

## CHAPTER 11. Cytology: The Vegetative System

Cytologists of the mid- and late-19th Century were preoccupied largely with the study of the cell from fixed and stained remains, but by 1900, the shift toward examining living material as well was firmly entrenched. The concept of two cell components -- nucleus and cytoplasm -- had been established prior to 1850, and by 1890, chromosomes were proven to be fundamental to mitosis (a process fully described by 1884). In the intervening years, knowledge of meiosis had accumulated, but ambiguities in the description and interpretation of its phases were to persist at least into the first decade of 1900. The chromosome basis of heredity was proposed about 1905, so that the most visible structures of the cell had been discovered, and there remained for the cytologist the task of sharpening the interpretation of cell anatomy within the limits imposed by light microscopy and chemical reagents. With the rapidly developing, large-scale use of the electron microscope, structural cytology entered a new era so impressive that the cell is now often described primarily as an electron image. To be sure, members of the Saprolegniaceae were pried into cytologically during this evolution of the field, but generally at a pace behind the investigations on other organisms.

The dimensions and practice of cytology have changed so prominently that the discipline has for some biologists taken on a new name -- cell biology -- and accorded a fresh meaning. So broad has the field become that its flavor of structural emphasis gives way at points to overriding elements of physiology, biochemistry, and molecular genetics. How to treat, within this expansive conceptual framework, the accumulated cytological knowledge surrounding the Saprolegniaceae has thus become a problem of considerable dimension. We are resisting temptation to follow the broadened concept, and will treat the information following more traditional lines.

This chapter is an account of the protoplasmic structure of the hyphae of water molds, and the next, the cytology of their reproductive elements. Both chapters take up the subject as it has been developed with the light microscope. Since the electron microscope has revealed so much more about the real nature of protoplasm and its inclusions than had traditional cytological methodology, our account is quite simply a history. This approach needs no defense. In prior pages we have dealt with the morphology of the Saprolegniaceae; here we consider primarily the nature and behavior of cellular and gross subcellular features in growth and reproduction. Chapters 13-15 recite what is known of the ultrastructure of the saprolegniaceous thallus.

### CYTOPLASM OF THE HYPHA

#### GRANULAR INCLUSIONS

It is not always possible to be sure that structures alluded to in early accounts of cytoplasmic features of the Saprolegniaceae were identified correctly. This is

particularly true of reports of granules. The first mention of such inclusions seems to be that by Pringsheim (1883a). He found structures in the base of older hyphae (and in some oogonia) of certain water molds, but was unable to stain them with chloriodide of zinc. Accordingly, he concluded that these clear bodies were composed of cellulose. In 1904, A. Meyer reported that methylene blue applied to hyphae of an *Achlya* (possibly *A. prolifera*) revealed volutin granules. Furthermore, he believed that the "microsomes" Hartog (1889) had found in certain species were proteinaceous and therefore identical to "β-Körner" or volutin granules. In 1890-91, P.-A. Dangeard published a lengthy paper on the histology of four species of Saprolegniaceae, and reported that some had granules tending to turn brown in iodine; these he alleged to be glycogen accumulations. Hartog (1896) mentioned a similar inclusion in the spores of water molds, but thought they were granules used during cyst wall formation. Errera (1905, 1906) also detected what he thought were glycogen granules in an unidentified species of *Achlya*. He maintained that some of these particulates were "paraglycogen" because they did not stain intensely, but it is evident that he was unable to interpret with certainty the granular nature of the hyphae.

In the mycelium of an asexual *Achlya*, Guilliermond (1920a, b; 1922) found various granular inclusions both in vacuoles and in the cytoplasmic matrix. Certain ones were refringent, varying in size according to location: small ones in young hyphae, large ones in older filaments. He assumed that these small bodies functioned in morphogenesis because they became prominent and very abundant in the hyphal tips that were to develop into sporangia, but disappeared as the spores germinated. Guilliermond supposed that the hyaline bodies were identical to the structures that P.-A. Dangeard referred to as microsomes. Lipid bodies also were present in the hyphae of the *Achlya* and these too, Guilliermond noted (1922), had been called microsomes.

