

CHAPTER 8. Morphogenesis: the Spore

The first recognizable mention of spores in the Saprolegniaceae is in Gruithuisen's account (1821:445, pl. 38, figs. 14-16) of "seeds" in "compartments" at the ends of filaments of "*Conferva*" *ferax*[†]. Hannover's exposition (1842) of the nature of spores and spore discharge in a watermold was far more detailed: records of daily observations on motility, encystment, and germination. It was Unger (1843, 1844), however, who first gave an accurate sketch of percurrent renewal, spore discharge and encystment. He saw one flagellum in the lateral groove of a secondary spore, and concluded that water flowed through this depression under the action of "vibrating organs." Thuret (1845) noted that the cilium (flagellum) insertion described by Unger was analogous to the "organs of locomotion" (two apically attached flagella) on "*Conferva*" spores (Thuret, 1843: pl. 10, figs. 1-3). In 1851 a full description of sporulation in *Saprolegnia ferax* (as *Achlya prolifera*) appeared in an account by Pringsheim. He observed spores that were by chance attached to one another by their flagella, and decided that these structures were "copulation threads."

The concept of two swimming stages in the watermolds -- "diplanetism" -- had its beginning with Leitgeb (1869-70) and his description of *Diplanes saprolegnioides*[†]. Later work was to show that, in fact, multiple swarmings and encystments may occur (Salvin, 1940, for example). Accordingly, the terms mono- and diplanetic, and mono- and dicystic (Coker, 1935), are not strictly applicable to the watermolds as E. A. Bessey (1950) so perceptibly argued.

Ordinarily, members of the Saprolegniaceae are thought of as expressing spore dimorphism to a very advanced degree. This is true of species of *Saprolegnia*, but in other genera one stage is suppressed. The primary spores are pyriform (de Bary, 1852), and are provided with two subapical flagella which, in motion, are oppositely directed. The secondary, reniform planonts (described as "bean"- or "kidney"-shaped, terms that Weston, 1917, firmly objected to) emerging from encysted primary ones are laterally biflagellate, with the posterior flagellum being the longer of the two (Crump and Barnton, 1966). Scanning electron micrographs published by M. F. Brown and Brotzman (1979:27, fig. 2) show the anterior flagellum of *Aphanomyces cochlioides* to be noticeably shorter and stouter than the posterior one. Further observations are needed to confirm whether this size difference occurs in all species of the genus. Any effects of SEM preparative techniques must be accounted for in such studies. Schussning (1948, 1949) introduced two terms to refer to these patterns of flagellar insertion: subakrokont for the subapically attached pair, and pleurokont for the laterally positioned ones. The secondary spores of *Aphanomyces cochlioides* and *Saprolegnia* sp. are not strictly reniform (Ho *et al.*, 1968). The anterior portion is flattened, and the two flagella are attached nearest to the forward end of the cell. The spore is perceptibly flattened on the side on which the lateral groove appears.

The secondary planont is the more vigorous swimmer of the two. It is this fact that perhaps led H. M. Ward (1883), Humphrey (1893), and Höhnk (1933) to conclude that the "purpose" of the primary spore of a watermold was to evacuate the sporangium, while that of the secondary one was to disperse the organism. Coker's

comment (1935:270) was contradictory: the “ ... first swimming stage in diplanetic species has nothing to do with emptying the sporangium and is of little use otherwise.” In any case, as Waterhouse (1962) has suggested, the difference in motility persistence and vigor between the two types of planonts is not the result of disparity in flagellum structure (Chapter 14). A simplified notion of the nature of primary and secondary spores occurred to H. M. Ward (1883). Since the latter were the most “constant” ones, he argued, they were probably the ancestral form.

One attempt has been made to define terms -- as they apply to the spores of *Aphanomyces euteiches* -- somewhat more precisely than has been the usual practice. Hoch and Mitchell (1972a) proposed that since the endogenous spores of this species lack flagella and do not exhibit amoeboid motion they should be referred to as primary spores. The extruded spore is then to be designated as the encysted primary spore. They argue further that the planont emerging from this discharged, encysted cell should be considered simply as a zoospore, and not be viewed as a secondary spore. This concept, of course, can be equally well applied to the spores of species of *Achlya* and other genera, were one inclined to do so. The motile spores of *A. euteiches*, however, are morphologically indistinguishable from the secondary ones of species of *Saprolegnia*. Thus, retaining the names primary and secondary in reference to the structurally distinct types of spores seems to us to have merit, even though one or the other of the two may be suppressed in some taxa.

As early as 1894 it had been discovered (A. Fischer) that some algal spores had two morphologically distinct types of flagella, “Peitschengeissel” or whiplash, and “Flimmergeissel” or tinsel. Vlk (1939) demonstrated that the secondary planonts of *Saprolegnia monoica*[†] and *Dictyuchus monosporus*, and the primary ones of *Saprolegnia* sp., also had these two types of organelles. There appeared quickly in publication several confirmations (for example, R. K. Saksena *et al.*, 1943) expanding Vlk’s observations.

THE MECHANISM OF SPORE DISCHARGE

Considerably more is known about the morphology of sporogenesis than about the physiology and biochemistry of differentiation and discharge. Nevertheless, lack of knowledge has not dampened speculation as to the cause of spore release in the Saprolegniaceae. A roughly chronological account of the literature on this aspect of morphogenesis reviews adequately the explanations that have been put forth.

Unger (1844), de Bary (1852, 1884), and Büsgen (1882) held the notion that discharge in water molds was the consequence of swelling of a gelatinous or mucilaginous substance in the sporangium. Rothert (1903) thought likewise, but believed that the expanding material was on the spore surface, and not between them. De Bary was not certain of the source of this substance, but thought it originated from the inner wall of the sporangium, from the spores themselves, or that it was a “free” substance between the spores. The possibility of an intersporal substance in sporangia of *Leptolegnia caudata* was suggested by A. C. Matthews (1932), who concluded that such a material might aid in discharge. Neither Strasburger (1880), Berthold (1886), M. C. Carlson (1920), nor Coker (1923), among others, accepted the explanation of a “swelling

substance,” and Hartog (1886-87, 1888a, 1895) vigorously opposed the idea that such a material existed. It should be recorded, however, that Coker (1912) had at one time accepted the gelatinous substance theory of discharge for the spores of *Achlya debaryana*. Since the cells were not flagellated on discharge, Coker proposed certain lines of evidence to support the “expanding substance” cause for spore ejection. First, the spores of *A. debaryana* were violently extruded through the orifice and were constricted as they emerged; they could not possibly leave the sporangium in this manner “... under [their] own steam...” (Coker, 1912a:321). Second, if the discharged cells were agitated in some fashion, they moved as a group, and this, Coker maintained would only happen if there was no expulsive substance present around the spores.

Another point of view held that discharge was affected by turgor. Like A. Braun earlier (1851), Cohn (1853), and Griffith and Henfrey (1875) inferred that water absorption into the sporangium, succeeded by swelling and stretching of the elastic wall, to force the spores through the exit orifice. Walz (1870a, b) thought it was the inner wall surface that distended to push the reproductive cells through the opening. The evidence to support this theory was that glycerine surrounding the sporangium favored spore discharge in *Saprolegnia monoica*[†]. Andrews (1921) fixed upon the notion that internal hydrostatic pressure indeed was the force behind spore release in the watermolds.

