

CHAPTER 7. Morphogenesis: The Sporangium

Eventually in the growth of hyphae of most water molds, primary sporangia are produced, followed by secondary ones formed either in a percurrent (S. J. Hughes, 1971b), sympodial, basipetal, or cymose fashion. Spore initials are delimited within the sporangium where they subsequently mature, and then are released (the discontinuous phase of growth according to Marchant, 1979). Rioux and Achard (1956) refer to the planonts and encysted spores of *Saprolegnia diclina* as planoconidia and cystospores, respectively, but this seems unnecessarily duplicative in view of the universal understanding that has emerged for spores of the Saprolegniaceae.

GROSS SPORANGIUM MORPHOLOGY AND DEHISCENCE

By the early 19th Century, sporangia had been observed on water molds, but considerable differences of opinion surrounded their interpretation. To Unger (1844), sporangia of "*Achlya proliferata*" (a species of *Saprolegnia*) were simply meristematic cells created by the formation of a septum. He described the rupture of the end of the "conceptacle", and the release of spores, and also noted empty cysts and internal proliferation. The released "germes", Unger believed, transformed into infusoria-like animals under favorable conditions. That he compared spores to infusorians is not at all unusual; Stilling zu Cassel (1841) thought that the "granules" (very likely they were spores) in *Achlya* hyphae were the eggs of such organisms. Areschoug's (1844, 1845) account of "*Achlya proliferata*" differs little from that of Unger. Earlier, Hannover (1839, 1842) published the first reasonably accurate description of spores and sporangia of a water mold on dead frogs and salamanders. He illustrated internal proliferation and an early stage in sympodial branching, described spore discharge through what he believed was an elastic orifice, noted that the spores were motile, and in time encysted. Hannover maintained that the spores and sporangia of the "Conferva" formed only in water and never displayed any aerial development. Curiously, De-La-Rue (1869), Fückel (1869), H. Hoffman (1867), and Bail (1860) evidently were unaware of Hannover's earlier publications as they described how water molds grew into terrestrial fungi. Schleiden (1842) made two pertinent observations on a fungus he identified as *A. proliferata*. First, he saw that the fungus had two kinds of "spores", large ones in spherical "sporangia" (oogonia, very likely), and smaller ones in filiform, terminal "joints". Second, he observed that in these terminal regions, spores vibrated for a short time before a minute operculum (!) was detached; the cells that did not escape germinated directly in the old "utricle", (the *utriculus matricialis* of Meyen, 1831). Some of the empty hyphal tips then became nearly filled with a new filament apex growing directly into them (Schleiden, 1842).

It is to Meyen (1839) that the first description of a dictyosporangium can be credited, although his terminology is quaint by current standards. Within the "fruit wall" (sporangium) the spores of Meyen's fungus (it cannot be identified) were produced in individual "mother-cells" (cysts). Moreover, he noted, the "mother cell"

wall and that of the sporangium were so delicate that the “germ filament” of the spore broke through them; this surely describes germination *in situ*.

The “germ cells” (spores) of *Saprolegnia capitulifer* (an *Achlya*) were not “born”), A. Braun (1851) said, until they left the “fruit clubs” (sporangia) and acquired motion. As the spores emerged, they clustered in a globular “capitulum”, and then each escaped from this mass leaving behind a membranous coat. Without a doubt Braun dealt with a mixed culture of species since he saw both achlyoid and saprolegnoid discharge and proliferation. Although Cohn (1853) identified a watermold found on individuals of *Daphnia pulex* as A. Braun’s (1851) species, one of Cohn’s specimens may have been an *Aphanomyces*, as is suggested by the substratum. As Meyen had discovered, Cohn also noted that spores on release would leave behind their “membranes” (cyst walls) to give the sporangium a parenchymatous aspect (dictyuroid). Cohn, too, derived his observations from a mixture of specimens. A most curious type of sporangium was described (but not illustrated) by Radais in 1898. He collected from bog drains and in water pipes a fungus which he identified as *Saprolegnia thureti*[†]. Secondary sporangia arose by internal proliferation, but protruded through the orifice of the primary sporangium and then branched profusely. Thus, a cluster of filaments (“arbuscule”) extended out from the apex of the primary sporangium. Radais thought these tufts of branches represented a last effort to “vegetate” by a poorly nourished plant that was unable to sporulate. He evidently saw typical dictyuroid discharge as well.