In 1916, P.-A. Dangeard declared that in the living hyphae of *Saprolegnia* sp. there were two "formations," stainable microsomes and oil droplets, and these moved about in the cytoplasm. Other refringent spheres did not exhibit such movement. As to metachromatic granules (a favorite "catch-all" to some early cytologists), Guilliermond (1920i) maintained that those found in fungi were not at all the same structurally and in origin as the ones of higher plants and animals. Metachromatic granules, P.-A. Dangeard insisted, were present only in vacuoles, and were probably artificial structures formed by condensation of colloids in the vacuoles under the effect of the fixative chemicals.

In species of *Saprolegnia*, P.-A. Dangeard (1931) identified another cytoplasmic inclusion, the cytosome: a spherical or elongate body in the cytoplasm of the hyphal tip, but having a more elongate and filamentous structure in older parts of the mycelium. He admitted to being unable to distinguish clearly between cytosomes and granules. There were in these same hyphae aggregates or clusters of lipid bodies which Dangeard called "ergastomes".

Murdia's (1938) observations on cytoplasmic inclusions in such species as *Achlya dubia*, *Aphanomyces cladogamus*, and *Thraustotheca clavata* were prominent by their

breadth but in essence merely demonstrated that granules of two types were to be found in intervacuolar spaces within the hyphal cytoplasm. There were small granular structures that fused during the application of stains, while other larger ones remained separate but were refractory toward staining. Some of the illustrations Murdia provided of the cytoplasmic inclusions in hyphae of these water molds hint at artificiality.

In the vacuoles of *Saprolegnia* sp. Guilliermond (1934a) found reticulate or lobed, refringent structures. These vacuolar bodies stained with cotton blue and ruthenium red, and thus obviously possessed callose and pectin, Guilliermond argued. Accordingly, he referred to these particles as "pectocallose" bodies. Additionally, Guilliermond located mucilaginous matter, which he identified as phospholipids or "galacto-lipid," in the vacuoles and granules. He also observed in intravacuolate refringent spheres, each with a central hilum and radiating striations. He compared these particles to clusters of starch grains, naming them spherocrystals. These inclusions (cruciform in polarized light) were present only in young hyphae, and as the filament aged were said to be transformed into vesicles. Guilliermond suggested (1934a, 1935) that spherocrystals were colloidal and represented some intermediate product accumulated in metabolism. From staining and solubility characteristics he concluded that these refringent spheres were lipid or phospholipid in nature. In a later paper (1938) published with Hurel-Py, Guilliermond again took notice of spherocrystals, confirming to his satisfaction his earlier observations. It is reasonable to assume that reagent impurities and chemical reactions between fixatives or stains and protoplasm must have accounted in part for the generous variety of hyphal inclusions seen by Guilliermond (and others) during these early days of cytochemistry.

## VACUOLES AND MITOCHONDRIA

An extraordinarily complicated and controversial period in the study of the cytology of the Saprolegniaceae extended from about 1912 into 1941 (and, it might be argued, beyond); the period was one well endowed with speculation liberally laced by disagreements in terminology and interpretation. Cytologists -- P.-A. Dangeard and A. Guilliermond most notably -- unable to agree in their interpretation of the nature, origin, and fate of particular hyphal inclusions, redefined old terms or created new ones when these differences seemed irreconcilable. Accordingly, although a cytologist would not now confuse vacuoles and mitochondria in saprolegniaceous hyphae, pioneers in the discipline certainly did so.

Vacuoles: -- In 1880, Schmitz described the internal structure of hyphae of a *Saprolegnia* (species?) as a perforated network with somewhat widened and thickened junctions in which the nuclei were suspended. He had, of course, seen the vacuolate nature of the cytoplasm. Later, Hartog (1888b) called attention to contractile vacuoles in water mold spores; these structures, he thought, expelled "plasmatic juice or cell sap" when the naked cell was exposed to water, and thus protected the spore from

“diffluence”. A much broader view of the cytoplasm and vacuoles was adopted by LeBlond (1919a, b), based on a study of the hyphae of an unidentified species of *Achlya*. He expressed the view that the sol and gel phases of living cytoplasm were but two aspects of a single colloidal substance. As the gel phase passed into the sol form, “alveoles” -- the vacuoles, of course -- of three size classes appeared, some having endogenous granules. Guilliermond (1930c) reviewed LeBlond’s work, and concluded that what the latter had believed was a “hydrosol” was in fact condensed colloidal material in small vacuoles.

