

CHAPTER 25. Phylogeny and Evolution

Not long after taxonomists recognized that among the saprolegniaceous fungi there were homologous groups possessing similar devices for insuring reproduction, they began industriously arranging these groups into hierarchical clusters to suggest relationships. Although the resulting phylogenetic schemes were fashioned around the relatively simple structure of the individual species, mycologists weighted differently the few available morphological similarities and differences.

The family unit itself has not escaped attempts at phylogenetic speculation, and at the suprafamilial level, concepts of relationships have touched upon the origin of the fungi as a whole and of the Oomycetes (Mastigomycetes) in particular. In fact, more attention has been paid to the phylogenetic derivation of the larger taxonomic units in which the water molds are placed than to the infrafamilial components. For example, Margulis (1968), who proposed that the eukaryotic cell arose through a series of specific symbioses with prokaryotes, suggested that animals and most fungi had a protozoan ancestry. On the other hand, Klein and Cronquist (1967) saw the Oomycetes as misfits in the otherwise cohesive unit of organisms recognizable as the fungi. These authors thought that oomycete ancestry was traceable to a different group of algae than that from which the fungi allegedly arose. Klein (1970) adopted essentially this same view.

Chiefly, the phylogeny of the saprolegniaceous fungi has long been thought to be woven closely with some ancestral alga, and the morphological resemblance of water molds to certain existing algal groups is usually advanced to testify to the soundness of this view. The propositions that derive the Saprolegniaceae (or Oomycetes in a broader sense) from algae has, of course, gone beyond simply selecting these chlorophyllous plants to be likely ancestors (C. E. Bessey, 1903; Klein, 1970). Now it is fashionable to remove the water molds and their kind from the fungi altogether (Shaffer, 1975) and make of them honest bedfellows of the algae or some other groups (Whittaker and Margulis, 1978). Most persisting schemes advocate a direct algal ancestry for the Saprolegniaceae even though as early as 1881 de Bary (also in de Bary and Woronin, 1881) proposed a derivation of these fungi from peronosporaceous types, and a resting place for the group (de Bary, 1884) among the Ascomycetes.

Early attempts at constructing phylogenetic sequences involving the Saprolegniaceae (and the Oomycetes) were of course not influenced by evolutionary thought or necessarily accompanied by the application of principles of progressive or regressive change. When, from a body of biochemical data, various relationships between water molds and other organisms were discovered, evolutionary tendencies were considered. To be sure, the biochemical evidence for evolution in the saprolegnians (and thereby for phylogenetic sequencing) is far from broadly based. Nevertheless, interest in the phylogeny of these fungi refreshingly has been advanced by the admission that reasons for relationships can be found in aspects of behavior beyond mere morphological expression. As these biochemical studies have shown, simplicity in form and absence of a generous fossil record have made it difficult to construct phylogenetic schemes on evolutionary grounds, but have not made the task

impossible. In recognition of what he calls "...non-fossil forms...", R. T. Moore (1971:55) proposed the term paromology to designate the relationships among fungi or trace their evolution. He would restrict the concept of homology to phylogenies established on fossil evidence.

The bulk of this chapter reviews two aspects of the phylogeny and evolution of the water molds. There is first of all a consideration of the various ancestral candidates that have been selected for the Oomycetes, and the relationship of the family to other groups of organisms and -- as some would have it -- to the fungi themselves! Second, attention is given to the evidence for relationships derived from biochemical analyses. The result will be a synthesis of the traditional and modern views. A substitute proposition to replace known phylogenetic schemes is not to be sought in this account, for precisely how to read the as yet very fragmentary record of morphological and biochemical similarities and differences among the water molds and other organisms is enigmatical.

MORPHOLOGICAL PHYLOGENY

The most popular schemes of phylogeny involving the suprageneric levels of the water molds have been those deriving from some algal ancestor. Other but less widely accepted views hold that members of the Saprolegniaceae are founded in other groups of organisms or are only indirectly ascendant (or descendant) from the algae. At one extreme in viewpoint is the contention that the Oomycetes are not fungi at all, but are a group associated with Protistan (or Protoctistan) assemblages (Whittaker and Margulis, 1978).

