beakes (1980c) explored the changes in cytoplasmic organelles during the formation, maturation, and germination of the zygotes. During oosphere differentiation in *Saprolegnia ferax* there is a steadily

increasing accumulation of lipid and lipo-phospho-glucan complexes (dense bodies), but not of other organelles (Beakes, 1980c). As maturation progresses, neutral lipid reserves are significantly reduced, there is a coalescence of dense-body vesicles, and a substantial decline in the abundance of most other organelles. At the onset of oospore germination, the osmiophilic ooplast matrix (dense-body vesicle-derived) disintegrates, and granules (Beakes refers to them as dense body-like) are released into the developing central vacuole and into vesicles in the peripheral cytoplasm. As Beakes (1980c) has pointed out, such cytoplasmic changes as the accumulation of neutral lipid and dense-body reserves, and a decline in the abundance of some organelles argues for the supposition that metabolism in the dormant oospores may well be held in check to some extent, and thus these zygotes are able to conserve endogenous reserves for germination. Attention is called at this point to J. Fletcher's (1979a) discovery that calcium concentration is associated with volume density increases in some organelles of *S. diclina* during oogenesis.

What subcellular events associated with nuclear behavior accompany oospore germination in the water molds? Beakes (1980d) has also provided the only substantive account of this subject (for the single, emasculate form of *Saprolegnia ferax*).

In *Saprolegnia ferax*, meiosis in the oogonial nuclei is synchronous through the first division but is then lost. Beakes (1980d) was unable to detect any well-defined synaptonemal complexes. Following oosphere delimitation, the fusion nuclei appear; these are pyriform organelles with a sparsely fibrillar nucleoplasm. The volume of the oosphere fusion nuclei is more than twice that of interphase nuclei, even though it would be expected that the DNA content is the same. Perhaps, as Beakes suggests, there is not always a correlation between nuclear volume and DNA content.

Prior to germ hypha emergence from oospores of the emasculate *Saprolegnia ferax* three or four nearby synchronous mitotic divisions occur, just as Trow (1899) had found for his variety *cambrica*[†] of *Achlya americana*. The volume of mitotic nuclei in *S. ferax* was approximately half of that of the meiotic ones. One of the early events in oospore germination, at least in the form Beakes investigated, is a series of nuclear divisions that are similar to those described in somatic hyphae by Heath and Greenwood (1976c). In some germinating oospores Beakes noted abnormally large nuclei having lobed profiles; he speculated that these might have been polyploids.

Evidence from Beakes' (1980d) study suggests that the sexual pattern in the antheridia-less *Saprolegnia ferax* probably was automictic (amictic pattern, according to Dick, 1972). In any case, the gross nuclear behavior in the sexual cycle in the isolate is virtually identical to that described by Howard and Moore (1970) for *S. terrestris*, and by Ellzey (1974) for *Achlya ambisexualis*.