It is Guilliermond who first singled out vacuoles in various plants (including a water mold) for detailed study (1920c; 1925b; 1930c, d; 1935). He described at length the origin and development of vacuoles in the hyphae of *Saprolegnia* sp. His concept of the nature of these inclusions was as follows. At the extreme tip of the hypha, vacuoles were present in the form of minute canals (“canaliculate”). As the filament elongated, these vacuoles fused into a network, and, in older parts of the hypha further anastomosed into a large, central canal. The first vacuoles to appear arose, Guilliermond (1930c) contended, from preexisting ones, yet he later (1932a, 1941) agreed that they could arise *de novo* in the germ hyphae from spores.

The young mycelium from germinating spores of the *Saprolegnia* sp. which Chaze (1924) had first isolated were vital-stained by Cassaigne (1931a, b). She traced a developmental sequence for vacuoles that was like that which Guilliermond had recognized, with but one exception; she believed all vacuoles arose *de novo*. According to Cassaigne, a notable characteristic of the vacuoles in *Saprolegnia* sp. was their extreme instability: fragmenting, contracting, expanding, and anastomosing. As to their origin she deduced that they appeared when the cytoplasm in the hypha secreted colloidal matter that was immiscible in itself. These colloidal nodules or centers subsequently imbibed water, and, in hydration, became vacuoles. Cassaigne’s view of the immiscibility of the vacuolar content with that of the cytoplasm was not at all at variance with Guilliermond’s (1922, 1927) proposition. The vacuolar content, he argued, could exist in three states of consistency: solid, semifluid, or liquid (by hydration). Thus the vacuole had a participatory function in endogenous osmotic change, and also served as a reservoir for important products of cellular metabolism. Guilliermond (1930c) held further that the vacuole contained a colloid with three characteristics: precipitable by neutral red, insoluble in alcohol or formalin, and was metachromatic. He of course considered the large, central vacuole occupying all but the tip of any coenocytic hypha to be an adaptation to the siphonaceous habit of the mycelium.

In a paper appearing in 1934, Dubitzky recorded some calculations of the volume and size of vacuoles in relation to the bulk of the protoplasm in the hyphae of *Saprolegnia mixta*<sup>†</sup>. As would be expected, the ratio of the volume of the vacuoles to that of the protoplast was low in the hyphal tips -- where vacuoles, at least such as can be seen with the light microscope, are scarce -- and quite high in the older portions of hyphae where the vacuole makes up the greater part of the hyphal volume. Dubitzky maintained that the hypha could be divided into three zones with respect to the

proportion of the cytoplasmic volume occupied by vacuoles. His proposed pattern of zonation was not adopted by his contemporaries or successors.

Mitochondria: -- Precisely when mitochondria were first seen in the hyphae of water molds is obscure, although it seems reasonable to conclude that the "leucoplasts" described by A. Meyer (1904) in *Achlya* sp. were these organelles. The next likely mention of mitochondria ostensibly is that in Rudolph's (1912: pl. 18, fig. 8) paper. The structures (in *Achlya* sp. filaments) that he called chondriosomes were alleged to be similar to ones in the protoplasm of *Vaucheria* species and cells of asparagus, and were probably mitochondria (Guilliermond, 1913, was of this opinion).

In 1916, P.-A. Dangeard published a treatment of cytoplasmic inclusions in the mycelium of an asexual *Saprolegnia*. Some important concepts emerged from this paper, and perhaps even prepared the stage for the grand entrance of Guilliermond's prolific papers published between 1918 and 1935 (a nearly complete bibliography of Guilliermond's work appeared in 1928 and 1934b).