ALGAL ANCESTRY

The very foundation of the history of the members of the Saprolegniaceae rests on their placement in the Vaucherias by Lyngbye (1819) and among the "Confervas" by Gruithuisen (1821). It should not be surprising, then, that these fungi subsequently continued to be thought of as derivations of algae, or at least as being closely related to them. Pringsheim (1858), among others, surely perpetuated this general view when he described the Saprolegniaceae as parasitic algae (these having dispensed with chlorophyll) and placed the family on a par with the Oedogoniaceae. It was Pringsheim also (1852) who saw a structural similarity between the germination of the zygospores of species of *Spirogyra* and sporangial discharge in a filamentous organism that he identified as *Achlya prolifera*. Perhaps he was observing a mixture of organisms when he derived this conclusion -- there is no way to be certain -- but doubtless he confused a water mold with an alga. Because he thought the primary spores of the then known saprolegniaceous fungi were morphologically similar to motile elements in the Chlorophycean algae, Lagerheim (1900) proposed that the water molds were derived directly or indirectly from this algal group. He evidently was not as satisfied with a possible origin of these fungi from some primitive (zoosporic) fungal group as was

Maurizio (1896b). Independent of Lagerheim, B. M. Davis (1900) concluded that the Saprolegniales departed ancestrally from algae. He held, however, that the degree of oogonium differentiation was a characteristic which was assumed after these fungi had made a recognizable appearance, and was therefore a feature uniquely derived independently of an algal ancestry.

The idea that members of the Saprolegniaceae could have descended from siphonaceous algae in general (and from the Vaucheriaceae in particular) surfaced again early in the 20th Century. In 1903, C. E. Bessey included the watermolds as a family of Hysterophytes (devoid of chlorophyll) in the "Siphoneae", and traced their origin to the Vaucheriaceae. Lotsy (1907), in establishing the Oomycetes (a group designation that included the family Saprolegniaceae) in the Siphomycetes, and seeing for this class an algal ancestry, very likely had in mind the Vaucherian type of siphonaceous algae.

It was B. M. Davis' (1903a) view that the "phycomycetes" were a cluster of independently-derived "phyla", related only through a common ancestor that produced coenogametes. He believed that the watermolds were only one group of organisms in which the sex cell type was derived from the coenogametous condition. This being so, he did not advocate a Vaucherian ancestry for these fungi, remarking that the only similarity between *Vaucheria* species and filamentous "phycomycetes" was that of the coenocytic habit. Much later, W. J. Koch (1951) was to demonstrate that the flagella on the spermatozooids (not zoospores) of *Vaucheria pachyderma* Walz. were nearly identical to those on the motile spores of *Saprolegnia diclina*. These two types of cells, he pointed out, had a single, anteriorly-directed tinsel flagellum, and a longer, posteriorly-directed whiplash one. Emphasis on this morphological resemblance, Koch (1951:129) wrote, might "... logically permit, though certainly not require, a derivation of at least some of the Phycomycetes from a Vaucheria-like ancestor."

Although Gäumann (1964) considered groups of uniflagellate fungi to have ancestral roots in the Flagellatae, he derived the Oomycetes from the Heterosiphonales (Chrysophyta: Botrydiaceae and Vaucheriaceae). According to his scheme, the Saprolegniaceae evolved in a line separate from that of the Peronosporaceae and Leptomitaceae, and possibly were the stock from which members of the Lagenidiaceae arose. Sparrow (1958) considered that there were two possible ancestral stocks for the Saprolegniales, one being some heterokont siphonaceous alga.

Speculation on the origin of saprolegniaceous fungi has also led to other algal autotrophic groups as possible progenitors. In 1942, E. A. Bessey published a phylogenetic scheme in which the Saprolegniaceae were at the apex of a line descending through the Leptomitaceae, Lagenidiaceae, and Olpidiopsidaceae to an ancestral heterokont unicellular alga. Klein and Cronquist (1967) recognized three possible phylogenetic lines (two based on chemical, functional, and structural grounds) for the flagellate fungi, but in each case, placed the Oomycetes in a direct line of descent from the Xanthophyceae. These authors suggested -- and their schemes so implied -- that the Oomycetes were not phyletically related to other fungi even though they were clearly derived from algae. Klein (1970) saw the Oomycetes as a branch arising from an

otherwise terminal group of algae, the Xanthophyta. He, too, traced the Oomycetes and other fungi (including the chytrids) along two divergent paths. The Chromophyte series (including such eukaryotic groups as the Xanthophytes and Chrysomonads) possibly served as ancestral stock for the Oomycetes, according to F. J. R. Taylor (1978). He cited as illustrative the gross similarity between the differentiated gametangia in *Saprolegnia* (species?) and those of the coenocytic Vaucherias. Taylor also suggested that one line of evidence for linking the Oomycetes with the Chromophytes was to be found in the fact that representatives of the various groups in the series have tubular cristae in the mitochondria. Cavalier-Smith (1978) derived the Oomycetes from the Xanthophytes on the basis of loss of plastids, and Leedale (1974), in his multikingdom scheme, removed the Oomycetes from the fungal kingdom, assigned them to the Kingdom Plantae, and traced them to the same ancestral stock line as the Phaeophytes, Chrysophytes, Xanthophytes, and Bacillariophyta.

