PART I

CRITERIA FOR DIAGNOSES OF FLAGELLATE FUNGI

M. W. Dick, *Straminipilous Fungi*

THE ZOOSPORE

Asexual reproduction: definitions of sporangia, sporangiospores and zoospores

For most of the organisms discussed here, the normal method of asexual reproduction is by spores ***originating from*** a sporangium. Exogenously produced conidia or chlamydospores are either not known or of minor significance. In eucarpic organisms with hyphae, the sporangia are separated from the assimilative mycelia by septa or plugs. For holocarpic organisms, in which all nucleated protoplasm is utilized in spore production, the definition of a sporangium becomes difficult: at maturity, the assimilative thallus is converted into a sporangium, frequently with little morphological modification other than the formation of an exit tube and dehiscence papilla.

The normal definition of a **sporangium** is a spore container or vessel, but in many of these fungi the protoplasm is not in the form of spores when inside the container, and a looser definition is required. Cleavage of the protoplasm to form the spores can take place after the protoplasm of the sporangium has been expelled, and therefore does not necessarily occur in any recognizable, walled, morphological structure.

In much of the mycological literature the terms sporangium and zoosporangium are used synonymously. A flagellate asexual spore is known as a **zoospore**, and a sporangium ***containing zoospores*** is a **zoosporangium**. Strictly, a spore is a walled structure, and only a spore formed ***within*** a sporangium is a **sporangiospore** (cf. *Thraustotheca* or *Eurychasmopsis* - Canter & Dick, 1994; Canter, Heaney & Lund, 1990). The term **planont** is therefore useful when the term sporangiospore is inappropriate. The original definition of a planont (Sparrow, 1943) is of any ***motile*** (and therefore usually naked) cell; it should be noted that this definition specifically ***includes*** gametes and zygotes as well as asexual spores. Thus the term planont is usually interpreted as a ***flagellate*** cell: the term **aplanospore** is used to include any naked, amoeboid or non-amoeboid ***mobile*** cell as well as a walled sporangiospore.

The fungal zoospore has been defined by Lange & Olson (1983) as follows: "true zoospores are formed in a zoosporangium, by mitotic nuclear divisions and directly give rise to a vegetative thallus". Such a definition is untenable for straminipilous fungi. In *Pythium* the zoospores are differentiated ***outside*** the sporangium. In the *Peronosporomycetes* there are two ontogenetic patterns of sporangial development: in the *Saprolegniales* (Humphrey, 1893) and *Pythiales* (Hemmes, 1983) the zoosporangial protoplasm is delimited with its full complement of nuclei, but in the *Peronosporales* (*Peronospora*, Trigiano & Spurr, 1987; *Bremia*, Tommerup, 1989; *Plasmopara*, Burruano *et al.*, 1992) nuclear division occurs in the immature sporangium. In *Phytophthora* (Maltese, Conigliaro & Shaw, 1995) and *Albugo* (Khan, 1976) nuclear division and nuclear abortion occur in the immature sporangium. Doubt is thereby cast on whether *Phytophthora* should be classified in the *Pythiales* or the *Peronosporales*. In *Saprolegnia* polyplanetism means that the development of the subsequent vegetative thallus may be ***indirect***.

The **zoospore** must be more loosely defined as:

> a motile (and flagellate or potentially flagellate) naked cell or planont, ***normally*** uninucleate, and ***usually*** functioning as a dispersal agent for the same phase of the life-history from whence it was generated.

Oertel & Jelke (1986) reported the formation of abnormal, multinucleate zoospores in *Phytophthora*.