To P.-A. Dangeard, the cytoplasm of the hypha of *Saprolegnia* sp. was constructed fundamentally of two parts, a nonstaining portion containing "microsomes" and oil deposits, and a staining portion. One segment of the cytoplasm, he maintained, consisted of "chondriosomes" having the appearance of vacuoles, but not moving about in the matrix. Dangeard argued that these structures were displaced positionally only as the hypha elongated. The chief proposition in his paper, however, dealt with the origin of chondriosomes. He considered that chondriosomes (he also used the term chondriome) were structures that prior and contemporary cytologists regarded as mitochondria or "chondrioconts". It was Dangeard's belief that chondriosomes arose by either of two means. In the germinating spores they developed as vesicles that participated in the formation of the germ tube vacuolar system, but in the hypha they were fragments of a preexisting vacuolar system. The large, central vacuole in a filament emitted very small and fine elongations, Dangeard proposed, that eventually acquired metachromatic granules, and subsequently fragmented into chondrioconts. If these "chondrioconts" became spherical, they were then the mitochondria. Dangeard's basic theory thus assigned to the vacuoles the function of producing mitochondria. He reiterated this view in 1931.

The terms used by P.-A. Dangeard (1916) require definition (P. Dangeard, 1958) if the publications subsequent to his are to be put in proper perspective. A "chondriome" was the total mitochondrial assemblage in a cell or tissue; a "chondriocont" was simply a filamentous element. Granular, spherical elements in the cytoplasm were mitochondria, but the rod-like structures were traditionally thought of as "chondriosomes".

The next event in the development of knowledge of the cytology of water molds was Guilliermond's rigorous attack on P.-A. Dangeard's conclusions -- stated in twenty-one papers (1918a- c; 1920a- d, g; 1921; 1922; 1924; 1925a; 1926; 1927; 1929a, c; 1930c, d; 1932a, b; 1935)! The first three publications in this long series although not dealing directly with representatives of the Saprolegniaceae relate all the essentials of

Guilliermond's disagreement with Dangeard. Basically, the latter took the view that chondriomes (*see* previous paragraph) were transitory elements and did not persist throughout the entire life of a cell. Guilliermond contended, quite to the contrary, that these subcellular structures were permanent, mitochondrial elements capable of assuming a variety of shapes (rod-like, spherical, undulant, branched) and probably playing some part in the elaboration of glycogen granules, oil droplets, and metachromatic deposits. These early papers suggest that Guilliermond was a bit uncertain of the relationship of vacuolar elements to mitochondria.

As Guilliermond's work progressed -- he used an unidentified *Saprolegnia* in much of the observational work -- his views became more firmly established (but not necessarily coordinately clearer). He reported, for example, that "chondrioconts" (simply elongate inclusions) could fragment to form vesicles, or even fuse into larger units. He also maintained that chondriomes and vacuoles were two separate structures, physically and "evolutionarily", within the water mold hyphae, and although mitochondria could take the form of chondrioconts, they did not degenerate into vesicles.

In 1926, Guilliermond added to his concept of the cytological features of *Saprolegnia* sp. a view of the "réseau de Golgi" (or "Holmgren canals" as they were known in animal cells). Initially, he recognized the structural similarity between net-like vacuoles in the hyphae and Golgi bodies, and, in fact, pointed out (1927) that the ability of the vacuoles to change shape at times gave them all the appearance of Golgi "networks". In 1929(c) Guilliermond proposed that the Golgi apparatus did not exist independently of the vacuoles and chondriomes. This conclusion was more adequately stated in 1935, based on further histochemical preparations of *Saprolegnia* sp. Chondriosomes, he wrote, assumed the appearance of Golgi bodies, under "proper" staining, but the only two subcellular structural features (save for nuclei, of course) in the cytoplasm of this fungus were the vacuoles and the chondriome system. The Golgi body apparently had no precise meaning to him and therefore was not a structural component of plant cells. He repeated this unorthodox view in 1941.

Guilliermond's concept of the mitochondria in filaments of individuals of *Saprolegnia* and *Achlya* was set out in brief form in his 1941 paper. He described the chondriosomes in the hyphal extremities as having the appearance of large mitochondria. Immediately behind the apex, these elements elongated to become rod-like organelles, and further along the hypha they converted into thin, undulant, often branched chondrioconts. These latter structures were but stretched mitochondria in his view.