According to Sagan (1967) the Oomycetes represent a branch from a continuous phylogenetic lineage, separate from the algae, but having a connection with the same basic line from which the latter arose. Eukaryotic cells, Sagan postulated, accrued from three free-living prokaryotic ancestral units, one being a "9+2 homologue". In the ascendancy leading to the Oomycetes two 9+2 homologous clones were evolved. One clone contributed a basal body that in turn gave rise to another such organelle, and from these structures two flagella eventually evolved. The second prokaryotic clone donated the extranuclear centrioles to the developing eukaryotic cells.

An ancestral pigmented flagellate analogous to members of the modern genus *Chrysomonas* (Pyrrophyta) was a likely base from which the Oomycetes descended, according to von Arx (1970). In 1967, however, this same author had held to the view that Oomycetes evolved from a siphonaceous ancestral stock in the Chlorophyceae.

ANCESTRY FROM A FLAGELLATE OR HETEROTROPH STOCK

The diplanetic condition (dimorphic planonts) was regarded by Atkinson (1909) as a fundamental character of the Saprolegniaceae. This feature, he argued, was not found among algae, and therefore the water molds could not have evolved from siphonaceous or confervoid algae. As further corroboration of his viewpoint Atkinson singled out the presence of fertilization tubes in the water molds, and the absence of any such structure among the known algal groups. Then, pointing to the occurrence of large, biflagellate spores in some chytridiaceous, uniflagellate fungi, he concluded that the biflagellate condition could have arisen if such a chytrid spore failed to divide yet was functional and gave rise to an individual in which this character was retained. Accordingly, he saw for the Saprolegniales an ancestry in the primitive, uniflagellate fungi, as did Moreau (1913-14, 1953), in agreement with P.-A. Dangeard's view. Moreau (1953) proposed that the Chytridiales, descendant from zooflagellate stock, consisted of two taxonomic groups, the Opisthomastigochytridiales and the Dimastigochytridiales. The Saprolegniales, whose members produced motile spores but not motile gametes, Moreau reasoned, would be traceable to the latter group.

De Bary (1881, 1884) fixed the origin of the Saprolegniales with the pythiaceus fungi, but E. J. Butler (1907) contended that this gave to the latter a primitive quality which they did not deserve. Instead, Butler suggested that the progenitors of the Saprolegniaceae resided with the monoblepharids, these in turn having been derived from some ancestral green alga. His argument to support this view was simply that the transition from the gametangial type of sexual reproduction characteristic of *Monoblepharis* species to that found in the Saprolegniaceae occurred before the ancestral form of these Oomycetes diverged from the algae. Scherffel (1925) saw in the biflagellate condition of representatives of *Ectrogella* a progression through *Aphanomyopsis* to the Saprolegniaceae on the one hand and to the Leptomitaceae on the other.

Separating the fungi from plants in a classification scheme, R. T. Moore (1971) proposed that the archetype for all the divisions in the "Super-Division Phycomycotera" was an ancient saprolegnoid organism. A dichotomy in progeny subsequently appeared such that two groups of zoosporic fungi evolved. The Oomycota, Moore proposed, developed a pattern of lysine synthesis distinct from that of other divisions (*see* section following). In any case, his phylogenetic dendrogram specifically singles out a saprolegnian ancestry for the present day Oomycetes. A separate subkingdom -- Fungi Inferior -- was proposed by R. T. Moore in 1974 (again referred to in a paper published in 1978) as a repository for the oomycetous forms.

One other major fungal group has been accorded the distinction of parentage for water molds. Because she found some members of the Blastocladiales to have spores with 1-3 flagella, Kanouse (1927a, b) believed it was logical to derive the Saprolegniales from the blastocladial order. This proposal has not enjoyed adoption. It is more than likely that failure of the sporangial protoplast to cleave fully in the Blastocladias that Kanouse saw resulted in chance abnormal spores with more than one flagellum. In any case, Kubai's (1978) analysis of the ultrastructure of the nuclear envelope and spindle pole bodies during mitosis suggests that the Hyphochytridiomycetes are like Chytridiomycetes in this respect and cannot serve as the bridge between blastocladial fungi and Oomycetes (LéJohn, 1974).