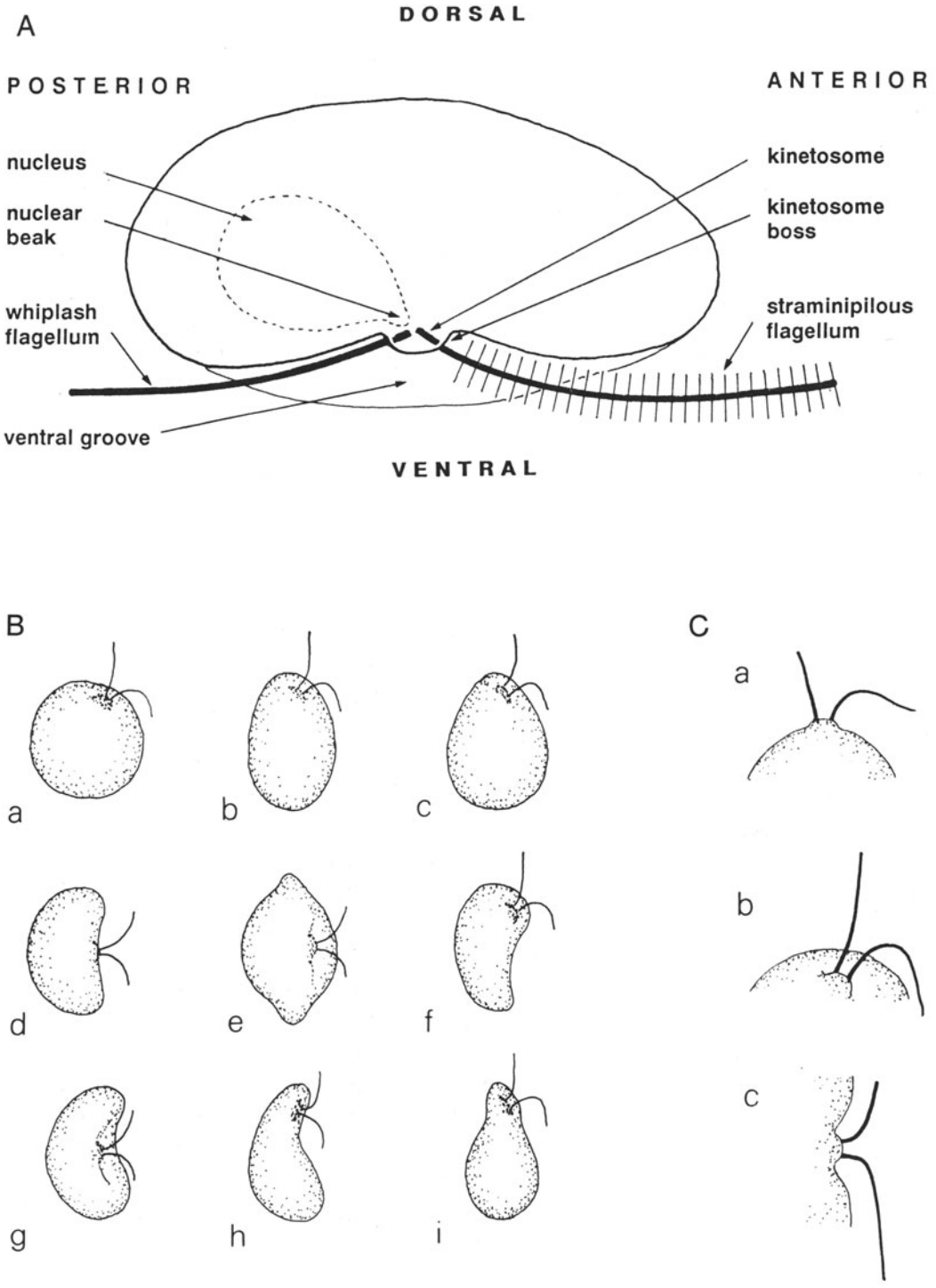

FIGURE I: 1. Zoospore orientation, terminology, shapes and flagellar insertion. A. Terminology; orientation of the principal-form zoospore. B. Zoospore shape, excluding flagellar insertion: a: spherical, anterior/posterior and dorsiventral axes equal; b: ellipsoid, anterior/posterior axis longer than the dorsiventral axis, symmetrical about the axes; c: ovoid, anterior/posterior axis longer than the dorsiventral axis which is greatest in the posterior half, symmetrical about the anterior/posterior axis; d: hemispherical, symmetrical about the dorsiventral axis; e: limoniform, ellipsoid with prolongation at the ends of the anterior/posterior axis; f: obovoid (or obpyriform), anterior/posterior axis longer than the dorsiventral axis which is greatest in the anterior half, asymmetrical; g: bean-shaped; h: grape-seed-shaped (arcuate pyriform); i: pyriform - ovoid, with a concave profile in the anterior half, symmetrical about the anterior/posterior axis. C. Flagellar insertion: a: apical; b: subapical; c: lateral. Fig. I: 1 A reproduced from Dick (1999: fig. 2 A) by permission of Academic Press.

A further modification of this definition from 'naked cell' to 'unwalled cell' would be necessary to encompass the thraustochytrids (but not the labyrinthulids) which have an investment of scales over the zoospore surface (*Althornia,* Jones & Alderman, 1971; *Thraustochytrium*, Kazama, 1974*a*; *Ulkenia*, Raghu Kumar, 1982*b*). In the *Chytridiomycetes*, at least, there is some evidence for a zoospore "cell coat" (Fuller, 1996: 168, and references therein). Compare also *Perkinsus* (*Labyrinthomyxa* of Perkins, 1976; regarded by Levine (1978) and Porter (1990) as an apicomplexan excluded from the *Labyrinthista*; (see PART V) and for molecular biology of this organism refer to Goggin & Barker, 1993; and Fong *et al.*, 1993.

Zoospore morphology and motile phases

Descriptions of zoospore morphology have become confused partly because of the definitions employed and partly because different structures or conditions have sometimes been described by the same words. Diagrams of zoospore symmetry and terminology are illustrated in Figure I: 1.

The phylogenetic significance of flagellar form and insertion was still debatable two or three decades ago, so it is not surprising that specific diagnoses and descriptions were imprecise regarding relative flagellar lengths and ornamentation. So few organisms have been studied by Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) that this systematic account must attempt an interpretation of the original descriptions in the light of reviews of ultrastructure, such as those by Barr (1981*c*), Barr & Désaulniers (1987*b*, 1989*a*) and Powell *et al.* (1985). The more structural/physiological/biochemical aspects, as opposed to the structural/taxonomic aspects, of zoospore organization are ably reviewed by Fuller (1996), with literature citations that hardly overlap with those given here.