That internal pressure (osmotic water potential, $\psi\pi$) in the sporangium (see Chapter 7) is a salient part of spore release seems no longer in doubt, if one can extrapolate to other water molds the results of a study on *Aphanomyces euteiches* by Hoch and Mitchell (1972a, 1973). They proposed that since the endogenous spores are not actively motile in this species, a hydrostatic pressure within the hypha must be involved in discharge. For such a pressure to develop a temporary obstacle to molecular diffusion would have to exist, and the spore wall itself (Hoch and Mitchell, 1972a) could be that barrier. When the spores are finally cleaved, the plasmalemma no longer is adjacent to the sporangium wall, and thus is not an intact obstruction; in fact, this membrane together with the tonoplast is incorporated into the wall of the spore during sporogenesis. New vesicles appear in the primary spores prior to and during expulsion, Hoch and Mitchell (1972a) found, suggesting that these subcellular units operate in some fashion to contribute to expulsion. Hoch and Mitchell (1973) have also demonstrated that the direction of endogenous spore movement could be controlled under the influence of externally applied osmotica. If the external solution were adjusted to -7.0 bars or less, spores moving toward the sporangial orifice during discharge would reverse direction. When the external $\psi\pi$ was raised the spores once again moved toward the exit pore.

Some investigators have suggested that primary spore motility at least assists in the discharge process, and very early, Robin (1853) remarked that the spores “purposely” became elongate so they could proceed with their two “cilia” through the sporangial opening. Perhaps motility is a supportive mechanism in *Saprolegnia* species (see section on galvanotaxis), but in watermolds where the primary stage is suppressed the involvement of flagella in discharge is less certain. Cornu (1872) argued that the escaping spores in specimens of *Achlya* had flagella, quite in contrast to de Bary’s (1884)

contention [emerging spores of *Aphanomyces* species were not flagellated de Bary earlier (1860) had maintained]. Humphrey (1891, 1893), Hartog (1886-87), Coker (1914, 1923), and Kauffman (1921) reported having seen flagella on the discharging spores of various species of *Achlya*. Rothert (1903) claimed to have detected these organelles on emerging spores of *Aphanomyces* species, but if *Aphanomyces euteiches* reflects the general condition in this genus there certainly is no ultrastructural evidence to support this view (Hoch and Mitchell, 1972a; for details, see Chapter 14). Evidently E. J. Butler (1907) was satisfied that flagella aided in spore discharge in species of *Achlya* and *Saprolegnia*, and Cotner (1930b) drew attention to the flagella on primary planonts of *A. conspicua* although she thought them to be too imperfectly developed to assist materially in evacuation. Earlier, Coker (1923) had decided that the flagella on emerging spores of *Achlya* species could not possibly provide the force necessary for expulsion. However, he also argued against the presence of a swelling mucoid substance as an aid in discharge (in achlyoid water molds) since he simply could not detect such a material.

Direct observations and experimental evidence from Borkowski's (1967) study reveals much about the nature of the discharge process and the involvement of flagella. He called attention to an often-observed but little-heeded phenomenon in planont discharge in species of *Saprolegnia* and *Achlya*, namely, that the emerging spores form an axial column as they travel through the sporangium toward the exit orifice. In *S. monoica*[†] the primary planonts leave the sporangium with the flagella trailing (Coker, 1923, had observed this in *S. anisospora*), and only when a spore has cleared the exit orifice does it turn and commence swimming. Moreover, individual spores left within the sporangium after the bulk of the planonts have escaped still tend to orient themselves into a central column even though the sporangium containing them is flooded with ethanol, chloroform, or calcium hydroxide. Borkowski concluded that discharge thus was passive in the sense that the flagella did not propel the endogenous spores.

The foregoing are not the only explanations of spore emergence that have been postulated. In particular, the theories advanced by Hartog and Humphrey warrant attention. Hartog disapproved of any hypothesis attempting to explain discharge on the basis of some expulsive substance, but it should be recognized that he was concerned chiefly with the process in species of *Achlya*. It is in his paper of 1886-87 that the first alternative theory appears: the rapidity with which the first few spores move toward the exit orifice could be attributed to a response to oxygen level. The ambient oxygen in the water outside the orifice simply was much higher than that within the sporangium, Hartog deduced, and was sufficiently "attractive" to account for the spores squeezing through the opening. He postulated, then, that the spores escaped by reason of "positive aerotaxy." Rothert (1888, 1890)¹ experimented with three species of

¹ The Polish edition of Rothert's paper appeared in 1888 not 1887 as has been widely cited. The German printing was issued in 1888, with a publication date of 1890. However, the paper appeared in the full volume of the journal series bearing the publication date 1892.

Saprolegnia (all are now recognized as forms of *S. ferax*) and *A. polyandra*, attempting to disprove Hartog's claim for aerotaxy. In reply, Hartog (1888a) admitted that he had perhaps emphasized this phenomenon too strongly, and the explanation could as well be "negative pneumotaxy," an escape response to the chemical stimulus of metabolic products of the spores themselves. Hartog was not certain what chemical stimulus was involved, yet in 1895 he again confirmed that discharge was the result of irritability and chemotaxy of the spores themselves. Gay and Greenwood (1966) hypothesized that the ultrastructure of sporogenesis in *S. ferax* supported the Rothert theory of discharge by means of turgor loss. This being so, how then could planonts escape from the sporangium? They suggest (Gay and Greenwood, 1966) that it is possible for spores to respond to an aerotactic/pneumotactic stimulus just as Hartog (1886-87, 1888) had theorized.

It appears that Humphrey (1893) aligned his views with Hartog's. The former observed achlyoid sporangia in which lateral openings developed after spore emergence through the apical orifice had begun. In such instances the spores nearest these lateral pores moved away from those streaming toward the apical opening, and issued through these new orifices. To Humphrey this meant that the "attractiveness" of the external force (he was unsure if it was oxygen) was stronger than the tendency of the spores to cling together.

We agree with Dick (1973) that there is as yet no adequate hypothesis to explain spore release in the Saprolegniales. Perhaps, as he suggests, pressure of hydration and differential viscosity could account for the process, but convincing evidence is not at hand.

ADELPHOTAXY

Why, upon release, do the primary spores of achlyoid sporangia cluster in a hollow sphere at the orifice? As in the case of the release mechanism itself, there seems to be no satisfactory explanation for this unusual phenomenon.

The concept of adelphotaxy was first advanced by Hartog in 1886-87 as an explanation of a form of irritability, but the theory was not developed and named by him until 1888(a). He explained that as the spores emerged from sporangia of *Achlya* species, they tended to align themselves with their axes parallel. A superficial excretion from each spore created surface tensions, Hartog (1895) suggested, that attracted and held together neighboring cells (but *see* next section). This expression of irritability was effective only for units in close proximity to one another; a spore physically removed a slight distance from the axial cluster would swim away rather than rejoin its "companions." Humphrey (1893) agreed with Hartog's hypothesis of adelphotaxy.

Somewhat later, J. N. Couch (1924a) examined again the matter of spore clustering in species of *Achlya* and *Aphanomyces*, noting that if the actively emerging reproductive cells were killed with osmic acid, they separated slightly but were still held loosely by a viscous material. Staining showed that many spores in the emerged mass were connected by delicate threads. These filaments were variously located at the ends of elongate spores, at the position occupied by flagella in a "typical" primary

spore, or at points intermediate between these extreme locations. The "cilia" seen by Cornu, Hartog, Coker, and others on emerging spores of *Achlya* species were these connecting protoplasmic threads, Couch believed, and the loose clusters of emerged spores in *Achlya* and *Aphanomyces* specimens owed their development to these threads. Hutchison (1940) also attributed achlyoid clumping to interlocking protoplasmic strands.