The origin of the saprolegniaceous fungi has been linked also to a variety of other supposedly ancestral organisms. The various organisms now considered to represent Oomycetes were traced by Scherffel (1925) to a beginning through an *Ectrogella*-like organism descended in turn from a monadine form (the *Aphelidium* group). Cejp (1933) followed Scherffel's view almost entirely in its basic concept but unlike Kanouse (1927b) and E. J. Butler (1907) excluded the blastocladial and monoblepharidaceous fungi from the oomycetous line, and gave to them a separate (but unidentified) origin. Sparrow (1958) hypothesized an ancestral biflagellate monadine origin for the Saprolegniales from which he derived the Ectrogellaceae, in contrast to Cejp's and Scherffel's view. On the other hand, Waterhouse (1962) thought it likely that the beginnings of the biflagellate fungi could be sought among the amoeboid protists. Although Whittaker (1969) assigned the Oomycetes to a subkingdom separate from that

of the "true fungi", he traced their evolution back to a common beginning, namely, to colorless, flagellated protistan unicells (Whittaker, 1959).

BIOCHEMICAL PHYLOGENY

Presumptive phylogenetic relationships based on structure and patterns of development ("life cycles") do not necessarily provide evidence on which generalizations can be made; morphological features of present-day forms unsupported by fossil evidence simply are an inadequate base. This being so, are there other characters of water molds which one may tap for measures of phylogenetic origin or derivation? Genetic data are not available; hence little can be expected from this quarter. The best clue to the availability of other parameters seems to be that provided by Bartnicki-Garcia (1970). He suggested that relatedness among fungi could be sought from three biochemical attributes: (1) chemical nature of cell components, (2) sequence of synthesis of some measurable end product, and (3) properties of enzymes. If it is assumed that the basic biochemistry of most living eukaryotes is identical (and the pen of the biochemist professes this to be so) then differences or similarities among fungi in one or more of these three attributes could assume phylogenetic significance.

NUTRITIONAL PARAMETERS

The first attempt to construct possible phylogenetic sequences through the medium of biochemical characteristics was that of Cantino (1950). His scheme, based on the nutritional and metabolic characteristics of the zoosporic fungi, was slightly modified from time to time (Cantino, 1955, 1966; Cantino and Turian, 1959) as new information came to hand. For example, digression (Cantino, 1950) of the genus *Brevilegnia* on a separate line from *Dictyuchus* and other Saprolegniales was based on the nonconformist physiological properties uncovered in *B. gracilis* by Bhargava's experimental work (1945a-c). It happens, however, that this species is a pythiaceous fungus, and therefore its characteristics are inconsequential when applied to relationships among the Saprolegniaceae. Nonetheless, Cantino's ideas at least aroused thought, and the search for biochemical similarities and differences among the Oomycetes and Saprolegniaceae took on respectability. The scheme that he proposed (Fig. 51) has not been improved upon, although it has been criticized (LéJohn, 1974) as being premature. It has one attribute that elevates it beyond most of the antedating schemes based on morphology: Cantino's system incorporates a strong (and welcome) evolutionary flavor.

Gleason and collaborators (1970) characterized the growth responses of four species of Saprolegniaceae to various amino acid and carbon sources (Chapter 16), and proposed a possible scheme of physiological evolution in the family taking place in several directions from a common ancestor. The saprolegniaceous forms were set apart from other zoosporic fungi by reason of their loss of the capacity to use sulfate sulfur; members of *Leptolegnia*, *Achlya*, and *Saprolegnia* were found to share this characteristic.

By the disappearance, additionally, of the ability to metabolize maltose, species of *Aphanomyces* and *Dictyuchus* evolved further along the saprolegniaceous line.

PATTERNS IN WALL CHEMISTRY

In 1921, F. von Wettstein reported on his analysis of the chemical nature of the hyphal wall of several species of Saprolegniaceae and, finding all of them to have cellulose (R. Wettstein, 1933) concluded that the Oomycetes were heterotrophic algae. Since that time, many analyses of the wall chemistry of various water molds have been reported (Chapter 6), and almost without exception have confirmed Wettstein's determinations, at least in part. The few exceptions, however, are quite critical ones if wall chemistry is to be used as a basis for phylogenetic speculation, and must therefore be considered at this point.

Parker *et al.* (1963), and Aronson *et al.* (1967) showed that native cellulose was not a major wall component in representatives of *Achlya*, and this was found to be true also for members of *Brevilegnia* and *Dictyuchus* (Parker *et al.*, 1963). *Saprolegnia* species similarly (Novaes-Ledieu *et al.*, 1967) are not equipped with substantial quantities of cellulose (reviewed by Bartnicki-Garcia, 1968). Dietrich (1975) concluded from the ratio of acetate to hexosamine fractions in the hyphal wall of seven species of Saprolegniaceae that a chitin fraction could be present. Should Dietrich's discovery be confirmed and found to be a characteristic of water molds, they must be allied more closely to the Hyphochytridiomycetes, among the zoosporic fungi, than has been thought possible in the past.