The extreme in the misunderstanding of the nature of sporangia in the family must surely be attributed to Nägeli (1844, 1846). He is alleged to be the first to recognize that spores formed around definite “centers” (nuclei). He applied this concept of free cell formation to the sporangia of *Achlya prolifera* (*Saprolegnia* sp.), but his accounts show beyond doubt that the “free” spores formed by this fungus were olpidioid sporangia (possibly of an *Olpidiopsis*). In 1880, A. Fischer recognized Nägeli’s “free cells” for what they were, but strangely Harper (1899) did not seize upon this error in summarizing Nägeli’s observations. Cienkowski (1855) also described the thalli of a holocarpic, olpidioid fungus as sporangia of *Achlya prolifera* (possibly a species of *Saprolegnia*), and evidently was not even aware that Nägeli previously had reported similar ones.

As knowledge of the Saprolegniaceae accumulated through the early part of the 20th Century, emphasis was devoted to variations in sporangia. Lechmere (1911b), for example, went to considerable length to describe three patterns of secondary sporangium morphology in *Saprolegnia thureti*. The first of these was the basipetalous mode, in which the successive secondary sporangia nested entirely within the primary one. At renewal, some sporangia developed a superimposed (Lechmere’s term) configuration: each succeeding sporangium emerged part way through the orifice of previously formed ones, giving an externally beaded appearance to the hyphal tip. In the third configuration of renewal, successive sporangia emerged part way through ones earlier delimited, but in no case did any emerge through the primary sporangium. Thus, the hyphal apex was “beaded” internally. These variations so painstakingly

described by Lechmere (1911a, b), and much later (1937a) mentioned by Wolf, are commonly encountered among members of the genus *Saprolegnia*.

Other studies in which sporangium variation was specifically emphasized are those by Höhnk, Salvin, and T. W. Johnson. Höhnk (1957) described one growth form of a saprolegnian (in mesohaline waters) in which the sporangia produces spores of several sizes; these cells either germinated *in situ* or escaped upon partial dissolution of the confining wall. Sporangium size and shape in a presumed species of *Brevilegnia* were so variable, Salvin (1942a) noted, that generic limits between his genus and *Thuaustotheca* were essentially negated. In another isolate of *Brevilegnia* T. W. Johnson (1950a) found sporangium morphology also to vary (length and shape, particularly), but not to the extent that generic boundaries were dimmed. Experimental culture manipulations to vary sporangial morphology -- that by Salvin (1942b) most notably -- is treated in a subsequent chapter.

SPORANGIUM DEVELOPMENT

A substantial body of literature has accumulated on the subject of morphogenesis of the asexual stage of various Saprolegniaceae. Part of that literature deals with subcellular aspects of sporangium development, but only in a small sampling of species (Chapter 14).

Most of the accounts that detail sporangium morphogenesis narrate remarkably similar observations of the various stages in the process (Fig. 12). The following papers are the most informative on this subject: de Bary (1884; various species), Bhargava (1950: *Isoachlya anisospora* var. *indica*†, Büsgen [1882: *Thraustotheca* (as *Dictyuchus*) *clavata*] M. C. Carlson (1920: *Saprolegnia* sp.), Coker and Hyman (1912: *T. clavata*), J. N. Couch (1927: *Brevilegnia subclavata*), P.-A. Dangeard (1890-91: *S. thureti*†), B. M. Davis (1903a, b: *S. mixta*†), Dayal (1961a: *Achlya aplanes* var. *indica*†), Hartog (1888a, 1895), Häyrén (1954: *S. dioica*†), Hine (1878: *Saprolegnia* sp.), Höhnk (1933: *S. torulosa*), Humphrey (1893), Lechmere (1910: *Saprolegnia* sp.), Leitgeb (1869-70: *Dictyuchus* species), Moreau and Moreau (1938: several species), Murdia (1939a: *A. dubia*), Pringsheim (1851: *S. ferax*), Rothert (1890: *S. monoica*†, *S. thureti*†, *S. monilifera*†, *A. polyandra*†), Schrader (1938: *T. clavata*), Schwarze (1922: *Saprolegnia* sp., *Achlya* sp.), Setzer (1941: *Isoachlya anisospora*†), W. L. Steffens (1976: *A. recurva*), Strasburger (1880), Thuret (1850: *S. ferax*), Unger [1844: *Saprolegnia* sp. (as *A. prolifera*)], H. M. Ward (1883: *A. polyandra*†, *A. apiculata*, *S. monoica*†), Weston (1917: *Achlya* sp., 1918: *T. clavata*, 1919: *Dictyuchus* sp.).

GENERAL PATTERN OF FORMATION

Sporangia are initiated when protoplasm streams toward the hyphal apex and accumulates there. This is a feature common to most water molds, including species of *Aphanomyces* (de Bary, 1860; Rothert, 1903, Sorokine, 1876), *Leptolegnia* (J. N. Couch, 1924a, A. C. Matthews, 1932), and *Geolegnia* (J. N. Couch, 1927, J. V. Harvey (1925a, b) in which sporogenesis differs in some particulars from the general pattern in members

of *Achlya* and *Saprolegnia*. In the usual cases of morphogenesis a central vacuole is visible (H. M. Ward, 1883, denied this for *Saprolegnia* sp.) in the hyphal tip even before the sporangium is delimited by a septum.