As early as 1907, E. J. Butler suggested that in both *Achlya* and *Aphanomyces* species, the first spores to emerge created an attractive force that -- perhaps by chemotaxy -- served to bring about a tension operating to evacuate successive ones from the sporangium. To Bonner (1952:154) the "...blastula..." of extruded spores from an achlyoid sporangium was the result of a simple morphogenetic movement. After experimenting with the loose, hollow sphere of emerged, encysted spores, Bonner deduced that the shape of the cluster was determined by the presence of a gummy adhesive between the spores, and the "... force of the spout of fluid issuing from the zoosporangium which essentially blows the cells out in the form of a sphere." After the secondary spores emerge from the primary spore cysts there remains, Bonner wrote, a "... spherical honeycomb of clear intercellular substance that presumably is the hardened cement which accounted for the original adherence of the cells." That the sphere or cluster of encysted primary spores in achlyoid sporangia is not always hollow has also been observed (Häyrén, 1942), but whether these are cases of extreme adelphotaxy or are due to some other cause has not been resolved. Working with *Achlya debaryana*, Armbruster (1980) demonstrated that if the apex of a sporangium was ruptured prior to spore release (the second cleavage was completed), the spores emerged, but did not form the characteristic hollow sphere at the opening.

GALVANOTAXIS

By means of a series of cleverly devised manipulations, Borkowski (1968a, b; 1969; 1970; 1972) experimented with the endogenous columnar (axial) orientation of spores that occur (in some species) as they move through the sporangia. He interpreted the results to mean that there is no expulsive substance associated with discharge.

To conduct the experimental work Borkowski (1968a) constructed on a microscope slide two opposing capillary pipettes filled at the narrow end with 3% water agar. To each of these tubes he attached an electrode so that a reversible current could be transmitted between them. He placed a sporangium between the electrodes, activated the current, and observed the movement of spores during evacuation. Spores moving in the endogenous central column (toward the exit orifice) were attracted to the negatively charged capillary pipette and the column was displaced as a mass to the "side" of the sporangium adjacent to the cathode. When the current was turned off, the spore column again became axial.

Once the spores had emerged through the exit orifice they moved toward the anode (+). Borkowski could move the axial column at will to any position within the sporangium by orienting the latter at various angles with respect to the electrodes. If

the negative charge was applied near the sporangium base, for example, the column of spores clustered at the base.

To explain this galvanotactic response, Borkowski (1968a) offered several observations and hypotheses. He inferred that there was a charge on the spore and sporangium wall. Under the influence of the artificially applied current the spore wall was changed from its normal negative charge to a positive one and the cell was therefore attracted to the negative pole. If the spores in fact carried a negative charge, they would be repelled from the sporangium wall and would accumulate into an axial column. This similarity of charge seems itself to be a contradiction because spores with a like charge would repel one another, and a central column could not possibly develop. Borkowski recognized this fact, and suggested that the spores do not repel one another because the surface of the pyriform cell is not isoelectric (this remains to be proven).

By what mechanism do the spores in the axial column in a discharging sporangium move toward the exit orifice? Borkowski (1968a) offered alternative explanations. Since the opening has no charge, the spores would move toward the region of least resistance, that is, in the direction of the exit pore. On the other hand, Borkowski proposed, the sporangium wall in the vicinity of the orifice could be different chemically from the rest of the wall and thus have a different electrical charge. There is, of course, no evidence to favor (or disprove) either of these provocative explanations.

Borkowski's (1968a) observations on disrupted spores show conclusively that there is a negative charge associated with the spore wall. Wall fragments of fractured endogenous spores flowed to the sporangium wall, and could not be removed from contact with it even by the external application of an electrical charge. Granules from the disrupted spores, meanwhile, moved axially toward the exit orifice, and did so considerably more rapidly than did intact spores. This event seems to refute the theory that favors a homogeneous swelling substance being in the sporangium. If such a material was present, spores and granules should move through it at approximately equal rates.

At particular pH levels the electrolytic properties of acid solutions applied externally to discharging sporangia disrupted axial alignment of the emerging spores in *Saprolegnia monoica*[†] (Borkowski, 1968b, 1969), *Achlya racemosa*, and *Aphanomyces* sp. (Borkowski, 1970, 1972). For *S. monoica*[†], for example, the lowest pH range in which axial alignment of spores occurred was 3.6-3.9. Below this level -- in sporangia in a solution at approximately pH 2.0-3.5 -- the central column of discharging spores disintegrated, but in the range of pH 4.0-6.5 was normal. Borkowski proposed that an acid environment diminished the magnitude of the negative charges between the spore and sporangium wall. The spore wall becomes positively charged before that of the sporangium reaches its electrical discharge point, and the spores break away from the column and flow to the sporangium wall. Although buffered acid solutions destroyed columnar orientation the spores reoriented themselves into an axial column soon after these solutions were replaced by distilled water (Borowski, 1969).

The characteristic spherical clustering of emerged spores in *Achlya racemosa* and *Aphanomyces* sp. is not disrupted by an electrical current applied while the spores are

leaving the sporangium (Borkowski, 1972). Encysted endogenous spores also responded to electrical impulses Borkowski (1969) observed. Such spores in *Saprolegnia monoica*[†] moved toward the cathode capillary tube when the current was activated, and were held against that portion of the sporangium wall nearest the cathode. When the external charge was removed, these encysted spores aligned themselves axially.

By micromanipulation, Borkowski (1970) inserted "foreign cells" -- sporulating sporangia of *Aphanomyces* sp., and cells of yeast and *Chlamydomonas* -- into empty sporangia of *Saprolegnia monoica*[†]. All such inserts reacted as did the planonts of the *Saprolegnia* itself: axial alignment, positive galvanotaxy (clumped toward cathode), and sensitivity to low pH. From these observations, Borkowski concluded that the theory of spore expulsion by means of some swelling substance was without foundation.

The Borkowski hypothesis of an electrical charge differential might also explain at least in part some results obtained by Johannes (1955b) in experiments on staining water mold hyphae. He noticed that the wall of ordinary hyphae of *Achlya racemosa* could not be stained with basic dye, except when gemmae developed in those hyphae. Staining was made possible, Borkowski suggested, by the charge differential conferred by an additional wall layer deposited as the gemmae formed.

SPORE DISCHARGE IN OTHER GENERA

THRAUSTOTHECA AND *BREVILEGNIA*

Characteristically in species of *Thraustotheca* and *Brevilegnia* the encysted primary spores are released when the sporangium wall ruptures. Largely through Coker's influence the pertinent literature leaves the impression that thraustothecoid and brevilegnoid spore release are different. Presumably the divergence lies in the totality to which the sporangium wall disintegrates. In *T. clavata*, the wall disappears except for an annulus-like remnant at the point of attachment of the sporangium to the hypha. In *Brevilegnia* species, on the contrary, the rupture of the wall is usually fragmentary and leaves remnants of material loosely adhered to spore masses at various points as, for example, in the vicinity of the sporangial apex. Such a distinction is unnecessary, since, in fact, the degree to which the sporangium wall disintegrates is inconsistent in specimens in either genus (*see* systematic account).