The chief components of the oomycete hyphal wall are alkali- and cuprammonium-insoluble glucans of β -1, 3- and β -1, 6-linkages, and as LéJohn (1974) cogently pointed out, cellulose is only a minor constituent. What had been considered to be solely a cellulose fraction is a cellulosic and noncellulosic form of β -glucan. This suggests that a refinement in terminology, at least, is necessary if wall chemistry is to be used as a peg on which to hang groups in an evolutionary sequence. Traditionally, however, the idea that Oomycetes have a cellulosic hyphal wall persists tenaciously, and shall have to be recognized.

Using a group average clustering program, Vaziri-Tehrani and Dick (1980a) constructed dendrograms based on wall-bound amino acids from certain water molds and other fungi. The dendrograms showed particular divisions of taxa reflecting dissimilarity levels. The authors postulated (p. 230) that these divisions could reflect either (1) "...sexually distinctive wall-bound proteins which are particularly abundant in heterothallic and diclinous species..." or (2) "...distinctive wall-bound proteins related to the ecology of the species." Vaziri-Tehrani and Dick (1980a) admitted that neither hypothesis "...necessarily involves any phylogenetic considerations or significance..." and (p. 225) the dendrograms "...do not provide clusters which correlate with taxonomic groups within the Oomycetes." The arguments for ecological niches by Vaziri-Tehrani and Dick (1980a:230), for example "...the eccentric *Achlya* species, are wholly aquatic...", are speculative at best, and are not necessarily borne out by habitat

type analyses which we have done on many species. A polygraph technique reported by Vaziri-Tehrani and Dick (1980b:237) "...effectively displays differences in amino acid ratios." They conclude (p. 237) that the differences to be seen among the various polygraphs (lysine:valine ratios and those for proline:valine, for instance) "...appear to correlate with [their] concepts of major taxonomic divisions..."

PATTERNS IN BIOSYNTHESIS

One of the most important contributions to the development of phylogenetic theory from biochemical parameters came with Vogel's (1960, 1964) publication on a survey of the biosynthesis of lysine by various organisms. *Achlya americana*, *A. bisexualis*, *Saprolegnia ferax*, *S. parasitica* (= *diclina*), and *Thraustotheca clavata* synthesized lysine via the α - ϵ -diaminopimelic (DAP) pathway, as did green flagellates (euglenoids) and bacteria. Chytrids, mucoraceous fungi, Ascomycetes, and Basidiomycetes produced lysine via α -amino adipic acid (AAA). From these findings Vogel (1964) derived two hypotheses. First, since bacteria possessed the DAP pathway for lysine manufacture; he argued that this was probably the most primitive system. Second, the animal-like characters of the euglenoids coupled with a pattern of lysine synthesis akin to that of the oomycetous fungi supported the notion that the latter group has affinities to the animal kingdom. In any case, Vogel stated, it was no longer possible to defend a monophyletic scheme for the origin and evolution of fungi as a whole.

By means of gas-liquid chromatography of saponified lipids from the mycelium of *Saprolegnia litoralis*, Shaw (1965) found that this species synthesized γ -linolenic acid, but not the α -isomer. Much has been made of this discovery. Since the thalli of higher fungi do not contain γ -linolenic acid, Shaw thought that the respective evolutionary pathways of these organisms might be different from those of the Phycomycetes. Erwin (1973) superimposed the information on polyunsaturated fatty acids onto the phylogenetic schemes proposed by Klein and Cronquist (1967) and Margulis (1968). He concluded that a correlation existed between the data from fatty acid analyses and the scheme put forward by Klein and Cronquist in which the Oomycetes were derived from the Chrysophycean algae. No such correlation appeared with Margulis' proposed phylogenetic sequence of major groups.

The sedimentation behavior patterns of four enzymes involved in tryptophan biosynthesis were determined by Hütter and DeMoss (1967) for 22 species of fungi (one of which was an unidentified *Saprolegnia*), a blue-green alga, and *Escherichia coli*. Five enzyme sedimentation types emerged, that of the water mold being, in common with the alga, type IV. All remaining fungi tested were representative of other sedimentation patterns. When this parcel of information was applied to Gäumann's (1964) phylogenetic proposal, the data correlated quite consistently: the Oomycetes (believed by Gäumann to have originated from an ancestral point different from that of the other fungi) were set apart by their particular behavior with respect to enzymes in tryptophan formation (Fig. 53). To Hütter and DeMoss this further supported the view that the

Oomycetes were not closely related to chytrids (these having a type I pattern of sedimentation).