Septum Development:

Following the accumulation of cytoplasm apically, a cross wall separates the sporangium initial from the parent hypha. Very little is known of this early developmental process, but the reports from those who have tried to detail it (Dayal, 1961a; Hartog, 1888a; Murdia, 1939a; Setzer, 1941; Weston, 1918) agree that a transverse, hyaline zone appears in the cytoplasm at the septum site prior to the materialization of the wall itself. According to Häyrén (1954), who studied development in *Saprolegnia dioica*[†], the septum begins as a ridge barely visible on the inner periphery of the hypha; within two minutes the expanding wall cleaves the cytoplasm. Earlier, Setzer (1941) reported -- incorrectly -- that the septum delimiting a sporangium of *Isoachlya anisospora*[†] formed simultaneously across the entire diameter of the hypha rather than in a centripetal fashion. In the immature sporangium "microsomes" appear in the cytoplasm in the vicinity of the septum, but are fewer on the sporangium side of the septum than on the hyphal side (Häyrén, 1954). Rothert (1890) reported that cellulose bodies accumulated in the region where the cross wall was to form then nongranular protoplasm ("hyaloplasm") appeared, and below this region (Weston, 1918) the septum developed. These granules, Rothert contended, were a soluble form of cellulose, that, when absorbed onto the hyaline disc, became the foundation on which the septum was built. After the septum was complete, the cytoplasm in the sporangium moved toward the crosswall (Rothert, 1890) and forced it toward the hyphal base into a concave configuration.

Only Hartog (1888a) seems to have paid serious attention to changes taking place at the sporangium apex during morphogenesis. Before the septum was formed (or sometimes during spore cleavage) a "process" developed, usually apically, on the sporangium wall. Hyaline, nongranular cytoplasm (a clear area) appeared just below the hyphal wall, and at that point pushed outward, Hartog stated, to create a short, cylindrical protrusion, the apical papilla. This bulge was filled with "hyaloplasm", but Rothert (1890) found that as differentiation proceeded the hyaline substance gave way to protoplasm that then filled the papilla. In 1976, W. L. Steffens called attention to the fact that apical papillae developed on sporangia of *Achlya recurva* at about the same time that septum formation was occurring, but in any event before sporogenesis began. Waterhouse (1962) regarded the exit papilla as a differentiated part of the sporangium wall. Hartog's observations (1888) appear not to have been expanded or improved upon by subsequent work.

Primary Sporogenesis:

Some time after the basal septum is formed -- Humphrey (1893) said it was within 20-30 minutes -- the first evidence of sporogenesis appears. Beginning at the tonoplast, clear cleavage lines push out toward the plasmalemma and become successively more prominent, thus delineating uninucleate clumps of cytoplasm. Bhargava (1950) demonstrated centrifugal cleavage by staining the central vacuole of the sporangium initial with a vital dye. As cytoplasmic segmentation proceeded, the dye flowed outward toward the wall. Subsequently, these fissure lines --- extensions of the tonoplast -- contact the plasmalemma and fuse with it to delimit polygonal units in the sporangial cytoplasm (Fig. 12). As the partitioning process advances, the basal septum in sporangia of most species curves upward slightly (convex toward the sporangium apex) just as Höhnk (1933), Humphrey (1893), and Schwarze (1922) had seen in the water molds they studied. On the other hand, J. V. Harvey (1925a) found that in young sporangia of *Geolegnia septisporangia*, the septum does not bend.

The manifestations of sporogenesis puzzled early mycologists, but not to the extent that they were necessarily cautious of their explanations. In a very early account of spore development, A. Braun (1851, 1853) described the young sporangium of *Saprolegnia ferax* as a structure filled with a granular mucilage surrounding what he thought was an internal cavity (vacuole). The parietal mucilaginous layer became sinuous, he said, the resulting furrows cut successively deeper, and the "mucilage", was cut into hemispherical masses that rounded up to become the "gonidia" (spores). Subsequently these were expelled from the "mother tube". Rothert (1890) saw cytoplasmic segmentation as the result of vacuolar swelling, and maintained that cleavage lines occurred all at once rather than slowly becoming successively more prominent. To Unger (1843, 1844), the clear strands between spore initials were a gelatinous matrix that contributed to cleavage, while Thuret (1850) believed the process to be just the result of cytoplasmic coagulation at many points.