Coker and Hyman (1912) simply reported that in *Thraustotheca clavata* the sporangium wall dissolved to release the spores. A few years later, Weston (1918) studied this species in detail, and concluded that there was no swelling substance in the sporangium to force the spores out when the wall ruptured. He favored A. Braun's (1851) view that the elastic sporangium wall was distended by the swelling of the spores themselves during sporogenesis. On the other hand, Weston (1918) concluded that Hartog's theory of adelphotaxy was not applicable to discharge in *T. clavata*: if a released spore was separated from its neighboring ones by a distance as much as one-half of its diameter, it did not retract to the mass when released. Murdia (1939a) found that the imbibition of water -- measured by the uptake of neutral red -- prior to spore

release in thraustothecoid sporangia of *Achlya dubia* caused the spores to swell so that the resulting pressure ruptured the sporangium wall.

LEPTOLEGNIA

Sporogenesis in *Leptolegnia caudata* is like that in the family as a whole (Chapter 7). At discharge, however, the spores of this species diverge prominently from the pattern in members of *Saprolegnia* and *Achlya*. The spores of *L. caudata* emerge (the causal mechanism is not known) as elongate or cylindrical cells (Coker, 1909; J. N. Couch, 1924a). Once outside the exit orifice, the spores fold back from a midpoint, assuming first an inverted V-shape (with respect to flagellar insertion) then gradually becoming pyriform with two subapically attached anterior flagella.

The physical movements by which the primary spores of *Leptolegnia caudata* convert from cylindrical to pyriform bodies have never been disputed, but the question of whether or not the spores are flagellated as they emerge from the sporangium is still not resolved. Coker (1909) maintained that the two flagella were attached laterally at a midpoint on the cylindrical spores while these cells were still in the sporangium. The observations recorded by J. N. Couch (1924a), however, show that Coker's description of flagellation was rather simplistic. Couch noted that prior to emergence the spores were connected at each end by one or two thin cytoplasmic strands. As the strands became perceptibly thinner, a papilla appeared on the spore at the base of each thread (the basal body of the flagellum, Couch presumed). As soon as the spore entered the water, it assumed the inverted V-shape, and the papillae were then prominently displayed. As the cell folded into the pyriform configuration, the terminal strands ("incipient" flagella) moved to the side of the spore and when the cell was fully pyriform these organelles were in their usual nearly apical position. The process by which flagellation is achieved by spores of *Leptolegnia* species awaits further exploration.

APHANOMYCES AND PLECTOSPIRA

Sporogenesis in the plant pathogenic species of *Aphanomyces* and *Plectospira* is not substantially different from that in other groups of water molds. In *A. euteiches*, however, the spore emergence process is evidently rather unique in some respects. It was reported by F. R. Jones and Drechsler (1925) that during discharge, the cylindrical spores elongated perceptibly and their speed increased measurably as they neared the exit orifice. In some instances, the moving spores near the orifice were about 2.5 μm in diameter, and 70-90 μm long. The ends of some discharging spores of *A. euteiches* were rounded, and Jones and Dreschler interpreted this to mean that these cells evidently were not pulled to the orifice by threads connecting them to previously emerged ones.

DICTYUCHUS

Leitgeb's description (1869-70) of discharge in *Dictyuchus* -- each planont emerging separately from its cyst -- has not been improved upon substantially. Nothing is known of the mechanism of release or why the primary spore cysts remain intact. Armbruster (1980) has carefully described some of the ultrastructural characteristics of the excysting spores in *Dictyuchus* species.

Coker (1935) defined two types of dictyucoid sporangia. In a false net sporangium the spores encyst in a rounded form and retain their position upon dissolution of the sporangium wall. The spores of a true net sporangium leave angular cysts, but the sporangial wall disintegrates before any of the spores swim from their cysts. That Coker's definitions do not entirely reflect actuality has been demonstrated by Padgett and Seymour (1974). They consider a false net sporangium to be one in which the entire surface of each spore is rounded such that intersporal spaces are prominent. Two types of true net sporangia may be recognized. In one, the sporangium wall usually remains intact, and the enclosed cells are tightly compressed so that there are no intersporal spaces. The sporangium wall might or might not persist in the second type of true net sporangium, but the spore cysts at the sporangium periphery are rounded (and there are intersporal spaces) while those near the central axis are angular and tightly appressed to one another throughout their respective surfaces. The ultrastructure study on cysts of *Dictyuchus sterile*[†] by Heath and Greenwood (1970a) is treated in Chapter 14.

SECONDARY PLANONTS

That most water molds -- species of *Aplanopsis*, *Pythiopsis* and *Geolegnia* excepted -- produce laterally biflagellate secondary planonts is firmly established. As Weston (1918) very early observed in *Thraustotheca clavata*, the secondary spores generally develop only when the primary spore cysts are kept in water; cysts immersed in a nutrient solution usually form a germ tube. This appears to be the pattern throughout the family. Almost nothing is known of the morphology or physiology of emergence of secondary planonts from the primary spore cysts, although ultrastructural studies (Holloway and Heath, 1977b) have done much to describe events in the conversion of primary planonts into secondary ones (Chapter 14).

Crump and Branton (1966) published an informative account of secondary spore release in *Saprolegnia* sp. as seen with ordinary brightfield optics. Excystment of the primary planont is eminent, they found, when vacuoles appear in the cyst cytoplasm. At least one of the vacuoles exhibits rhythmic contraction just prior to the spore's emergence. The cytoplasm then exits through the end of a small protuberance on the cyst wall and becomes spherical, but remains attached to the rim of the exit orifice while the flagella are forming. The flagella first appear as two small knobs; these gradually elongate to become the organelles of locomotion (the details of this transformation are unclear; see Crump and Branton, 1966; Holloway and Heath, 1977a, b). The motion of primary spores that are about to encyst is noticeably erratic.

Hartog (1895) believed that the planonts emerging from primary spore cysts were positively aerotactic, while the germ tubes from encysted secondary spores were

chemotropic. Evidently Hine (1878) observed secondary planont exit in *Achlya* species, but did not see flagella. Instead, he deduced that the oscillatory movement of the released spore resulted from an endogenous and exogenous temperature differential. Höhnk (1933) noticed that the end of the secondary spore first to emerge from the cyst was vacuolate, and that flagella were not evident until the planont was freed from the cyst. Armbruster (1980) has unequivocal ultrastructural evidence that the encysted primary spores of some species of *Brevilegnia* and *Dictyuchus* have the flagellar root system (kinetosomes) polymerized before the protoplast emerges during excystment. Drechsler's (1929: fig. 16 Pd) illustrations of some partially extruded secondary spores of *Plectospira gemmifera* show them to be biflagellate; such spores he viewed as abnormal.

Investigators seem to have concerned themselves merely with the physical nature of variations in the emergence process. For example, F. R. Jones and Drechsler (1925) described a pythiaceous type of secondary spore emergence in *Aphanomyces euteiches*. A papilla protruded from the cyst, the protoplast flowed into the resulting evagination, and then "matured" into the reniform stage within this vesicle-like expansion from the cyst. Abnormally large encysted spores of *A. cochlioides* may, when germinating into the secondary planont stage, produce more than one biflagellate spore (Drechsler, 1929). Repeated encystment and excystment of the secondary planont is a well-documented phenomenon in some of the Saprolegniaceae.