Contemporaries of Guilliermond were concomitantly also developing concepts of mitochondria in the Saprolegniaceae. Milovidov (1928) performed a series of cytochemical tests on the "chondriosomes" (mitochondria) of *Saprolegnia*, concluding that they were proteinaceous, contained albumin, and were identical to those found in animal cells. He also experimented with the effects of certain environmental factors on the appearance of these inclusions. High temperature, narcotics, acids, and desiccation, among other factors, induced morphological changes in these inclusions

such that they appeared to have an “amoeboid mobility.” Within limits the chondriosomes of the *Saprolegnia* were said to be able to modify their shape reversibly, and prolonged exposure to  $\beta$  and  $\gamma$  radiation did not alter them physically (Milovidov, 1930).

Policard and Mangenot (1922) exposed a specimen identified as *Saprolegnia* sp. to various temperatures, and found that the mitochondria were disrupted at 48-50 °C; Milovidov (1929) had secured irreversible fragmentation of these structures in his *Saprolegnia* sp. at 46 °C. In 1931 Famin repeated the work by Policard and Mangenot, but raised the temperature of incubation of his cultures of *Saprolegnia* sp. as high as 66 °C for 18 hours. He found that the optical refringence of mitochondria increased in hyphae as the external temperature rose to 42 °C, then decreased to a minimal level at 50 °C. The chondrioconts (filamentous elements) in the same cytoplasmic system fragmented into barely visible vesicles at temperatures approaching 55-60 °C. It is not surprising -- as seen from the vantage point of biochemistry -- that Famin could report greatly diminishing affinity for stains, by mitochondria, as the temperature was raised.

Joyet-Lavergne (1928a, b; 1929; 1932; 1933; 1934) attempted to explain the chemical nature of mitochondria also in an asexual *Saprolegnia*. By means of known histochemical reagents -- hydroquinone and pyrogallol, among others -- he demonstrated that the mitochondria contained glutathione, and hence participated in metabolism. Furthermore, he found that the colorless derivatives of various dyes (methylene blue, Nile blue, and Janus green, for example) stained the mitochondria and the nucleoli of *Saprolegnia* sp., and he interpreted this to mean that these subcellular structures were capable of performing oxidation/reduction reactions. Wurmser (1932) took exception to Joyet-Lavergne's conclusion, pointing out that the specific reactions the latter reported did not prove that mitochondria had oxidative properties.

The figures provided by Murdia (1938) to depict filamentous mitochondria in *Achlya dubia* and some species of *Aphanomyces* are not convincing. The regularity and regimentation of these mitochondria as he illustrated them create an image of artificiality -- unless, of course, the mitochondria were in fact regularly disposed along microtubules (which he could not detect).

## VITAL STAINING

The use of vital stains classically was an accepted method of detecting cytological features in plant and animal cells. Water molds were not exempt from an occasional forced dip into some foreign chromatic fluid in attempts by cytologists to bring out vacuoles clearly, and to seek out the origins of subcellular structures. Guilliermond, Joyet-Lavergne, and Johannes contributed most heavily to this aspect of cytology of the Saprolegniaceae.

Although he tried several stains for their ability to bring out sharply certain cytological features of the hyphae of *Saprolegnia* sp. (and cells of various other organisms), Guilliermond settled on neutral red as being most useful (1923; 1929b, d, e; 1930a-c). He found that this stain penetrated especially well into vacuoles, and his