According to Hartog (1888a) cleavage in normal sporangia began at the "protoplasmic investiture" (plasmalemma) where fractures developed and proceeded toward the vacuole until they opened into it. Thus, in his view (1886-87), protoplasmic clumping preceded vacuolar extension. Hartog also (1888a) observed, however, that in "poor sporangia" (those having a very large, central vacuole), the protoplasm simply heaped up, and concomitantly thinned out between the clumps. In any case, as the protoplasmic centers condensed they excreted (Hartog, 1895: 673) a liquid into a "...network of splits..." that separated the spores.

The account by B. M. Davis (1903a, b) of sporogenesis in *Saprolegnia mixta* does not differ in essential details from those of most other mycologists of the time. He contended that when the cleavage furrows reached the sporangium wall turgor was "relieved" through loss of water, and the spore origins in effect collapsed on one another. It appears, however, that Davis' chief concern was to point out that sporogenesis was unlike oogenesis because in the former process there was no coenocentrum. What Davis referred to as a coenocentrum, of course, was clusters of astral rays. Indeed, as Heath and Greenwood (1970c) have demonstrated so adequately by electron microscopy, Davis was wrong on yet another point: there are astral rays

associated with the centioles adjoining each nucleus in sporangia of *Saprolegnia* species.

Several other concepts of spore development have been expressed. While Häyrén (1954) agreed that spore cleavage began at the tonoplast, he thought that hyaline cytoplasm formed around each initial, and H. M. Ward (1883) observing cleavage in *Achlya polyandra*^t concluded that there was no membrane around the spore initial, only a “watery substance”. The spores of *Saprolegnia* sp., M. C. Carlson (1920) said, were delimited by a coalescing vacuolar ring that formed circumferentially at some distance from each nucleus. Even de Bary’s views (1884) of sporogenesis in the water molds differed radically both from those of his contemporaries and his successors. He contended that the spores were delineated by partitioning plates made up of rows of soft, homogeneous, hygroscopic, granular material. Berthold’s (1886) belief that spores in the water molds appeared through free cell formation was, like de Bary’s idea, somewhat removed from actuality. According to Berthold, sporangial protoplasm simply accumulated around vacuole centers, the resulting heaps continued to enlarge, pushed out into the central vacuole, and then subsequently contracted from the sporangium wall. The position of the spores in the sporangium was said to be determined by the number of new centers of attraction toward which the protoplasm was drawn from its peripheral position. Sporangium formation and oogonium maturation, Berthold concluded, were identical processes in which three steps occurred: separation of the protoplasm to a circumferential location and the vacuole to a central one, disjunction of the peripheral wall of protoplasm from the sporangium wall, and the subsequent division of that protoplast into a number of conglomerates which gradually disassociated from one other. Others, such as H. M. Ward (1883), also thought that spore cleavage occurred at many centers in the sporangium.

Whether there is nuclear division in the sporangium initial before sporogenesis commences, as de Bary maintained (1884), or not (Bhargava, 1950) is an unsettled point. Nuclei in the hyphae of *Leptolegnia* sp. divided, J. N. Couch (1924a) reported, prior to the delimitation of the sporangium; subsequently some of the nuclei disintegrated. On the other hand, B. M. Davis (1903a, b) had reported that there were no nuclear divisions in the sporangium. The cytological evidence for the opposing views is treated in Chapter 12.

The Homogeneous Stage:

At some point after the sporangial protoplast cleaves into polygonal units, these incipient spore initials suddenly disappear (bright field optics). Most investigators of sporogenesis in the Saprolegniaceae, save primarily for M. C. Carlson (1920), agree that there is such a homogeneous stage, but there is wide disparity in resulting explanations. Some of the diverse viewpoints put forth to explain this phenomenon follow.

De Bary (1884) merely touched upon the homogeneous stage in sporangium maturation, saying that the partition plates between spore initials simply disappeared. Two years earlier, on the basis of some observations on *Dictyuchus clavatus*^t, Büsgen (1882) stated that the transitory cell plates separating the initials were absorbed but

later became converted into an “expulsive substance”. In effect, Büsgen contended that the first formed spore initials simply fused. According to H. M. Ward (1883) a watery substance was secreted by the first-formed spore initials and this served to separate them. When the liquid was absorbed, the homogeneous stage resulted. Somewhat later in development, he asserted, the spores went through a “... more energetic separation ...”, and thus the second cleavage came about.