THE CYSTS

Save for exploration of the subcellular nature of spore cysts in some of the water molds (Manton *et al.*, 1951; Heath, 1976), these structures have essentially been ignored. One study only, that by Hallett and Dick (1976), treats spore cysts at all extensively, and the results from it have taxonomic implications. They compared cyst sizes from colonies of *Saprolegnia anisospora*, *S. parasitica*[†] and *S. ferax* grown at six temperatures. From very precise graphic analyses they determined the mean dimensions and volumes (and the standard deviations of the resulting data) of cysts from these species. The cyst diameter data from all isolates of *S. anisospora* plotted into bi- or trimodal curves, indicative of the existence of two or three size classes. The data provided by Hallett and Dick agree in general with Coker's (1923) analysis of the species. He found the spore cysts of *S. anisospora* to segregate into three size classes: large (30 x 12 μm), intermediate (22 x 11 μm), and small (12 x 9 μm). Coker also divided the smaller size class into two "subclasses", but the information from the study by Hallett and Dick does not support such a separation.

Their data (mean diameters) showed that three spore classes exist in this species: 12.9 μm (I), 16.6 μm (II), and 21.1 μm (III). Generally, the larger spores (II and III) were more frequently formed by sporangia on mycelium propagated at increased incubation temperatures. Moreover, the three isolates of *S. anisospora* used in the analysis responded differently in terms of their predominating cyst size at the various temperatures. As had Coker, Hallett and Dick found that sporangia usually if not

always contained spores of only one size class. The plotted data for cyst sizes in *S. parasitica*[†] and *S. ferax* gave unimodal curves.

SPORE MOTILITY

It was not until the appearance of J. N. Couch's paper of 1941 on spores of *Achlya* spp., *A. caroliniana*, *Dictyuchus monosporus*, and *Saprolegnia ferax* that flagellar action and resulting spore motion was precisely defined (see review by Kole, 1965). The flagella on both the primary and secondary planonts of these water molds participated in motility, and the posteriorly directed one did not simply function passively in determining direction. Since it has been proposed that each flagellum beats in one plane only, it may appear (J. N. Couch, 1941) as though the trailing one is inactive. If the anterior flagellum of a primary planont is lost the cell swims erratically; it does not do so if the posterior one is missing. When the trailing flagellum is lost, Couch observed, the spore moves smoothly through the water and without any spiral or rotational motion. According to W. E. McKeen (1962) the posterior flagellum propels the spore while the anterior one acts as a rudder.

The observations by J. N. Couch (1941) were essentially confirmed in 1966 by Crump and Branton, working with an asexual *Saprolegnia*. In their specimen the two flagella of the secondary planont beat in almost mutually perpendicular planes. Comments by W. E. McKeen (1962) dealt with the position of these organelles with respect to the spore body. He called attention to the fact that the axes of the flagella in secondary planonts (*S. parasitica*[†] and *Achlya americana*) are aligned centrally, as it were, to the spore axis. The flagellar axes of the primary spores of *S. parasitica*[†], however, are acentric, that is, positioned at an angle to the centerline of the spore. McKeen postulated that an acentric configuration in the primary spore would account for its slow and awkward swimming motion. He also pointed out that while planonts are in motion, 20-50% of the flagellum length is pressed against the spore body, and the full length of the organelle is not seen until the spores are killed.

The review of planont behavior by Hickman and Ho (1966) substantiates the commonly accepted observation that the unimpeded forward motion of "normal" primary and secondary spores in water traces a spiral configuration. Although Lowndes' (1943) study of the mechanics of flagellar motion was not with saprolegniaceous spores, the model he constructed establishes that a body propelled by a rotating flagellum (as opposed to one beating in a single plane) would be drawn through the water and at the same time turned on its long axis. Lowndes studied the motion of a laterally biflagellate, dorsiventrally flattened spore of a cryptophyceous alga, and noted that its motion was the same as that described by a monoflagellate cell: a helical configuration through the water. Even a slight rotational motion in the anterior flagellum of a water mold spore would impart the spiral pathway ordinarily prescribed by this cell. The action of the trailing flagellum might then impart the usual somewhat erratic nature to the spore's track. It has been suggested (W. E. McKeen, 1962) that if the lateral groove (as in secondary planonts) were positioned at an angle to

the longitudinal axis of the cell the spore would be rotated as it moved through the water.

FLAGELLAR RETRACTION

For a planont to encyst, its flagella either must be absorbed during the process or cast off prior to completion of encystment. The earliest detailed record of the fate of these organelles on watermold spores appears to be that of Rothert (1894). He reported that the flagella of the primary planont in *Saprolegnia monoica*[†] and *Saprolegnia* sp. were retracted as encystment began, while those of the secondary spore simply dropped off. Rothert noticed a loop on the flagella of the secondary planonts, and a ring on those of the primary ones, but assumed that these configurations were merely instances of the flagella curling back upon themselves. Evidently H. M. Ward (1883) saw a bulb on the end of each flagellum in the motile spores of *Achlya polyandra* but inferred that the organelle was simply elongating as it unrolled from the terminal knob.

The flagella on spores of *Aphanomyces euteiches* were first described precisely by Cunningham and Hagedorn (1960). They recognized a minute "bubble" at a bend in some flagella, but in other instances, the organelle assumed a paddle-like aspect, just as Ferris (1954) had described. These configurations were assumed to be evidence of retraction. Manton and her associates (1951) detected a bubble-like structure on the flagella of some spores of *Saprolegnia ferax*, and thought perhaps this might represent the gathering of the flagellar sheath as it was being stripped off during retraction. Secondary spores of *Achlya americana* and primary and secondary ones of *S. parasitica*[†] are said to produce a bead-like profile at some point on each flagellum, and that subsequently these organelles are detached but may retain autonomous movement (W. E. McKeen, 1962). This of course is contrary to Rothert's earlier view.

Detailed observations on the fate of flagella were provided by Crump and Branton (1966) in their study of spore behavior in *Saprolegnia* sp. (asexual). The flagella of the primary planont were not retracted in a consistent manner at the onset of encystment. In some instances the flagella simply became successively shorter (one more quickly than the other) as they were absorbed by the encysting spore. Holloway and Heath (1974: fig. 6) recognized the difference in retraction rate, but could not determine if it was the tinsel or whiplash one that withdrew the more rapidly. In other instances, Crump and Branton observed a bead formed on only one of the flagella of the primary planont, but nevertheless both organelles retracted. In the primary planonts of *S. terrestris*, a somewhat similar pattern of retraction occurs (Holloway and Heath, 1974). A "bead" appears at the base of each flagellum, travels to the end of the organelle, reverses direction, and as it moves toward the spore surface the flagellum is concomitantly shortened and subsequently absorbed. The ultrastructural aspects of flagellar absorption as Holloway and Heath interpreted them are treated in Chapter 14.

The locomotive organelles of secondary planonts of *Saprolegnia* sp. are cast off (Crump and Branton, 1966) rather than being retracted as in the case of the primary spores. A "bead" forms at the base of each flagellum and migrates to the tip. The organelle is then rolled up as the bead on it reverses direction. When the retracting bead

reaches the spore wall, the flagellum is cast off. The available evidence, then, points to retraction for the flagella of primary spores of saprolegniaceous fungi, and disjunction for those of the secondary ones. Ultrastructural studies on polymerization and depolymerization of flagellar proteins are needed if flagellum formation and retraction are to be traced and fully understood.