Saprolegnia ferax produces cyclosteroid isomerase, and converts cycloartenol and lanosterol in its biosynthesis of sterol. From this fact, Bu'Lock and Osagie (1976) concluded that the Oomycetes were allied with algae rather than "true" fungi. Chemotaxonomic characteristics of the Oomycetes -- lysine pathway and tryptophan enzymes -- they emphasized, argued forcefully for removing this group from the fungi.

The most direct and extensive application of biochemical information to the subject of phylogeny in the Saprolegniaceae comes from the experimental work of LéJohn (1971b, 1974, 1975b) and Wang and LéJohn (1974a-c). These investigators explored various aspects of enzyme regulation in as many as 37 species of the family.

Glutamate dehydrogenase functions in a strategic position in cell metabolism, connecting oxidative metabolism to the biosynthesis of glutamate amino acids. In the Saprolegniales only the NAD-linked type III glutamate dehydrogenase is present (LéJohn, 1971b, 1974), and the *d* (-) form of NAD-linked lactate dehydrogenase is synthesized. Also in these fungi the NAD-linked isocitrate dehydrogenases are absent (LéJohn, 1974), although Wang and LéJohn (1974c) report isozymes of NADP-specific isocitrate dehydrogenase in water molds. Considering the activator and inhibitor properties of the type III glutamate dehydrogenases LéJohn (1971b) suggested that an evolutionary trend could be seen among the zoosporic fungi (Fig. 52). As is evident, his concept affords the Hyphochytridiomycetes a prominent part in the derivation of saprolegniaceous fungi, but this is not necessarily a role supported on morphological grounds.

LéJohn (1974) considered that the Oomycetes are unique with regard to NAD-specific glutamate dehydrogenase, for unlike the higher fungi, they do not also have the NADP-linked analogue. He proposed that two evolutionary tendencies might be recognized with regard to this property. The Oomycetes are perhaps evolving, LéJohn suggested, toward an NADP-linked dependency, and at some earlier point in evolution the higher fungi may have achieved this ability from ancestral Oomycetes. Alternatively, the possession by Oomycetes of only the NAD-dependent glutamate dehydrogenase system came into existence coincidentally with their evolution, and thus they are possibly unrelated to the fungi (LéJohn, 1974).

The investigation by DelValle and Asensio (1978) of ATP-dependent hexose kinases in a variety of microorganisms is alleged to add to the accumulating evidence for specific biochemical divergences among the major groups of zoosporic fungi. They found that *Saprolegnia litoralis* (the only oomycete tested) produced two types of hexose kinase activity, one type catalyzing glucose phosphorylation, and the other functioning in the phosphorylation of fructose and mannose. The Chytridiomycetes (represented solely by *Allomyces arbuscula* E. J. Butler) and the Hyphochytridiomycetes (*Hyphochytrium catenoides* Karling as the one example), on the contrary, have but one hexose kinase system. Many more representatives of these three fungal groups must be analyzed before the findings reported by DelValle and Asensio can be evaluated properly for their impact on relationships.

The parameters of cell wall chemistry, tryptophan biosynthesis, and the lysine pathways were favored by Bartnicki-Garcia (1970) as phylogenetic markers. To what degree do these parameters mesh with Gäumann's (1964) scheme of phylogeny based on morphology? As Figure 53 shows, there is some degree of correlation, save principally in the selections of the presumed ancestral forms.

Olive (1975) has applied the biochemical parameters of relationship to taxonomy at the higher levels. He recognizes two classes, Oomycetes and Hyphochytridiomycetes, in the Phylum Pantonomycota. The fungi included in this phylum are in part characterized by the DAP route for the biosynthesis of lysine, and hyphal walls typically containing cellulose.

PATTERNS IN NUCLEOTIDES

Are evolutionary and phylogenetic trends to be found in the regulatory nucleotides (DNA and RNA) of the Saprolegniaceae? Although the DNA base composition data assembled by Storck and Alexopoulos (1970) and B. R. Green and Dick (1972) show some coincidental taxonomic groupings according to the ranges of GC content they do not appear to correlate with any supposed phylogenetic tendencies (LeJohn, 1974).