It was Rothert (1888, 1890) who gave the first full analysis of the homogeneous stage in sporogenesis, and attempted to explain it. He advanced the view that disappearance of the spore initials occurred when they expanded because of sudden changes in the vacuole (his “primordial utricle”) and the plasmalemma (Wandbeleg). As long as these membranes were intact, Rothert asserted, there could be no transfer between the sporangial cavity (vacuole) and the surrounding water. When the vacuolar membrane stretched during first stage cleavage, the “utricle” broke, and fluid escaped to the outside (it was attractive to bacteria, Rothert claimed). The spore wall, which had been distended as the result of internal pressure resumed its natural position when liquid left the sporangium, and hence the entire structure became smaller. The sudden upward curvature of the basal septum during the homogeneous stage, Rothert argued, was evidence of turgor loss (Setzer, 1941, reached the same conclusion in her study). Hartog was somewhat vague in explaining (1888a) the homogeneous stage, but he too, thought that cell sap must escape at this time (it could not do so as long as the protoplasmic “investiture” was intact); Schwarze (1922) agreed. When this enclosing membrane ruptured, Hartog proposed, a segment of it formed around each spore. Indeed, he stated unequivocally that he observed a fragment of membrane between two spore origins split and separate such that each spore acquired half of the membrane. Thus, Hartog’s theory (1886-87, 1888a, 1895) that plasmolysis led to the homogeneous stage was not unlike that of Rothert. Hartog (1895) went further, however, because he contended, in opposition to Rothert, that the polygonal initials fused with one another during the homogeneous stage. Hartog explained that as cell sap escaped from the central vacuole, water entered, vacuoles were induced in each of the initials, and the “spore origins” swelled up and fused together.

Murdia (1939a) held that the homogeneous stage in *Achlya dubia* came about as Rothert believed, that is by rupture of a peripheral protoplasmic membrane. Humphrey (1893) also favored Rothert’s explanation, emphasizing that the accompanying sudden upward curvature (toward the apex) of the basal septum, and flattening of the end of the apical papilla were evidence of turgor loss. Weston (1919) found that the sporangia of *Dictyuchus* sp. shrunk at the homogeneous stage and the cytoplasm became vacuolated. The decrease in sporangium size in *Thraustotheca clavata*, Weston suggested (1918), resulted from the sudden release of cell sap in the vacuolar clefts between the polygonal spore initials. Working with the same species R. K. Saksena and Bhargava (1946) also detected shrinkage in the sporangium at the time of the homogeneous stage in spore cleavage. They attributed the size change to rupture of the vacuoles and the expulsion of liquid --“cell sap” they termed it -- adjacent to the

sporangial wall. Once the second cleavage began, some of the cell liquid was again absorbed into the cytoplasm. It may be recalled that these two investigators, as well as Bhargava (1950), saw vital stain in the sporangium vacuole flow outward in strands as the polygonal spore units were cleaved. At the onset of the homogeneous stage the dye disappeared from the sporangium and later reappeared in the vacuole of each discharged spore.

Gay and Greenwood (1966) demonstrated that the homogeneous stage in *Saprolegnia ferax* is accompanied by collapse of the discharge papilla, a change of the septum from a concave to a convex attitude toward the sporangium apex, a ten percent decrease in sporangium volume, and swelling of the incipient spores. These changes parallel in striking fashion the principal ones recorded by Rothert (1888, 1890) and Hartog (1888a) nearly eighty years earlier. By way of explanation of these physical changes in the sporangium at this stage in sporogenesis, Gay and Greenwood hypothesized that turgor loss was the result of leakage of vacuolar sap. Concomitantly, the incipient spores, at that point lying free in the sporangial cavity after the plasmalemma and tonoplast had fused absorbed water and expanded.

Secondary Sporogenesis:

It is obvious that the homogeneous stage has neither been characterized nor explained in its entirety. That it is accompanied by turgor loss (Hartog, 1886-87, argued that the reduction in turgidity was the result of the homogeneous stage and not its cause) and the appearance of numerous small vacuoles seems established, but the details are obscure. In any event, this step in sporogenesis is followed by the reappearance of spore initials which successively become more distinct as they round up within the sporangium. Little is known of this phase in development since it seems to have been noted only in passing, as it were, and its features still remains unexplained. Humphrey (1893), for example, simply said that the spores contracted. He and Hartog (1895) agreed that the lines of the second cleavage were in the same position as the ones formed prior to the homogeneous stage, and that the number of spores was not increased between the first and second cleavage (*see* Chapter 14).

During what he called the second contracting phase of the spores (*Achlya* sp. and *Saprolegnia* sp.) Schwarze (1922) argued that there was further loss of turgor. Harper (1899) had proposed earlier that furrowing of the sporangial cytoplasm was comparable to the rupture of a drying colloidal mass. Schwarze accepted this comparison for the cases at hand, and also agreed with Harper's contention that desiccation was less in the vicinity of nuclei than in other parts of the cytoplasm. By way of explanation, Schwarze suggested that nucleic acids had a greater affinity for water than did the surrounding cytoplasm. Häyrén (1954) decided that the change between the polygonal and the spherical spore stage was the result of water loss and consequent rounding up of the spores. The observations by Dayal (1961a) on a variety of *Achlya aplanes*[†] are in part diametrically opposed to earlier accounts. He discovered that the polygonal spore

initials did not take up a stain as they formed, but the second cleavage spores clearly did so.