The term 'flagellum' has received different definitions in recent years, sometimes being restricted to prokaryote appendages (Margulis *et al.*, 1990). The term 'cilium' has a long historical documentation, and has been preferred by some recent authors (e.g., Cavalier-Smith, 1991, 1993) but no consensus has been achieved. I prefer to retain 'flagellum' for cells with only one or two (perhaps a few) relatively long appendages for which the length of the quasi-sinusoidal flagellar beat is appreciably shorter (say, a third to a quarter of) the length of the flagellum. The term 'cilium' is more suitable when the appendage is replicated over the surface of the organism, often with interconnecting kinetids, and/or for those organisms in which the length of the quasi-sinusoidal wave is more nearly equal to or shorter than the length of the appendage (e.g., ciliates and *Chlamydomonas*). For straminipilous fungi and plasmodiophorids the term 'flagellum' is more appropriate.

It should be noted that substantial differences in zoospore volume and concomitant cytoskeletal complexity may influence zoospore shape and ultrastructure. Differences in zoospore functions between the life-histories of different but related species will also be reflected in biochemical biodiversity and complexity.

In the great majority of the peronosporomycetes there is only *one* zoospore form, this is the **principal zoospore** form, which is reniform or bean-shaped with flagella laterally inserted in a groove (Dick, 1973, 1990*a*, 2000*c*). The flagella are inserted on a protuberance, here termed the **kinetosome boss**, which may form a dyke bridging the flagellar groove (Dick, 1997*a*). The angle of divergence between the two flagella (and their subtending kinetosomes) is approximately 130°-150° (Barr, 1983). The principal zoospore exhibits polyplanetism (see p. 20) in both the *Saprolegniomycetidae* and *Peronosporomycetidae*. Whether the polyplanetic sequence involves mitosis is not established: if there is mitosis, the cyst is functioning as a microsporangium (Willoughby, 1977), but even if there is no mitosis the cyst may still be equivalent to a microsporangium (Pearson, Pike & Dick, 1991). In a small minority of taxa (*Saprolegnia*, *Protoachlya* and perhaps *Pythiopsis*) the more or less ovoid zoospore formed within the zoosporangium may develop flagella that are apically (or near-apically) inserted (Holloway & Heath, 1977*b*; Barr & Désaulniers, 1987*b*;

see also Money *et al.*, 1987). This zoospore, which has been termed an **auxiliary zoospore** (Dick, 1973, 1990*a*, 2000*c*), is a poor swimmer and there is no good evidence that it can ever show polyplanetism. It has often been asserted (e.g., Barr, 1983, Barr & Désaulniers, 1989*a*) that apical flagellar insertion represents the primitive state: there is no evidence to justify this statement (see p. 32). Certainly in *Saprolegnia* and *Protoachlya* any zoospore produced from a first-formed, auxiliary sporangiospore cyst is of the principal form, and the auxiliary zoospore may be merely an ecological adaptation.

The terms **primary zoospore** and **secondary zoospore** have different definitions for the plasmodiophorids compared with their use in much of the earlier literature on straminipilous fungi. These terms should be restricted to the occurrence of zoospores in different phases of the life-history, as in the plasmodiophorids, where the primary zoospore is derived from the resting cyst and the secondary zoospore is derived from the zoosporangium. A further source of confusion has recently been introduced by Glockling & Beakes (2000*a*) who have used the term 'secondary spore' as a synomym for the glossoid spore (gun cell) in *Haptoglossa*. The terms 'primary zoospore' and 'secondary zoospore' should not be used when referring to the *Peronosporomycetes*.

Of the lagenidiaceous fungi, *Lagenidium giganteum* has zoospores of the principal form (Domnas, Jaronski & Hanton, 1986; Brey & Lebrun, 1987). However, in *Salilagenidium callinectes* (Couch) M. W. Dick *loc. cit.* (Bland & Amerson, 1973*a*; Gotelli, 1974*b*), although the zoospore is described as bean-shaped, the flagellar attachment was stated to be sub-apical without any groove. Similarly, *Haliphthoros milfordensis* Vishniac (Overton, Tharp & Bland, 1983) has an ovoid zoospore with sub-apical flagellar insertion. The flagella appear to be inserted on a kinetosome boss, but again there is no indication of a flagellar groove. Schnepf, Deichgräber & Drebes (1978*d*) describe the zoospore of *Lagenisma* as bean-shaped, with lateral flagellar insertion in a "shallow sinus". The zoospore shape and flagellar insertion are not clearly depicted in the TEMs of a fourth marine genus, *Ectrogella* (S. Raghu Kumar, 1980*b*). In *Olpidiopsis saprolegniae* (A. Braun) Cornu var. *saprolegniae* (Bortnick, Powell & Bangert, 1985) the zoospore is elongate-ellipsoid (pyriform) with a narrower tip, the flagella are inserted sub-apically and there is a distinct, but small flagellar groove (M. J. Powell, pers. comm.). This, much smaller, zoospore apparently swims in a manner equivalent to that of the principal zoospore form of the *Peronosporomycetes* (Bortnick *et al.*, 1985). In *Lagena* the zoospore is ovoid or broadly pyriform with the flagella apically or sub-apically inserted (Barr & Désaulniers, 1987*b*) (after motility and prior to encystment the flagellar insertion readjusts to an apical position - D. J. S. Barr, pers. comm.). A TEM section of the zoospore of *Crypticola* (Frances, Sweeney & Humber, 1989) depicts an obpyriform zoospore with sub-apical flagellar insertion on a kinetosome boss. The zoospore of *Blastulidium* (Manier, 1976: fig. 3) is ellipsoid with sub-apical flagellar insertion. In contrast to these genera *Ducellieria* (Hesse, Kusel-Fetzmann & Carniel, 1989) has zoospores which are ellipsoid with distinctly lateral flagellar insertion in a groove.