An oogonial strain of *Achlya ambisexualis*, among other fungi, was analyzed by Lovett and Haselby (1971) for the weight of its 25S RNA fraction (species). The data from all electrophoretic determinations supported the hypothesis, they claimed, that the Oomycetes had an origin independent of all other fungi. Ojha *et al.* (1975) sought out nucleotide sequence homologies (absorbance-temperature profiles, and buoyant density gradients) among three uniflagellates -- *Allomyces arbuscula*, *A. macrogynus* (Emerson) Emerson and Wilson, and *Blastocladiella emersonii* Cantino and Hyatt -- and the two biflagellate species, *Achlya radiosa* and *Saprolegnia ferax*. The results of their determinations showed *S. ferax* to be "distantly related" to the species of *Allomyces*. Measuring the polyadenylic acid-RNA fraction in the mycelium of *Achlya ambisexualis* Silver and Horgen (1974:254) found it to be smaller than that of mammals, but very similar to that of yeasts. Of this they wrote that "...evolution has resulted in progressively longer poly(A) pieces on the mRNA molecule in organisms with greater phylogenetic complexity."

There seems little recourse but to conclude from what is known of nucleotide constituency that there is not as yet in this factor a parameter of much heuristic value in determining phylogenies.

ULTRASTRUCTURAL PHYLOGENY

Is it possible to resolve any problems in the phylogeny of the Saprolegniaceae through an exploration of their subcellular machinery -- a micromorphological approach, as it were? While commendable conceptual progress has been made in the field of comparative ultrastructure of water molds, there has been little impact on

projecting relationships at any taxonomic levels. The subject, however, is not entirely *in vacuo*.

In 1951, W. J. Koch called attention to the similarity in structure and position of the flagella on the spores of *Saprolegnia diclina* to those on the spermatozoids of *Vaucheria pachyderma*. Sagan (1967), as we have seen, regarded the 9+2 flagellar structure as a fundamental homologue in the evolution of Oomycetes.

Some aspects of subcellular endogenous structure also have been cited as indicative of evolutionary tendencies. Those members of orders of biflagellate fungi that have been examined have dictyosomes, according to R. T. Moore (1971:51), while the uniflagellate species do not (nothing is positively known of the Monoblepharidales in this respect, Moore remarked). The presence of dictyosomes, he suggests, is accompanied by a more precise degree of subcellular organization than is evident in fungi lacking these organelles.

Although he predicted it was not possible to evaluate meaningfully subcellular structural patterns as phylogenetic markers, Heath (1975c) did point to certain characters in the Oomycetes that were at least suggestive of relationships. In the nuclear division of leptomitaceous fungi, extensions of the nuclear membrane ("horns") beyond the centrioles are lacking. This, Heath suggested, might imply that these zoosporic fungi were not closely related to the Oomycetes. The orientation of centrioles at 180° -- as in certain saprolegnians (Heath and Greenwood, 1968, 1970c; Heath, 1974a, b) -- could possibly represent a common ancestry among those organisms having this feature. If this is so, then the Oomycetes might be accommodated on the same evolutionary branch as the plasmodiophoraceous fungi (except that there is no mastigonemate flagellum in members of the Plasmodiophoromycetes). Finally, Heath (1975c) has singled out the concertina-like collar at the base of the flagella (Heath and Greenwood, 1971) in spores of *Saprolegnia ferax* as a possible indicator of relationships. This organelle recalls certain algae (Xanthophyceae, Chrysophytes). Cavalier-Smith (1978) emphasizes the importance of flagellar diversification in phylogeny, and while he uses this feature as a prominent part of the delineation of seven monophyletic kingdoms of eukaryotes, he still derives the Oomycetes from a xanthophycean ancestry.

Some of the ultrastructural events and structures associated with mitosis and meiosis in the fungi also have been cited as indicators of inter- and intra-group relationships. Kubai (1978) noted that there are two divergent mitotic types in the Mastigomycotina. In members of the Chytridiales, for example, the nuclear envelope is open during mitosis. The saprolegniaceous fungi (representatives of *Saprolegnia* and *Thraustotheca*), on the other hand, are characterized by a closed nuclear envelope during division. All Oomycetes seem characterized by centric mitosis (Fuller, 1976), and in at least one species of Saprolegniaceae, *S. terrestris* (Howard and Moore, 1970), meiosis takes place within a single, intact nuclear envelope, just as is known to occur in some red algae, and in *Saccharomyces cerevisiae* (Moens and Rapport, 1971).

Drawing upon ultrastructural characterizations (Heath, 1978; 1980b, c) of fungal mitosis, Heath (1980a) has attempted within some rather severe limitations to suggest relationships among taxa. Variations in the pattern of mitosis among fungi, as shown

by a phenogram constructed by application of a cluster analysis technique, indicate that the Oomycetes (Oomycota) cluster with the Ascomycetes, Basidiomycetes, and Dictyostelids, and are remote from uniflagellate fungi. The highest degree of similarity in the patterns of mitotic events where known in species of *Achlya*, *Saprolegnia*, *Thraustotheca*, and *Aphanomyces* is with members of *Phytophthora*. Heath's application of numerical taxonomic methodology to characteristics of fungal mitosis is quite preliminary since some taxa used in the cluster analysis lacked substantial portions of information on the pattern of mitosis. In a very detailed review article, Heath (1980d) speculates further on the evolution of mitosis and its use as an indicator of phylogeny.