Spore Emergence:

Within one-half to several hours (depending on culture conditions) after their appearance in the sporangium the spherical spores (second cleavage) begin a slight movement. This motion increases in intensity, the terminal exit papilla dissolves (in some cases more than one functional exit apparatus is produced), and the spores escape. The first detailed description of the discharge process seems to have been that of Thuret, in 1850, although Hannover (1839, 1842) a few years earlier, had observed motile spores. In the case of *Saprolegnia ferax* spores near the sporangium apex began a feeble motion, Thuret observed, and then a small apical protuberance appeared and ruptured. The first cells to escape moved extremely rapidly he found, and this phenomenon has been confirmed many times since in species of *Saprolegnia*. Thuret also described spore shapes: the emergent spores were "turbinate" (pyriform) and biflagellate, and, he thought, closely resembled monadines and infusorians.

Other than Thuret's report, only that by Hartog (1888a) comments to any extent on the "structural" changes in the sporangium at the moment of discharge. According to Hartog spore emergence could occur in one to three ways (he of course maintained that the spores of *Achlya* species were flagellated on discharge). In some cases, the apical spore pushed against the papilla wall, the wall dissolved and the cell emerged. In other instances, Hartog said, the sporangium wall disintegrated prior to any contact with a spore, or the papilla apex was pushed off like a lid (operculate!). This latter mode has not again been observed although the sporangium apex dehisces in species of *Calyptralegnia*. Spore discharge mechanisms are considered in Chapter 8.

VARIATIONS IN SPORANGIUM DEVELOPMENT

The general pattern of spore release just described can vary as, for example, in species of *Dictyuchus* where the individual planonts emerge from cysts that persist within the sporangium. In fungi assigned to *Brevilegnia* and *Thraustotheca* the sporangium wall ruptures or dissolves to release encysted primary spores. Moreover, there are variations among the watermolds in some aspects of spore formation itself.

Aphanomyces species: -- de Bary (1860) first described spore cleavage in members of this genus. He observed that during sporogenesis the protoplasm in the sporangium segregated into cylindrical segments with vacuoles between them, and each spore initial became separated from the sporangium wall by hyaline protoplasm. As the cylindrical spore initials moved apart the cytoplasm between them collapsed, de Bary said, to form only a thin, connecting thread. An alternate view is that proposed by Scott (1961a). He believed that the separation of cylindrical initials in *Aphanomyces* sporangia is accomplished by thin, transverse bands of hyaline protoplasm that

function as cleavage planes. In any case, sporogenesis occurs rapidly in species of *Aphanomyces*, and there is no homogeneous stage. Prior to discharge the spore initials contract to short, cylindrical segments (with rounded ends) or even spherical bodies (Scott, 1961a). The connecting strands of protoplasm disappear during this condensation.

Sorokine (1876) described the spore initials in *Aphanomyces stellatus* as being fusiform, and lacking protoplasmic connection between them, but he was principally occupied with the fact that in his fungus spore behavior was often like that in *Dictyuchus* or *Aplanes* (as that genus was then circumscribed). Segmentation of the sporangium cytoplasm (a central vacuole is absent in the sporangium initial in species of *Aphanomyces*) was accomplished, Hartog (1895) decided, by the appearance of elongate vacuoles compressing the cytoplasm into patches, a view paralleling the earlier one of de Bary (1860). Rothert (1903) regarded the spaces between spore initials in the sporangia of *Aphanomyces* sp. as mere openings left by a contracting ring-like segment of protoplasm; the protoplasmic membrane subsequently collapsed to form connecting strands between adjacent spores. In contradiction of both de Bary (1860) and Rothert (1903), J. N. Couch (1924a) believed there was no gelatinous or protoplasmic material between the spore initials and the sporangium wall in *Aphanomyces* specimens. However, Couch did agree with Rothert on the nature of the connecting strand between initials: these were remnants of the collapsed protoplasmic membrane.

Leptolegnia species: -- Only J. N. Couch (1924a) has recorded any substantial observations on sporangium and spore development in members of *Leptolegnia*, although A. C. Matthews (1932) described some aspects of sporogenesis in connection with flagellation in *L. caudata*. In species of this genus, the spores are in a single row in the sporangium, and are elongate on emergence; they do not cluster at the orifice as in *Aphanomyces*. The following account is taken from Couch's study, supplemented by our published observations.