In most other genera the shape of the zoospore and insertion of the flagella are less well established, relying on light microscope observations. When first-formed, the zoospore of *Syzygangia zygnematicola* M. W. Dick (Dick, 1997*b*) is apparently of the principal form, but it becomes more or less spherical when swimming (Ivimey Cook, 1935). Living material of dehiscing sporangia of *Myzocytiopsis lenticularis* (G. L. Barron) M. W. Dick (Dick, 1997*b*), a parasite of nematodes, also developed initially quiescent biflagellate zoospores which were nearly hemispherical with flagella inserted in the middle of the concave side (Glockling, 1994; Glockling & Dick, 2000). Glockling & Beakes (2000*b*) have described a range of zoospore forms and zoosporogeneses for *Myzocytiopsis*. In *Haptoglossa* (Barron, 1980) and *Myzocytiopsis humicola* (G. L. Barron & Percy) M. W. Dick (Barron & Percy, 1975; Dick, 1997*b*) the zoospores (photographs of living cells) are narrowly obpyriform with sub-apical flagellar insertion; in *Ciliatomyces* (Foissner & Foissner, 1986*b*, 1995) the zoospores (drawn) are also obpyriform with sub-apical flagellar insertion (***transverse*** sections ***below*** the region of flagellar insertion are described as reniform, due to a shallow groove). There are no TEMs of zoospores of the marine genera *Sirolpidium* or *Pontisma*, but

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Sparrow (1934) has described the zoospores as narrowly pyriform, with a curved axis, and flagella inserted laterally on the attenuated anterior part.

In the plasmodiophorids the zoospore is subspherical and the flagella are apically inserted (Barr & Allen, 1982; Kole & Gielink, 1962). *Endesmosarca*, a genus possibly related to the plasmodiophorids, has an ellipsoidal zoospore with sub-apical flagellar insertion in a groove (Erdos & Olive, 1971; Olive, 1975). *Nephromyces* is a posteriorly biflagellate protoctist with some fungal characteristics (Saffo, 1981; Saffo & Fultz, 1986). Uniflagellate taxa tend to have flagella inserted apically (hyphochytrids - Cooney, Barr & Barstow, 1985) or posteriorly (chytrids - Lange & Olson, 1979) but there are exceptions such as *Rozella*, in which the flagellum is inserted at the base of a lateral, posteriorly directed canal (Held, 1975) (see p. 246).

Flagellar length is difficult to measure, and may be liable to misinterpretation because of partial withdrawal (Lange & Olson, 1983). The semi-conservative replication of flagella in heterokont biflagellates (Andersen *et al.*, 1991; Beech, Heimann & Melconian, 1991) presents a further complication, but its significance for zoosporic fungi is unclear. There may also be some lack of synchrony in the withdrawal of the two flagella (Holloway & Heath, 1974). Nevertheless, photographs of living cells of *Haptoglossa* and *Myzocytiopsis* indicate a very different proportionality in flagellar length with respect to body size, compared with *Ducellieria*, which has much longer flagella. Zoospores of other taxa are illustrated in Fuller & Jaworski (1987).

The ***shape*** of the zoospore, the ***precise pattern*** of flagellar insertion, and the ***relative lengths*** of the flagella to each other and to the zoospore all need to be documented, if possible, when describing new taxa.

Diagrams of representative ranges of the size and form of biflagellate zoospores are illustrated in Figures I: 2A and 2B on the following pages.