SPORE BEHAVIOR

It is well known that among the Saprolegniaceae one species may have more than one spore discharge pattern and, moreover, taxa of two or more genera may share similar patterns of spore release. Salvin's (1942a, b) study on species of *Thraustotheca*, *Brevilegnia*, and *Dictyuchus* gives excellent examples of the extent of such variation. Indeed, the spore discharge pattern characteristic of *Achlya* has been found (Batko, 1971) to occur in a member of a genus (*Pythiogeton*) in a totally different order of Mastigomycetes. A few additional examples -- divested of taxonomic implications -- illustrate spore release behavior more comprehensively.

Lechmere (1910:318) reported that over a five-month span he found an unidentified *Saprolegnia*, to exhibit asexual reproduction characteristic of six genera of water molds " ... on the same mycelium..." One may reliably assume, as did Coker (1923) that Lechmere had a mixed culture. Perhaps observations on mixed cultures could account as well for some of the quite peculiar morphological expressions exhibited by another unidentified *Saprolegnia* reported by M. I. Collins (1920). She described (and illustrated) dictyucoid sporangia that appear suspiciously like hyphae infected with *Dictyomorpha dioica* (Mullins, 1961). More peculiar are the "dictyuplanes" sporangia of Collins' *Saprolegnia* sp. in which the spores in a part of the sporangium emerged as in *Dictyuchus* species, and the remainder germinated *in situ*. She found also that encysted spores in a "sporocyst" (her term for sporangium) often blocked the exit orifice, and hyphal renewal was then sympodial as in species of *Achlya*. The figure (M. I. Collins, 1920: fig. 11) that depicts this renewal mechanism suggests internal rather than lateral proliferation of the hyphae. Collins also described aplanoid sporangia in which the germ tubes grew for some distance, and then swelled at the end. She thought it possible that the aplanoid condition arose from a *Dictyuchus* type of discharge, but the reasoning in support of this suggestion is obscure. Collins' report of single sporangia having two concurrent mechanisms of spore discharge is matched only by W. G. Smith's account (1878a: fig. 105) of spore emergence in *S. ferax*. He illustrated a sporangium with some spores emerging from the apical orifice as biflagellate cells while others at the base were germinating *in situ*. Such a condition seems to be little more than the result of artistic license. There are no illustrations available to confirm Horton's observation (1921) of sporangia in an *Aplanes*† sp. (probably an *Achlya*) in which some spores clustered at the orifice while others in the same asexual cell germinated *in situ*.

One of the pioneer reviews of spore behavior in the water molds (*Achlya racemosa* and *Saprolegnia torulosa*) was that published in 1933 by Höhnk. He reported that encystment and swarming accompany a high water level while reduced amounts of

water promote amoeboid motion. Germination on the other hand, is associated primarily with nutrient concentration. The important point to emerge from Höhnk's (1933) work is the proposition that there is a correlation at the generic level between the species composition of habitats and the availability of water. With respect to spore swarming among the members of the family five patterns emerge (Table 20). Collection data -- insofar as that reveals habitat preferences -- show that water molds having swarming types I and II (spores swimming immediately on discharge) occur chiefly in water. Species with type III behavior, that is, the spores cluster at the exit orifice upon release, are said to occur in swampy areas. Representatives of genera in type IV have a reduced level of spore activity at discharge (the sporangium dissolves, for instance, to release encysted cells), water molds of type V have no motile stages. Höhnk believed that fungi of categories IV and V, therefore, were forms representative of a transition to a terrestrial existence. Thus, the progression among the water molds -- expressed by their patterns of sporangial dehiscence -- from predominantly aquatic to predominantly terrestrial habitats is accompanied by a tendency for spore behavior to convert from a planetic to an aplanetic mode. Statistically significant data to support these general conclusions are absent. Weston (1919) suggested that some genera in the family consisted of individuals displaying a gradual decrease in the extent of their asexual cycle. Höhnk's later (1933) proposition of type grouping of genera (Table 20) does not digress basically from Weston's concept.

Water level alone evidently is not the only factor influencing spore behavior in the Saprolegniaceae as Höhnk's hypothesis would seem to indicate. Alabi (1972) demonstrated a shift in the manner of sporangium dehiscence in *Achlya dubia* in response to temperature. In the cool, rainy season in Nigeria, isolates of this species most often exhibited the achlyoid method of spore release, while specimens collected in the warm, dry season produced such sporangia initially, but subsequently developed the thraustothecoid type principally. When mycelium of *A. dubia* was exposed experimentally to water at 28-32 °C, higher percentages of thraustothecoid sporangia were formed than when colonies were incubated at lower temperatures. Other investigators also leave no doubt that temperature is a strong influence in spore release and subsequent behavior. Murdia (1939a) observed that 27-30 °C was the optimum range for spore formation and release in *Achlya dubia*, but that 33 °C prohibited these processes. Working with a variety of *Isoachlya anisospora*†, Bhargava (1950) found 25 °C to be the most favorable temperature for sporogenesis and discharge, while 35 °C was clearly inhibitory. He noted incidentally that spores were discharged from colonies incubated in media with a wide range of pH: 4.5-9. The study by Salvin (1941), however, is far more revealing of temperature effects than either Murdia's or Bhargava's accounts.

Salvin used two *Saprolegnia* species, *Achlya flagellata*†, *Thraustotheca clavata*, and *Dictyuchus* sp. in his investigation. Primary and secondary spore behavior in these fungi was followed in hanging drop preparations in which temperature was controlled by a device held on the microscope stage. There were substantial differences in the response behavior of the two spore types. At 25 °C the primary planonts of *S. delica*† swam faster than the secondary ones of the same or any other species used. This is

somewhat surprising in view of the degree of motility usually ascribed to the pyriform spores: they are much less vigorous swimmers than the secondary ones. Salvin also found that the laterally biflagellate planonts of the isolates maintained motility over a longer period of time and over a wider temperature range than did the primary ones. Arrhenius equation calculations showed that the primary planonts had a higher temperature coefficient than did the secondary spores. Salvin (1941) suggested that this dissimilarity reflected fundamental differences in biochemical constitution of the two spore types.

Numerous additional cases of variation in spore release and dispersal are cited by Coker (1923); these are primarily applicable to taxonomy. In his 1914 paper there is a valuable historical account of early observations on spore behavior.

REPEATED EMERGENCE

A common phenomenon in individual water molds is that of repeated encystment and excystment -- a behavior pattern induced even by relatively unrefined environmental manipulations -- of released spores. Weston (1919) washed broth-grown colonies of *Dictyuchus* sp. in water, and then placed tufts of mycelium from them in hanging drops. Some of the released planonts encysted, then excysted again and subsequently secondary spores emerged. By successively transferring a nonsexual *Achlya* through changes of cool, well-aerated water (hanging drops), Salvin (1940) induced the secondary spores to undergo five successive excystments. The motile phases of these spores retained the same size throughout the induced encystment/excystment manipulations, but the oil reserve in each diminished. Successive emergence could not be induced in *Thraustotheca clavata*. The maximum number of repeated encystments and excystments observed in *A. racemosa* is four; in *S. torulosa* it is but three (Höhnk, 1933).

TACTIC RESPONSES

Pfeffer (1883, 1884) furnished some of the pioneer work on tactic responses. He observed that spores from parasitic species of *Saprolegnia* (both the identity and the alleged parasitic nature of the fungi are unknown) were "directed" in their movement by their particular hosts. When spores from a "parasitic" *Saprolegnia* species were put in the vicinity of a fly leg in water, they swam toward that object, and clustered heavily about the torn end. Further experiments (Pfeffer, 1884) demonstrated that planonts of *S. ferax*(?) were attracted to certain concentrations of meat extract placed in capillary tubes. As Pfeffer was not aware of chemical contaminants in media the results of his experimental work are subject to differing interpretations.