The sporangium initial in specimens of *Leptolegnia* has parietal protoplasm and a central vacuole. At the onset of spore formation, enlarged masses of protoplasm collect in two rows adjacent to the sporangium wall (as seen in optical section), but connected by thin cytoplasmic strands. Subsequently, the parietal protoplasm loses its segmented appearance -- the homogeneous stage -- and migrates to the center of the filamentous sporangium as an axial "ribbon". The spore initials now begin to contract along this column, and migrate to align themselves in a single row. As a group, the developing spore initials move toward the sporangium apex, and then recede. As a result of this shift in position spaces are created between each incipient spore. A parietal membrane connects the young spores in such a fashion as to give to them the appearance of having oppositely-directed polar flagella. Just before discharge, the spores swell, become nearly cylindrical, and push against one another. The apical spore in the row is thus forced firmly against the sporangium apex which suddenly gives way, and the motile cells rush out. Spore behavior on emergence is treated in Chapter 8.

Geolegnia species: -- The two known species of *Geolegnia* do not produce planonts, and although there are basic similarities in the process of sporangium and spore development in the two, there also are differences of some magnitude. Knowledge of sporangium development in the genus comes largely from J. N. Couch's study of 1927, but J. V. Harvey's (1925a) earlier brief descriptions of sporangium and spore development do not differ substantially. In the early stages of sporangium delimitation in both *G. inflata* and *G. septisporangia* there is a central vacuole and parietal protoplasm, and during sporogenesis a homogeneous stage occurs.

A hypha of *Geolegnia inflata* that is to produce a sporangium becomes inflated at intervals, a basal septum is formed, and the protoplasm in the bulbous regions aggregates into several dense, parietal clumps as the central vacuole pushes out toward the inner periphery of the wall. These protoplasmic masses, J. N. Couch (1927) suggested, were homologous to the polygonal spore initials in sporangia of *Achlya* and *Saprolegnia* species. Shortly, the incipient spores fuse, vacuoles appear in the cytoplasm, and the sporangium becomes almost homogeneous internally. There then follows a period in which the protoplasm -- with considerable active motion -- condenses in each of the inflations (*see* systematics section) of the sporangium. As condensation proceeds, the cytoplasmic strand connecting two adjacent clumps of protoplasm becomes successively thinner, and finally ruptures as the two spore initials pull further apart and round up. The spores resulting from cleavage are multinucleate, a condition quite unlike that in species of other genera in the family.

In *Geolegnia septisporangia*, the incipient sporangium displays parietal protoplasm and a large, central vacuole, but because the protoplasm continues to stream into the initial prior to septum formation, it soon obliterates the vacuole. The basal septum forms and a large, irregular vacuole appears, surrounding a central core of protoplasm, that may cleave into two or more parts. The homogeneous stage then occurs, and subsequently "second" spore initials materialize, their number being the same as that of the protoplasmic clumps prior to the homogeneous stage. At first the initials are faintly delimited, but they become increasingly distinct as they round up into uni- or multinucleate spores.

Sommerstorffia spinosa: -- This species (Arnaudow, 1922-23, 1923) is not typically saprolegniaceous because its thallus consists of delicate, rather poorly developed, tubular, sparingly-branched hyphae that are largely confined within parasitized rotifers. In its spore discharge pattern, however, *S. spinosa* resembles *Achlya* species and (because of the filamentous nature of the much-prolonged sporangium exit tube) members of *Aphanomyces*. Up to a point, at least, sporogenesis in *S. spinosa* also relates the genus to the Saprolegniaceae. Karling (1952) gives the only complete account of development in the species; Sparrow's (1929) treatment is largely taxonomic.

The young sporangia (part of the lobed, endogenous thallus) are highly vacuolate, and possess a central vacuole that extends out into each of the lobes (Karling, 1952: fig. 1B, C). Spore delimitation is usually centrifugal, with localized extensions of the central

vacuole pushing out into the parietal protoplasm. This certainly is typical of saprolegniaceous fungi in general. However, cleavage is sometimes centripetal in *S. spinosa*, a condition that is not characteristic of other water molds. Sporogenesis evidently proceeds without an intervening homogeneous stage, also unlike the development pattern common to most other members of the family.

PHYSICO-CHEMICAL ASPECTS OF SPORANGIUM DEVELOPMENT

Several environmental factors accompanying the culture of water molds influence the initiation of sporangium development as well as its rate and duration. This aspect of morphogenesis is reviewed comprehensively elsewhere (Chapter 19), but some indicative remarks are in order at this point.

Cornu (1877a) was of the opinion that oxygen was a major factor controlling sporogenesis, but other investigators did not agree. One of the early studies -- reasonably complete but by modern standards crude -- on the influence of nutrition on sporangium development was that published by Götze in 1918. He divided the maturation process into four stages: formation of the clavate end to the hypha, cytoplasm accumulation and septum development, spore cleavage in which the units were tightly appressed, and a final stage of well-developed adjoining spores within the intact sporangium. He demonstrated that particular concentrations of meat extract in the medium could curb these stages. For example, the second stage in sporangium formation occurred normally in meat extract at 0.75%, but was prevented from further change by increasing the extract concentration to 1%.