FIGURE I: 2. Zoospore outlines, drawn to the same scale. Composite drawings taken as far as possible from light microscope and TEM illustrations (principal sources indicated in brackets). Note the presence or absence of the flagellar groove and the kinetosome boss (which is relatively more prominent in the smaller zoospores). The presence and arrangement of tubular tripartite hairs is diagramatic, based on a shaft length of 1μm for the tripartite tubular hair, except where it is known to be longer (the straminipilous flagellum is interrupted by '?' where this feature has yet to be verified); the acronema has been indicated where known to occur.
Figure I: 2A: a: *Saprolegnia* sp. principal form zoospore (Crump & Brandon, 1966); b: *Saprolegnia* sp. auxiliary form zoospore (Crump & Brandon, 1966); c: *Salilagenidium callinectes* (Bland & Amerson, 1973*a*); d: *Lagenidium giganteum* (Domnas *et al.*, 1986); e: *Ectrogella perforans* (C. Raghu Kumar, 1980*a*); f: *Haliphthoros milfordensis* (Overton *et al.*, 1983); g: *Myzocytium megastomum* (Canter, 1947); h: *Eurychasma dicksonii* (Sparrow, 1934); i: *Crypticola clavulifera* (Frances *et al.*, 1989); j: *Ducellieria chodatii* (Hesse *et al.*, 1989); k: *Gracea gracilis* (Dick, 1997*a*, 2000*d*); l: *Rhizidiomyces apophysatus* (Fuller & Reichle, 1965); m: *Sirolpidium bryopsidis* (Sparrow, 1934); n: *Pontisma lagenidioides* (Sparrow, 1934). **Fig. 2B**: a: *Lagena radicicola* (Barr & Désaulniers, 1990*a*); b: *Phytophthora palmivora* (Hemmes, 1983); c: *Haptoglossa mirabilis* (Barron, 1980); d: *Ciliatomyces spectabilis* (Foissner & Foissner, 1986*b*); e: *Woronina pythii* (Fuller & Jaworski, 1987) f: *Polymyxa graminis* (Barr & Allan, 1982); g: *Plasmodiophora brassicae* (Aist & Williams, 1971); h: *Endemosarca ubatubensis* (Olive, 1975); i: *Nematophthora gynophila* (Kerry & Crump, 1980); j: *Olpidiopsis saprolegniae* (Bortnick *et al.*, 1985); k: *Olpidiopsis varians* (Martin & Miller, 1986*b*); l: *Blastulidium paedophthorum* (Sigot, 1931); m: *Syzygangia zygnematicola* from zoosporangium (Ivimey Cook, 1935); n: *Syzygangia zygnematicola* from oospore (Ivimey Cook, 1935); o: *Rozella allomycis* (Held, 1975); p: *Dictyomorpha dioica* (Mullins, 1961); q: *Myzocytiopsis lenticularis* (*loc. cit.*); r: *Labyrinthula* sp. (Amon & Perkins, 1968); s: *Thraustochytrium* sp. (Kazama, 1974*a*); t: *Lagenisma coscinodisci* (Schnepf *et al.*, 1978*d*).