Stange (1890), Miyoshi (1894), and F. Müller (1911) studied chemotactic responses of spores from water molds in a far more detailed manner than had Pfeffer. They tested individual chemicals in various concentrations, using spores from fungi in the *Saprolegnia ferax* group, among others. The chemicals were placed in capillary tubes, and these put in distilled water along with sporulating cultures of the fungi. Miyoshi

(1894) experimented with inert, perforated materials as a means of separating spore suspensions from test chemicals. Both he and Stange (1890) looked for degrees of spore attraction or repulsion, or whether the spores simply were “indifferent” to the chemicals (according to H. Ziegler, 1962, Stange found that only the secondary planonts were chemotactic; Stange’s account does not clearly make such a distinction). The results of their tests are in general agreement, although there were some chemicals not tested by both investigators. Spores of the water molds were not stimulated by KNO_3 , KClO_3 , MgSO_4 , and KH_2PO_4 , or were repulsed by these compounds. Meat extract and lecithin induced positive chemotaxy, but planonts showed no response to glycerin, leucine, lactose, fructose, glucose (at concentrations of 0.01 and 0.001%), gum arabic, or nitric, sulfuric, and hydrochloric acids. Negative chemotaxy was stimulated by such compounds as saccharose, glucose, alcohol, and various salts. Among the compounds that in certain concentrations “attracted” spores of *S. ferax* were NH_3 , phosphoric acid, asparagin, prune extract, and phosphates of calcium, sodium, potassium, and lithium. In general, F. Müller’s (1911) study yielded results much like those reported by Stange and Miyoshi. He stated, however, that the planonts of the three water molds he investigated were insensitive to heavy metals. The chief criticism to be directed at the work of Stange, Miyoshi, and Müller is that they did not eliminate the influence of oxygen as a chemical excitant, and, like Pfeffer, were probably unaware of the contaminating influences of trace chemicals in their media.

A curious tactic spore behavior was reported in 1927 by Lounsbury, but has not since been confirmed. From sporangia of a nonsexual *Isoachlya*[†], clumps of encysted spores were produced, and during erratic swimming movements planonts in the culture water struck and adhered to the masses of encysted spores. Larger “spore balls” thus were accumulated over a period of time.

Using the capillary tube method employed by Pfeffer (1883, 1884) and others, F. G. Fischer and Werner (1958a) tested solutions of a number of chlorides of alkaline and alkaline earth metals for their ability to stimulate chemotactic responses in planonts of several species of Saprolegniaceae. These investigators observed, in confirmation of Pfeffer’s work, that natural materials -- dead insects and their parts -- were spore “attractants,” and that certain combinations of amino acids with inorganic salts imitated the positively chemotropic inducement afforded by these natural substrates. Chlorides of potassium, sodium, rhodium, ammonium, cesium, and lithium (in a descending order of effectiveness) elicited chemotropic responses, and the ability of these substances to do so apparently was by the presence of amino acids. Younger spores were more noticeably chemotactic than were older ones, and on an individual species basis, there were discernible differences in response (F. G. Fischer and Werner, 1958a; see Table 21).

Although the chemical immobilization (F. G. Fischer and Werner, 1958b) of planonts is not strictly taxis, the reversibility of paralysis certainly touches upon such a response. Nicotinamide (10^{-7} M) immobilizes the flagella of *Saprolegnia mixta*[†] immediately, but the ability of the organelles to move can be restored if the spores are washed free from the chemical. Compounds such as sulfanilic acid, α - and β -aminopyridine, α -picolinamide, and n-diethylnicotinamide, structurally similar to

nicotinamide, are ineffectual in immobilizing the planonts. Nicotinamidenitril and nicotinamide ethyl ester elicit negative chemotaxy (F. G. Fischer and Werner, 1958b).

Two further analyses of planont chemotaxis have been published, a study by Ho (1975a) of spore response to nutrients, and that by N. Singh (1975) on planont movement in relation to absorbed ions. Ho has shown that the planonts of an asexual *Saprolegnia* cluster quickly around the cut surface of a hempseed put into a suspension of those spores. Moreover, if agar discs containing hempseed extract are submerged in the spore suspension in place of the seeds themselves, the spores actively swim to these discs as well. Plugs of YpSs or potato dextrose agar elicited no such response, Ho reported. However, the motile spores of *Saprolegnia* sp. were attracted to freshly cut root pieces (1-2 mm long) of *Oxalis stricta* L. and *Raphanus sativus* L., but not to roots of *Oxalis stricta* L. and *Raphanus sativus* L., to roots of *Lemna minor* L. In his experiments with tactic behavior N. Singh (1975) placed (on coverslips) resin particles to which were adsorbed ions of H⁺, Na⁺, NH₄⁺, OH⁻, Cl⁻, formate⁻, and acetate⁻. A spore suspension of *S. ferax* was affixed to the coverslip to provide a hanging drop preparation, and any tactic responses were then determined microscopically. There was no spore movement in response to the presence of OH⁻ but slight positive chemotaxis was elicited by hydrogen, sodium, and ammonium ions. Motility was preserved by spores in the presence of formate and acetate.

A penetrating analysis of spore behavior in the water molds is incorporated in the unpublished thesis by H. E. Prescott (1966). While his observations remain unconfirmed by repeated experimentation, his methodology represents a novel approach to the study of spore behavior (in *Achlya bisexualis*), and certainly his theories are provocative. Prescott hypothesized that a motile spore moves in response to a relatively simple "program" that "directs" it from a lower to a higher concentration of nutrients. Experimental evidence was collected by observations of spore movement in an ingenious slide chamber that restricted the cell to two-dimensional movement. Spore motion was photographed through precisely defined gaps in a rotating disc such that the path of the swimming cells was recorded on film at 1/12-second intervals. The movements thus detected were then analyzed mathematically to test for random and nonrandom behavior.

Prescott had noticed that spores of *Achlya bisexualis* in unrestricted swimming described a right-handed helix, attained speeds of 150-300 $\mu\text{m second}^{-1}$, and, on encountering sufficiently "intense" nutrient concentrations, made turning movements. He found also that spores rapidly accumulated around bits of hempseed in water. Observations and calculations demonstrated that a motile spore restricted to two dimensions and responding to a concentration gradient turned only to the right or only to the left and at an angle of approximately 120° at each directional shift. From a hypothetical model predicated on direct measurements and visual sightings Prescott predicted that a planont turning, for instance, only to the right and at this particular angle would eventually reach an area of high nutrient concentration from any distant point in relation to that source (Fig. 13). Thus, movement was not random, and a planont was not "required" to turn directly toward a nutrient source to reach it. For the Prescott model to be at least an approximation of actuality several requirements are

necessary (Prescott, 1966). First, the spore must move at a constant speed, in a straight line, and then execute a relatively precise degree of deflection; Prescott demonstrated that in fact this is the pathway the planonts take. Second, the cell during movement must adapt (that is, cease to respond to a previous change in concentration), and must “measure” continually the concentration gradient it encounters. Since the swimming cell meets successively different concentrations during any given time interval, it must, thirdly, record those concentration signals over time, and thus has to possess some “time-keeping” mechanism. Further work along the lines developed by Prescott, especially investigations directed at testing his theory in a three dimensional configuration, should be revealing.