INFRAFAMILIAL PHYLOGENY

Infrafamilial taxa of the Saprolegniaceae have not escaped phylogenetic speculation. In retrospect, it seems natural that the morphological parameter first seized upon as a base for phylogeny was the motile spore. Humphrey (1893) regarded the diplanetic (=dimorphic) character of the motile spores as a primitive condition, and therefore placed *Saprolegnia*, *Leptolegnia*, and *Pythiopsis* at the base of a phylogenetic series of genera (Fig. 54). Elements of the genus *Achlya* then persisted, according to his scheme, as the base for the remaining genera. Humphrey argued that suppression of only the primary spore, as in *Achlya* species, was carried to an extreme -- elimination of both motile spore types -- in *Aplanes*. Moreover, he held the view that if those elements of ancient *Achlyas* possessing some dictyosporangia changed in such a fashion that the sporangium wall disintegrated, thraustothecoid species would evolve. Should the spore walls coalesce and multiple exit pores persist, *Dictyuchus*-like ancestral forms would arise. Representatives of the genus *Aphanomyces* were merely degenerate *Achlyas*, Humphrey thought.

Unlike Humphrey, E. J. Butler (1907) regarded the primary spore as having evolved later than the secondary one, and saw a greater phylogenetic role for the presumably ancient species of *Aphanomyces* and *Pythiopsis*, than had Humphrey: these genera represented links between saprolegniaceous and pythiaceous fungi. According to Atkinson (1909), diplanetism (=dimorphism) reached a high degree of specialization in the Saprolegniaceae, even though this characteristic ultimately disappeared in what were then recognized as aplanoid species. In contrast, to E. J. Butler's (1907) view, Höhnk (1933:59) believed that the primary planont of the Saprolegniaceae was, in terms of evolution, the earliest spore form to be developed, but considered that only the laterally biflagellate planont had "... phylogenetic value for this group of fungi." Accordingly, Höhnk visualized an evolutionary progression from a primitive *Achlya* through ancestral *Isoachlyas* and species of *Saprolegnia* to the advanced group of *Pythiopsis* species. He regarded the multiosporous condition as more primitive than the single-egged one, hence believed that he had additional evidence for the primitiveness of the genus *Achlya*.

Both Apinis (1929a) and Cejp (1933) supported Scherffel's view (1925) that the saprolegniaceous forms were derived in an ascending (progressive) fashion from an

Ectrogella ancestor via some form resembling an *Aphanomyopsis*. Apinis thought that the subfamily Achlyeae -- he recognized taxa at this level -- was derived from *Aphanomyopsis*, and by further reduction in the number of motile stages of the spores, representatives of the subfamily Geolegnieae (and some Brevilegnias) were evolved. Cejp (1933) evidently followed Apinis' scheme for the derivation of the Geolegnias from ancestral Achlyas, but believed that *Aphanomyces* was an important link between the Achlyas and *Aphanomyopsis* (Fig. 55). The subfamily Saprolegnieae also was derived from *Aphanomyces* by Cejp (1933), as were certain parasitic forms which he assigned to the order Saprolegniales.

Although he was not able to predict the phylogenetic end point for species of *Achlya*, J. R. Raper (1959:113) suspected that they were in a state of evolutionary transition. He cited in evidence the varied sexual reactions -- sometimes interfertile -- between homothallic (monoecious) and heterothallic (dioecious) isolates, and the success of induced interspecific and intergeneric matings.

Do biochemical analyses provide any clues to evolutionary sequences among the genera of the Saprolegniaceae? Only Wang and LéJohn (1974a-c) have explored this possibility at all extensively. Their data on electrophoretic isozyme mobility patterns of glutamate, *d* (-) lactate, malate, and isocitrate dehydrogenases from various water molds show some degree of consistency among species of a few genera, but there are enough anomalies to suggest that evolutionary patterns cannot be extracted with confidence (Wang and LéJohn, 1974c). Experimental data on enzyme regulation show that the pythiaceus fungi are more sensitive to allosteric control by guanosine triphosphate and are also more complex in their regulatory isozyme properties than are members of the Saprolegniaceae (Wang and LéJohn, 1974a, b). It would seem that too little is yet known of biochemical parameters among a large number of species of water molds to permit decisions on relationships and phylogeny among genera.