The influence of external factors on sporogenesis has since Götze's time been much more closely examined and precisely identified. Using a replacement culture technique with *Achlya* sp. Griffin (1966) demonstrated the importance of electrolytes on sporangium (and gemma) differentiation. Calcium (0.1 mM) is required for a sporangium to be differentiated on a hyphal tip. In cultures containing 0.01 mM CaCl_2 the characteristic spore ball at the exit orifice is not formed and some of the primary spores swim away sluggishly instead of encysting immediately upon discharge. EDTA alone (in concentrations as low as 0.001 mM) is said to prevent sporangium formation in *Achlya* sp., but in the presence of CaCl_2 this inhibitory effect is nullified. Calcium is required for all stages of production and discharge of spores, and salts of magnesium, sodium, or potassium cannot substitute for it in its role in sporangium induction (Griffin, 1966).

O'Day and Horgen (1974), among others, have found a temporal sequence of morphological differentiation in hyphal tips of *Achlya bisexualis* exposed to the sporangium-inducing influence of calcium ions. The sequence of events leading to sporulation is as follows. At zero hour, the hyphal tip is subulate, a shape typical of purely vegetative filaments in most water molds. Hyphae of colonies 2-3 hours old become rounded at the apex, and during the third to fourth hour of age cytoplasm concentrates in the tip. In the age period of 4-4.5 hours, the septum is formed, and between 4.5 and 6 hours, spore differentiation is completed, and discharge effected.

Thus, there appear to be six recognizable stages in sporangium formation in *A. bisexualis* and also in an *Achlya* sp. studied by Timberlake *et al.* (1973). Moreover, protein and nucleic acid content of developing sporangia can be related to the sequential pattern of differentiation. Using labeled exogenous amino acids, Timberlake and his associates found that the maximum rate of uptake occurred approximately two hours after sporangium induction began, and the maximum rate of incorporation at about 3.5 hours. During the first hour after induction the rate of protein synthesis increased to a level twice that in hyphae at zero hours. Subsequent to this surge in amino acid uptake during the first hour after sporangium induction, protein turnover and degradation maintained a high and constant level. During cytoplasmic differentiation (sporogenesis) the acid-extractable amino acid content of the vegetative mycelium remained constant.

Water potential is extremely influential in sporangial morphogenesis in *Aphanomyces euteiches*, as has been well demonstrated by Hoch (1972) and Hoch and Mitchell (1973). They measured the water potential of mycelium of this fungus ($\psi\pi$; in energy units, 1 bar = 10^{-6} dynes cm^{-2} , or about 0.98 atmospheres) submerged in solutions amended with various osmotica such as sugars, salts, and polyethylene glycol. While the $\psi\pi$ of the mycelium of *A. euteiches* was about -8.0 bars, its water stress was approximately -3.0 bars when sporulation took place. Sporangia in colonies held at a $\psi\pi$ of ≤ 5.0 bars did not differentiate into spores in several osmotica: sugars, KCl, CaCl_2 , and polyethylene glycol. Those sporangia produced by mycelium kept at ≤ -4.0 bars formed primary spores, but these cells did not extrude until a $\psi\pi$ of ≤ -3.0 bars was reached. Actively swimming spores, Hoch and Mitchell noted, were most abundant from colonies propagated in osmotica at $\psi\pi$ of -0.5 bars, but also occurred at -0.2 bars. The nature of the osmoticum was influential in determining the $\psi\pi$ at which stages in sporangium morphogenesis occurred. In mycelium grown in mannitol, *i*-erythritol, glycerol, and *i*-inositol, spore delimitation occurred as low as ≤ -11.0 bars, and was not inhibited until a $\psi\pi$ of ≤ -7.0 bars was reached. Hoch and Mitchell also observed that the direction of spore movement during emergence from the sporangium could be dictated by controlling the $\psi\pi$ of the medium (*see* Chapter 8).

Biochemical analyses of proteins and nucleic acids in *Achlya* sp. (Griffin and Breuker, 1969) confirm that a large fraction of the hyphal cytoplasm does in fact flow into the incipient sporangium. Timberlake and his associates (1973) noticed that the amount of protein and amino acids in the spores of *Achlya* sp. was a very substantial fraction of that measurable in the vegetative hyphae from which the spores were cleaved. It has been shown also that the lipid content of the hyphal tip is elevated as the sporangium is formed (S. W. T. Law and Burton, 1976a).