RESPONSES BY PATHOGENS

A sizeable fraction of the accumulated information on tactic responses by planonts of water molds has come from studies on species of *Aphanomyces* pathogenic to vascular plants. The implications of taxis to phytopathology problems are treated elsewhere (Chapter 27; see also, general remarks by Schroth and Hildebrand, 1964).

Among the pioneers in studies of motile spore responses in plant pathogenic water molds was C. M. Haenseler (1925). From the results of experimental work on infection of pea roots (*Pisum sativum* L.) by *Aphanomyces euteiches* he concluded that the distance of planont migration in soil was probably less than $\frac{1}{2}$ inch (13 mm). Later, Cunningham (1961), and Cunningham and Hagedorn (1962a) tested *A. euteiches* for chemotactic response to roots of a number of plants in addition to peas. In instances where the spores of this water mold showed positive chemotaxis, with but one exception (root sections of alsike clover, *Trifolium hybridum*) they were attracted to and settled on the regions immediately behind the root cap. The influence of root exudates on tactic responses by planonts of *A. euteiches* was explored by Morrison (1972). At some concentrations (but not all) of pea root exudates motile spores were tactically stimulated. The magnitude of attraction elicited by exudates from various pea cultivars was not the result of differences in the major components of those root fractions, but simply attributable to the fraction concentration.

The motile spores of *Aphanomyces cochlioides* (Chapter 28) do not accumulate in easily visible masses on beet roots according to Winner (1966b). He found that planonts did encyst in the vicinity (0-1.6 mm) of the root tips.

A far more extensive study than that by Winner on tactic responses by *Aphanomyces cochlioides* was reported in 1966 by P. V. Rai and Strobel. They investigated specific potential chemotactic stimulants added to agar in capillary tubes. Having previously identified in root extracts of sugar beets nine carbohydrates and organic acids (e.g., gluconic acid, glucose, melibiose), and eight amino acids (e.g., alanine, lysine, glycine) they tested these compounds and some crude extract and neutral fraction products obtained in resin exchange columns. The crude extract of beet roots proved to be the strongest excitant of a chemotactic response by spores of *A. cochlioides*; a mixture of those amino acids identified in the beet root exudates did not elicit a tactic response but supported germ tube growth. The planonts showed slight

positive chemotaxis to the neutral fraction, but this portion of the extract had little influence on germination. The organic acids in the root extract also stimulated chemotaxis. Combinations of the various fractions had no additive effect in terms of inducing increased chemotactic response. Results of tests using individual sugars and organic acids were varied. Gluconic acid was a strong stimulator of chemotaxis, melibiose was neutral, and raffinose and ribose elicited negative chemotaxis. The data supplied by Rai and Strobel admit rather readily the conclusion that the crude beet root extract contains all the necessary "ingredients" not only for positive chemotactic response, but also for spore germination. Whether in the planted sugar beet field chemotaxis plays a major part in inoculation by spores is still obscure.

Tactic responses are also displayed by *Aphanomyces raphani* (Chapter 28). Planonts of this fungus are attracted to the roots and hypocotyls of host and also of some non-host plants (Yokosawa *et al.*, 1972). Basic, acidic, and neutral crude extracts (separated in ion exchange columns) from cabbage (*Brassica*) served as attractants to the planonts of *A. raphani*, with the basic ones being the more effective in this respect. Individual amino acids extracted from the basic fraction by Yokosawa and his associates (1974), however, did not elicit an effective response from the spores. In a continuing study of *A. raphani*, Yokosawa and Kuninaga (1979) identified the planont attractant from cabbage leaves as indole-3-aldehyde. This substance functioned as a stimulant of motile response in concentrations of 0.001–0.1 ppm. Four related indole compounds also elicited a tactic movement, but only when in a saturated solution.

The planonts of *Aphanomyces astaci* do not react in the same manner as those of some of the plant pathogenic species in this genus. Motile spores of the crayfish parasite display no chemotactic response (Unestam, 1966b) or only a very weak reaction (Unestam, 1969b) to such materials in the medium as crayfish blood, bits of the host animal, or B-vitamins. Unestam (1969d:232) viewed this general inactivity with respect to exogenous chemicals as an advantage to the fungus in that its spores would not be wasted, as it were, on "... meaningless..." substrates.

Fulton's review (1906) covers the early literature on the subject of chemotaxis. Reviews by Carlile (1966), P. M. Robinson (1973), and P. M. Robinson *et al.* (1968) constitute the chief general references on methodology and implications of chemotactic spore behavior.

TROPIC RESPONSES

The first experimental study of chemotropism in a watermold was that by Wortmann, published in 1887. His contribution was simple: if hyphae of *Saprolegnia* (sp.?) were placed in a hanging drop containing a suitable substratum, those hyphae would grow "energetically" toward that substance. Spores, Čelakovský (1897) found, also reacted chemotropically. When sporangia of *Dictyuchus monosporus* were oriented in wet mount preparations at various angles to the edges of the coverslips, germ tubes from those spores grew toward the nearest edge. To Čelakovský this was evidence of an aerotropic response.

Germ tubes from spores (and antheridial branches also) of several species of water molds respond chemotropically to localized concentrations (in agar blocks) of amino acids (Table 21). Few of the species tested by F. G. Fischer and Werner (1955) did not react to some degree. While individual amino acids failed to provoke tropic responses, combinations of a minimum of 5-7 *l*-amino acids were effective. The results reported by F. G. Fischer and Werner (1955) have since been confirmed -- and considerably expanded upon.

Musgrave and associates (1977), using germinating encysted spores of *Achlya bisexualis*, explored the possible influence of casein hydrolysate and several amino acids on chemotropic response. They performed three tests. In the reorientation test, the position of a block of agar containing the test compound (casein hydrolysate, single amino acids, or mixtures) was changed after germ tubes had begun to grow toward it. Subsequently, the casein hydrolysate diffused into the agar from a different direction to that in which the germ tubes were growing, and the incipient hyphae reoriented toward the repositioned source. Any lateral branches that developed originated only on the side of the germ hypha toward the diffusing chemical. In perforation experiments, agar containing the test chemicals was separated from agar lacking the chemicals by a centrally perforated mica disc. The latter was seeded with a spore suspension on the surface. When the germ tubes appeared they grew (where the diffusion concentration was sufficiently high) to the opening in the mica barrier, and toward the casein hydrolysate (or amino acid mix). In a third set of experiments, the surface of a strip of agar was seeded with a spore suspension. The strip was then cut, the two halves separated, and an uninoculated block containing the diffusible test substance was inserted between them. During incubation, germ tubes from spores grew toward the inserted block. Single amino acids (except cysteine) were ineffective in attracting germ hyphae except when casein hydrolysate was present as a nutrient for the germinating spores. Musgrave and his collaborators (1977) suggested that sensing "receptors" possibly were present in the endogenous transport system of the germ hyphae. In germlings of *Achlya* sp. spores, at least, all amino acids are taken in by active absorption (D. P. Singh and LéJohn, 1975).

Some single ions also have chemotropic influence on germinating spores, as N. Singh (1975) demonstrated. Anions of acetate (absorbed on resin particles) induced a negative chemotropic response by germ tubes from encysted spores of *Saprolegnia ferax*; the response to the formate anion was positive. Singh found that germination occurred in the presence of the cations of hydrogen and sodium, and the ions of chlorine. The OH⁻ ion elicited no response whatever.