theory of the *de novo* origin of vacuoles in *Saprolegnia* sp. was developed from observations on specimens so treated. Dead hyphae would not absorb the dye, and Guilliermond concluded that it was a specific stain for vacuoles (Guilliermond and Gautheret, 1938a). Basic dyes or their leuco forms (Guilliermond and Gautheret, 1938b) were the only ones to function properly as vital stains, and some alkaline and many acid stains were toxic (Guilliermond and Gautheret, 1940, 1946). Even suitable vital stains reacted differently in watermold hyphae in response to the medium on which the specimen was grown, and in *Saprolegnia diclina* the stained vacuoles could progressively reduce a few vital dyes to their colorless forms. There was no certain explanation for this latter response, but Guilliermond and Gautheret (1937) proposed two alternative possibilities. They suggested that decoloration came about either through destruction or rejection of the stain by the mycelium. Gautheret (1939) concluded from a study of neutral red vital staining in *S. diclina* that the hyphae of this species were not susceptible to the decolorizing effect of changes in pH. Guilliermond and Obaton (1934) had earlier proposed that during its growth *S. dioica*<sup>†</sup> simply did not acidify the medium, and thus was able to take up neutral red more intensively than those fungi which did lower the pH of the medium. For neutral red to act properly as a vacuolar stain, then, the pH of the medium had to be at or near neutrality.

Guilliermond (1941) maintained that species of *Saprolegnia* were rather intermediate in cytological qualities between all other fungi on the one hand and the phanerogams on the other. Nonsaprolegniaceous fungi absorbed neutral red only when their growth was arrested, but the cells of phanerogams sequestered the dye during growth and retained it throughout their development. *Saprolegnia* species accumulated neutral red during growth but did not necessarily retain it.

Although Guilliermond and Gautheret had noted an effect of pH on vital staining, their experimental work was indeed on a minor scale compared to that performed by Johannes (1939, 1941-42, 1955b) working with *Saprolegnia* species, and *Achlya racemosa*. He explored very thoroughly the process of vital staining by means of a special apparatus through which he could percolate various dyes and buffer solutions, and at the same time control ambient temperature. Thus it was possible to vary the microenvironmental conditions at the time of staining, and then to trace (over time) the reactions to the stains within actively growing hyphae. Johannes employed such chemicals as neutral red, berberin sulfate, potassium fluorescein, rhodamin S, B, and 3B, and acridine orange.

As Guilliermond and Gautheret had found, Johannes (1939, 1955b) also observed that pH had a severely limiting effect on the absorption of stains by the hyphae. Some dyes, like neutral red, were effective only at or near neutrality; others stained the hyphae only in acid conditions, while still others were effective over a wide range of pH. The cytoplasmic membrane of watermold hyphae did not stain in neutral red, but if filaments were immersed in berberin sulfate at an acid pH, their membranes (plasmalemma) were faintly fluorescent. Certain dye derivatives (Johannes, 1941-42) -- rodamine G and sulforhodamins, for example -- did not react as vital stains. Rhodamin B and 3B proved to be excellent vital stains for mitochondria. None of the rhodamin

compounds reacted with vacuoles or cytoplasmic membranes. Subsequently, Johannes (1955b) studied the staining properties of acridine orange and neutral red on the hyphae and reproductive cells of *Achlya racemosa*. Neither stain persisted in the mycelium, and none was carried forward into new growth. He found that any staining process applied to the vacuoles or cytoplasm caused irreversible damage to the hyphae, although nuclear staining was reversible at highly acid pH levels.

Johannes' (1939, 1955b) extensive work on vital staining of water molds uncovered a provocative side issue with respect to the sexual apparatus in an unidentified *Saprolegnia*. When neutral red was applied to the mycelium bearing oogonia and antheridia, the only portion of the wall of either of these specialized hyphal branches that took up the stain was the pit "membrane" at points where an antheridial cell joined the oogonial wall. This selective staining, Johannes maintained, indicated that the pit was in fact the gametangial wall. He came to this unusual conclusion by analogy from positive selective staining of the adjoining walls at the juncture between coenogametangia in, for example, *Phycomyces blakesleeanus* Burgeff. Johannes (1955b) found that the only other points at which neutral red selectively stained wall material in specimens of *Achlya racemosa* (and a second water mold not identified) were the septa delimiting gemmae and sporangia. These rather precisely limited staining reactions have not been corroborated, and reasons for them are obscure.

Vital stains applied directly to intact hyphae have limited usefulness particularly if the object is to intensify the visibility of nuclei. Using the mycelium of *Achlya bisexualis* and *A. ambisexualis*, Schafrick and Horgen (1978) developed a method by which nuclei (and the hyphal matrix) can be stained and examined free of a confining wall. Hyphae are treated at ambient temperature with Driselase, a commercially prepared enzyme complex extracted from *Irpex lacteus* Fries. Shortly after treatment, the hyphae extrude protoplasts, and the preparations are then stained with the fluorescent dye mithramycin. This method is likely to have application in biochemical and physiological studies of mycelium as well.