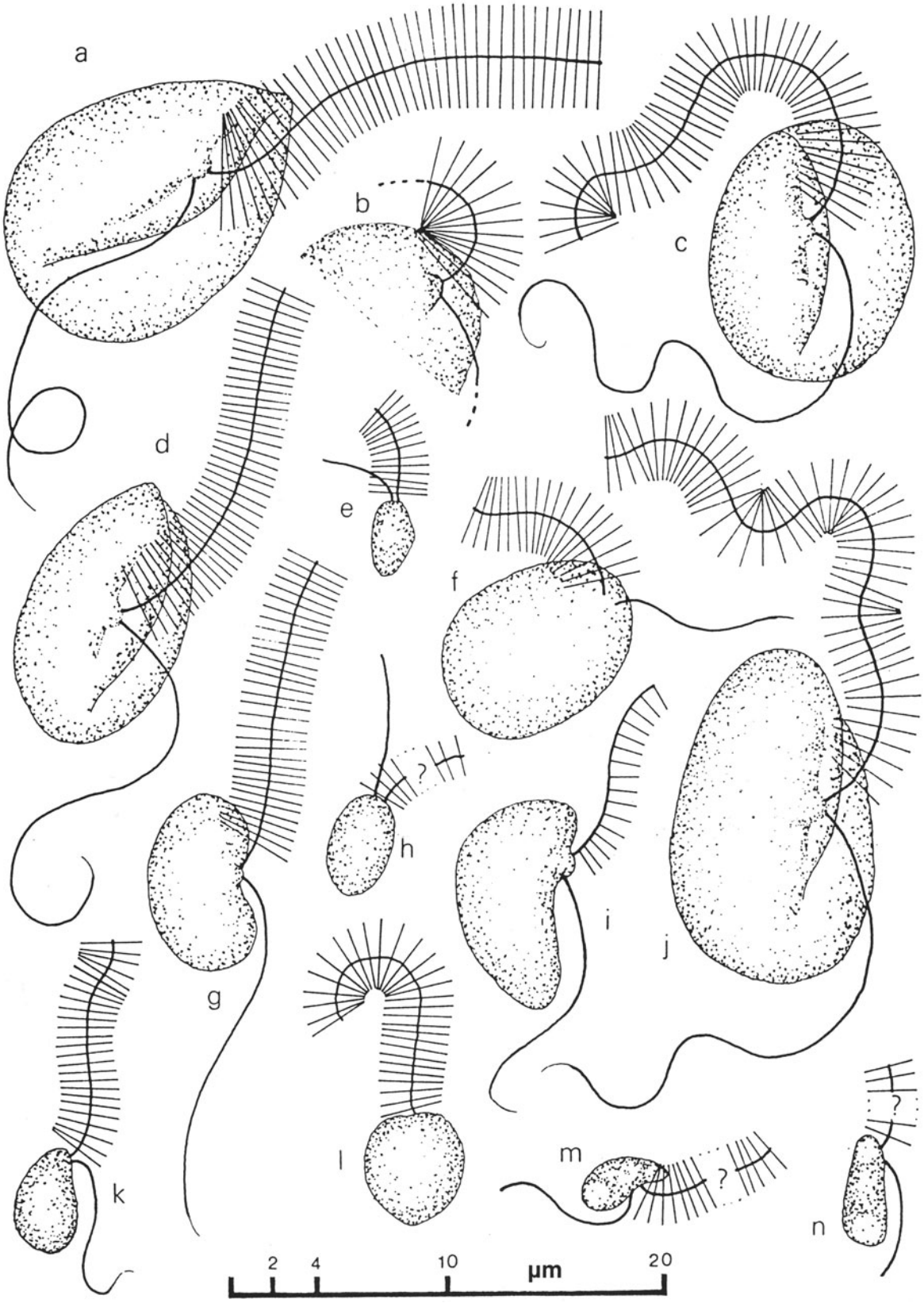

FIGURE I: 2A: a: *Saprolegnia* principal form; b: *Saprolegnia* auxiliary form; c: *Salilagenidium*; d: *Lagenidium*; e: *Ectrogella*; f: *Haliphthoros*; g: *Myzocytium*; h: *Eurychasma*; i: *Crypticola*; j: *Ducellieria*; k: *Gracea*; l: *Rhizidiomyces*; m: *Sirolpidium*; n: *Pontisma*.

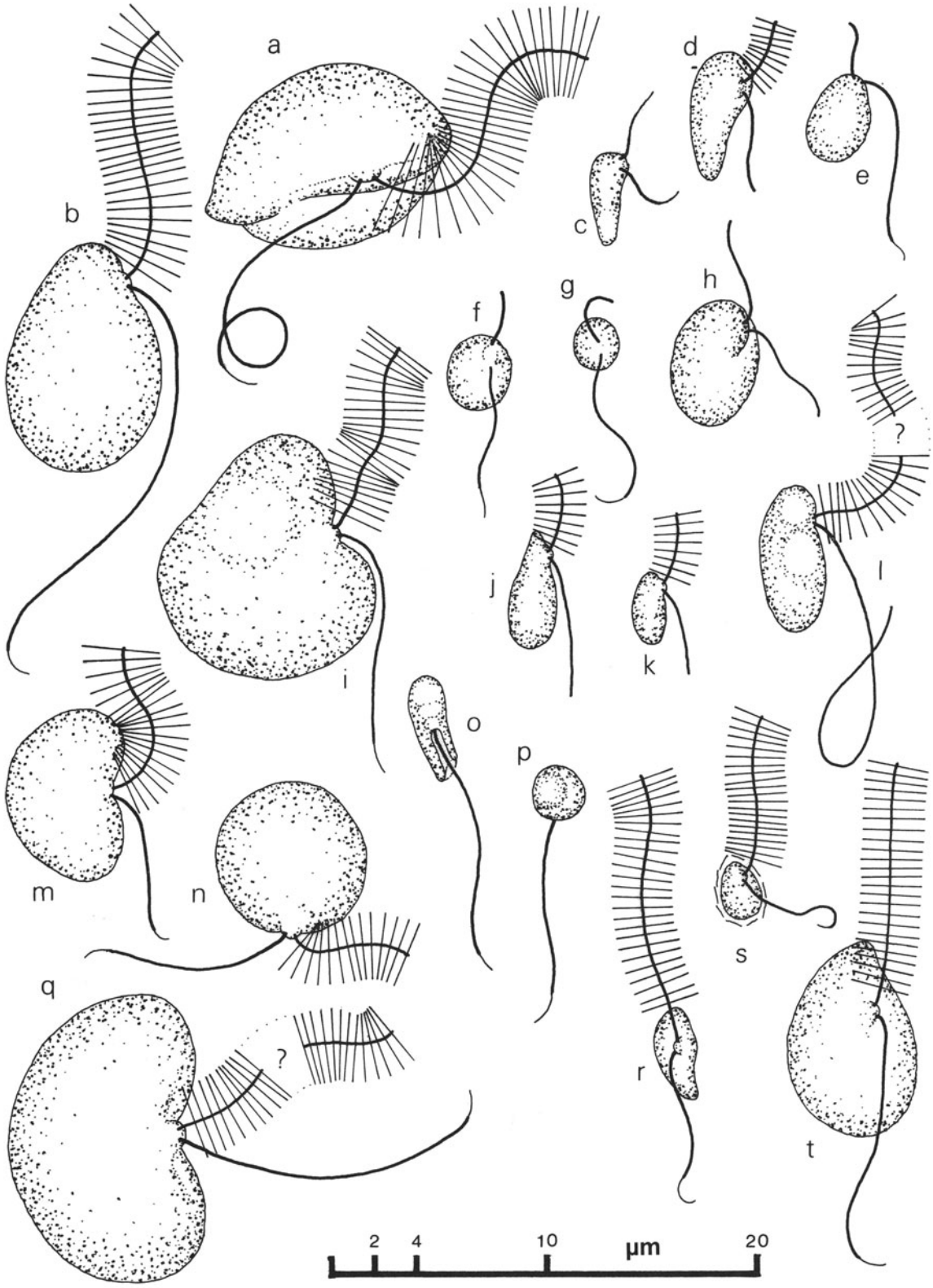

FIGURE I: 2B: a: *Lagena*; b: *Phytophthora*; c: *Haptoglossa*; d: *Ciliatomyces*; e: *Woronina*; f: *Polymyxa*; g: *Plasmodiophora*; h: *Endemosarca*; i: *Nematophthora*; j: *Olpidiopsis saprolegniae*; k: *O. varians*; l: *Blastulidium*; m: *Syzygangia* (zoosporangial); n: *Syzygangia* (oosporic); o: *Rozella*; p: *Dictyomorpha*; q: *Myzocytiopsis*; r: *Labyrinthula*; s: *Thraustochytrium*; t: *Lagenisma*.