## MEMBRANE REGENERATION

It has long been known, of course -- and early attempts at isolation of saprolegniaceous fungi were grounded in this fact -- that hyphae can be severed and still function as propagative units. In 1935, Gröhrock published a paper on experiments with "membrane" regeneration (hyphal wall, not plasmalemma) in *Saprolegnia mixta*<sup>t</sup>. He reported that wall formation could be induced by plasmolytic shock, and when hyphae were immersed in increasing concentrations of glucose repeated wall deposition occurred. He observed that a similar proliferative development also could be induced in the spores. By artificial wounding (either by severing or burning) Gröhrock could induce wall regeneration in hyphae, oogonia, antheridia, and sporangia. In the latter, the new wall could reach a thickness of 20  $\mu\text{m}$ . Without corroboration, his observations must remain in the realm of curious events.

## THE NUCLEI

Work of a strictly cytological nature on the saprolegniaceous hypha was of course not limited to the most prominent subcellular inclusions, vacuoles and cytoplasm. Some investigators selected the nuclei of water molds for intensive study, but a few of the very earliest to do so were evidently not always able to recognize this organelle. Moreover, a circumstance was to occur in the late 1800's that would divert attention from nuclear structure and behavior in the vegetative phase of saprolegnian development to the process of fertilization. This was the controversy arising between Pringsheim and de Bary, and subsequently joined by Hartog and Trow, the latter being drawn in almost as an innocent bystander. The somatic nucleus simply did not command much attention at this time, and it was therefore somewhat ignored.

Although Hartog was preoccupied with the sexual process in the water molds, he was one of the earliest (1889b) of the biologists to comment on somatic nuclei in hyphae of water molds. In actively growing filaments the nuclei were fusiform or oval, he noted, but in others they were spherical. Much later, F. E. V. Smith (1923) was to report that a transition could be seen in the hyphae (of two species of *Saprolegnia*) from spherical to elongate nuclei. It was P.-A. Danegard's (1890-91) contention that nuclei were so numerous in hyphal tips as to give the apex the appearance of a single mass of endogenous chromatin. Somatic nuclear division, he maintained, was direct (amitotic). Trow believed that the central, dark-staining body in the nucleus of certain water molds was a chromosome, but P.-A. Dangeard (1894-95) disagreed, saying it was the nucleolus. Later (1899), Trow concluded that this central body in the nucleus was neither a chromosome nor chromatin. Trow further thought that the fine network of strands which nearly every cytologist saw in nuclei did not change shape (during division?), and in this respect the nuclei of water molds were unlike those in plants in general. In accord with prior observations by earlier mycologists, J. R. Raper (1936) reported that the chromatin in the vegetative nuclei of *Achlya bisexualis* was scattered in clumps along the inner periphery of the nuclear membrane, and was connected to the central nucleolus by fine strands.

The first complete description of nuclear division in a water mold was that published in 1895 by Hartog. Briefly, his account of the process in *Saprolegnia monoica*<sup>†</sup> was as follows. At the beginning of division the central "nuclein mass" separated into four short, rod-like structures at the equator. These divided to give eight "rods" that then separated into two lunate groups, and ultimately fused into two new nuclei. These nuclei were then separated by the constriction of the nuclear membrane, a structure maintaining itself intact throughout the division process. Hartog obviously enjoyed keener vision than most workers to follow, but he was certainly perceptive with respect to the persisting nuclear membrane.

Divisions in *Saprolegnia torulosa* and *S. monoica*<sup>†</sup> were amitotic, F. E. V. Smith (1923) reported (thus confirming Trow's observations), but he saw no chromosomes in stained preparations. The Moreaus (1937b) decided that nuclear divisions in the

Saprolegniaceae could be either mitotic or amitotic; Murdia (1938), however, found them to be consistently of the latter type. Divisions of somatic nuclei in the mycelium of *Achlya aquatica*<sup>†</sup>(?), *A. prolifera*, and *A. racemosa* were reported by Dayal and Thakur Ji (1973) to be entirely amitotic. They could not detect any spindle fibers, chromosomal elements, or metaphase plate configurations in their fixed and stained (Giemsa, Feulgen, and iron-alum hematoxylin) preparations.

What was alleged to be amitotic division of the somatic nuclei in certain Saprolegniaceae was described in some detail in 1960, by Bakerspigel. According to him, chromatin in the nuclei of *Saprolegnia ferax*, *S. parasitica*<sup>†</sup> and *Achlya racemosa* arranged itself into two separate portions at the very onset of the division process (Dayal and Thakur Ji, 1973, also reported this initiating morphological change). After the chromatin had condensed, the nuclear membrane constricted, and in doing so pushed the two chromatin masses apart. Bakerspigel thought that the nucleolus shared in the elongation of the nucleus prior to membrane constriction. He saw no spindles or metaphase plate configurations in any dividing nuclei. A somewhat different description of somatic nucleus division appears in Flanagan's account (1970) of the process in *A. klebsiana*<sup>†</sup> and *S. ferax*. Contrary to the prevailing view that the nuclear envelope persisted during division, Flanagan held that it did not, and he contended, moreover, that chromosomal filaments were visible in division figures as were metaphase plates. From the evidence of his own preparations, Flanagan concluded that mitosis in the hyphae of these two species resembled that in the somatic cells of other eukaryotes. He reported, however, that there were no telophase figures in his material and he thought that anaphase was an exceptionally brief stage. It also must be noted that Flanagan's observations suggested an extranuclear separation of chromatin.

Observations at variance with some of Flanagan's conclusions are those by Lie and Laane (1978) on somatic nucleus division in *Achlya radiosa* as seen by a Feulgen-fluorescence staining process. They demonstrated that division was intranuclear, but there was no symmetrical distribution of chromatin on a metaphase plate (Flanagan reported metaphase figures in the nuclei of water molds he investigated). According to Lie and Laane, the middle region of chromatin in an elongating nucleus of *A. radiosa* was much more intensely fluorescent than the surrounding chromatin matrix, and the onset of anaphase was signaled when this bright central spot became duplicated. During the division process, two to six brightly fluorescing bodies were present, and these at times were linearly arranged. At anaphase what were possibly individual chromosomes appeared as highly visible, bright regions within the fluorescing chromatin masses. The nucleolus was visible during interphase and in early stages of nuclear elongation. Subsequently, this organelle disappeared to reappear in the interphase of the two daughter nuclei. The centrioles were clearly exogenous to the nuclear envelope (see Chapter 13). Lie and Laane postulated that separation of daughter nuclei in the hyphae of *A. radiosa* was the result of interacting forces generated in spindle development and nuclear envelope elongation.

In an investigation of the effect of colchicine on nuclear division in *Saprolegnia delicata*<sup>†</sup> and in a *Saprolegnia* invaded by *Olpidiopsis incrassata* Cornu, Slifkin (1967a,b; 1968) confirmed Bakerspigel's (1960) observations. Since colchicine did not inhibit the division of somatic nuclei she concluded that the process was amitotic. Slifkin also explored and characterized the ultrastructural events taking place during nuclear division (Chapter 13).

A significant discovery by Heath (1980c) may invalidate certain prior concepts of some events in vegetative mitosis in water molds if corroborated for a representative spectrum of species. Heath treated the mycelium of *Saprolegnia ferax* with mithramycin, and then studied chromatin organization. Nuclei in the hyphal tips had no detectable condensed chromatin even though most of these organelles were in various stages of mitosis. The results of this study suggest that no condensed heterochromatin forms at any stage in division. Presumed meiotic nuclei in antheridial cells of *S. ferax*, however, do contain condensed chromatin. Heath's finding contradicts prior reports of mitotic chromosomes (Flanagan, 1970, among others) in some water molds. If Heath is correct, one can suppose that the methods used by other investigators to pretreat the hyphae may well have led to artifacts, which were then interpreted as chromosomes.