Polymorphism, dimorphism and polyplanetism

There has been much debate about these terms in relation to fungal zoospores (Sparrow, 1958, 1960; Dick, 1973, 1990*a*; Martin, 1977; Ross, 1979: 61-62). They are defined again here:

Polymorphic spores
: The existence of sporangiospores in more than one form, either co-existing during sporangiogenesis or showing a sequential progression after release, with or without quiescent phases. Spores which show amoeboid changes in shape are polymorphic.

Monomorphic spores
: The existence of sporangiospores in only one form, but for the peronosporomycetes and other straminipilous fungi it is essential to qualify this term by stating whether the zoospore is of the principal or auxiliary type.

Dimorphic spores
: The occurrence of zoospores with two morphologically and ultrastructurally distinct kinds of flagellar insertion. These types of zoospore are formed sequentially, each always followed by an encysted phase, and with the cysts formed from each kind of zoospore also often morphologically distinct.

Polyplanetism (diplanetism)
: A sequence of two or more motile flagellate phases with interspersed mobile aplanosporic phases; the aplanosporic phase may be naked or as a walled cyst. The motile phases may be monomorphic or dimorphic.

Polyplanetism of the principal-form zoospore has been well documented for the *Saprolegniaceae* (Weston, 1919; Salvin, 1940; Cerenius & Söderhäll, 1984; Dieguez-Uribeondo, 1994) and all of these terms have been used principally in relation to this family, but polyplanetism also occurs in the *Pythiaceae* (Hallett & Dick, 1986). The principal-form zoospore of the *Saprolegniomycetidae* and *Peronosporomycetidae* is not known to be polymorphic. Polyplanetism is not documented for *Olpidiopsis saprolegniae*, but *Pleocystidium parasiticum* C. Fisch (≡ *Olpidiopsis schenkiana* Zopf) is reported to be polymorphic (Sparrow, 1960: 947). In other groups these criteria are seldom noted, but there are several references to polymorphic zoospores in lagenidiaceous fungi (e.g., Ivimey Cook, 1935 - *Syzygangia zygnematicola*, in which there is a change from an initially laterally flagellate condition to a pyriform-spherical shape when swimming; and Barron, 1976*c* - *Myzocytiopsis anomalum* (G. L. Barron) M. W. Dick (Dick, 1997*b*) in which the (naked-?) aplanospore changes slowly into a lens-shaped zoospore). Polyplanetism is recorded for *Myzocytiopsis parthenospora* (Karling) M. W. Dick (Karling, 1944*c*; Dick, 1997*b*) and *Syzygangia oedogonii* (Scherff.) M. W. Dick (Karling, 1981*a*; Dick, 1997*b*). In the plasmodiophorids polymorphic zoospores are frequently noted, but polyplanetism has not been reported for either the primary or the secondary zoospore. In some of the fungi of lagenidiaceous affinities the term diplanetic may have been used as if equivalent to dimorphism of the zoospore, even if the first naked aplanospores to be released are non-flagellate (cf.'achlyoid' discharge). The uninucleate protoplasts within the sporangium may or may not be flagellate; if flagellate, they may retract (?) their flagella upon emergence and become naked spheres or amoeboids; they may resume flagellate motility with or without an intervening encystment stage. In this respect comparisons with *Dictyomorpha* (Mullins, 1961) are relevant (see p. 252). Frequently, precise details are obscure: if '*Lagenidium*' zoospores encyst immediately upon emergence they may not do so in a hollow spherical cyst ball with the cysts cemented together as in true 'achlyoid' behaviour. Contrast the inferences for *Syzygangia oedogonii* (Karling, 1981*a*: 118) and *Myzocytiopsis parthenospora* (Karling, 1981*a*: 132). It is unclear whether flagella are present prior to 'achlyoid' encystment (cf. *Protoachlya*) or absent (cf. *Achlya sensu stricto*, see Money *et al.*, 1987).

In his earlier work Sparrow (1960) placed some reliance on descriptions of polyplanetism in the placement of peronosporomycete genera and families, but later (Sparrow, 1973*b*, 1976) he placed less emphasis on this character. I am in partial agreement with Sparrow (1976), in that I do not consider the descriptions to be sufficiently rigorous to justify the coupling of behaviour (within the definitions given above) with taxonomic hierarchies.

Body shape: reniform, bean-shaped, pip-shaped and grape-seed-shaped zoospores

All the terms describing body shape: reniform, bean-shaped, pip-shaped and grape-seed shaped zoospores, imply an asymmetry about the long axis of the zoospore. The descriptive problems arise because of:

1. the relationship of the asymmetry to the presence or absence of a groove parallel to the long axis
2. the extent to which the anterior and posterior parts of the zoospore have a different outline
3. the point of insertion of flagella

Asymmetry is primarily due to the construction of the cytoskeleton; that is, differences in the microtubular array from the kinetosomes. The possibility of morphological change, due to a change in the cytoskeleton, during maturation of the zoospore has not been considered. Such a change may account for the reniform to pyriform polymorphism noted in *Syzygangia zygnematicola* (Ivimey Cook, 1935), and revision of the characterization of this first-formed zoospore as a principal-form zoospore might then be necessary.

Interpretation of most descriptions involves a considerable amount of conjecture. 'Kidney-shaped' (reniform) and 'bean-shaped' are synonymous and commonly imply that there is a long axis groove; a similarity in shape of the anterior and posterior ends, and lateral insertion of flagella (in the groove). 'Pip-shaped' and 'grape-seed-shaped' commonly imply an axis of curvature, possibly with a shallow groove on the concave (ventral) side; a difference in shape between the anterior and posterior ends, and an apical or sub-apical insertion of flagella. However, different authors have attached different emphases to these terms. Sparrow (1960) tended to use 'bean-shaped' and 'grape-seed-shaped' as synonyms primarily to imply ***any*** asymmetry in contrast to the more or less spherical zoospore of the chytrids. Karling (1981*a*) tended to use the terms to distinguish sub-apical to lateral flagellar insertion from apical to sub-apical insertion. 'Lateral' insertion can be interpreted as 'sub-apical' if the zoospore is elongate. For example, see the attachment of flagella in *Olpidiopsis saprolegniae* (Bortnick *et al.*, 1985): either form of words could be used, yet the flagellation is typically heterokont on all ultrastructural criteria. Another example of the kind of contradictions that can occur is found with descriptions of *Sirolpidium*: Sparrow (1934: 10, plate 2Hb) "[flagella sub-apically] attached to the anterior, attenuated portion of the spore"; Sparrow (1960: 966, fig. 77 I) "[flagella] anteriorly attached"; Karling (1981*a*: 57) "laterally inserted flagella". The ratio length:breadth is also a factor that has determined morphological description, the more elongate zoospores being described as 'grape-seed-shaped' or 'pyriform' (i.e., 'Conference' pear), while 'bean-shaped' and 'kidney-shaped' can indicate a variety of length:breadth ratios.

Since a degree of longitudinal asymmetry is present in both peronosporomycetous and plasmodiophoromycetous biflagellate zoospores, I attach more importance to dimensions, when they are given, than to light-microscope descriptions of body shape of these small (*ca* 5·5 μm diameter, <75 μm^3) or very small (*ca* 3·5 μm diameter, <25 μm^3) zoospores. Similarly, when descriptions have been based on light microscopy without accompanying photographic evidence, lesser weight is given to indications of point of flagellar attachment. Table I: 1 provides information on the diversity of zoospore/zoospore cyst volume found in straminipilous fungi, and Figure I: 2 provides an indication of the size ranges found in different taxa (often ***within*** a genus).

Inclusions

Vacuoles and refractive granules are occasionally mentioned in descriptions, but most descriptions make no mention of these inclusions and others are not readily interpretable. In light microscopy refractive granules are more likely to be recorded than vacuoles (note the photographs of *Haptoglossa* in Barron, 1980: fig. 10, and *Myzocytiopsis humicola* in Barron & Percy, 1975: fig. 11, which appear to show posterior refractive inclusions). Particular attention should be paid to this feature when mentioned, because refractive granules are not characteristic of peronosporomycete zoospores, though they are mentioned in descriptions of plasmodiophoromycete zoospores. The ultrastructural basis of these refractive granules has not been explored.

TABLE I: 1. *Calculations of mean zoospore/zoospore cyst volume for a miscellaneous range of taxa to give an indication of inter-generic variation (based on published cyst diameters - spheres, $4/3\Pi r^3$ - or mean zoospore dimensions assuming an ellipsoidal body - $4/3\Pi r_1 r_2^2$). Most members of the* Peronosporomycetidae *and the* Saprolegniomycetidae *have zoospore cyst diameters of 8-12μm; inter-specific differences have taxonomic value. Lagenidiaceous fungi (nomenclature as given in* PART V*) usually have zoospore cysts with much smaller diameters.*

	volume in μm^3
Peronosporomycetidae	
Pythiogeton autossytum Drechsler	1767
Pythium anandrum Drechsler	1288
Phytophthora cinnamomi Rands	606
Pythium aquatile Höhnk	434
Pythium tracheiphilum Matta	203
Pythium angustatum Sparrow	113
Basidiophora entospora Roze & Cornu	29
Saprolegniomycetidae	
Saprolegnia anisospora de Bary (large cysts)	4905
Saprolegnia anisospora (medium cysts)	2399
Saprolegnia anisospora (small cysts)	1200
Verrucalvus flavofaciens P. Wong & M. W. Dick	755
Saprolegnia ferax (Gruith.) Thur.	668
Aphanomyces amphigynus Cutter	113
Leptolegniellaceae	
Aphanomycopsis bacillariacearum Scherff.	696
Leptolegniella exoospora W. D. Kane	243
Aphanomycopsis desmidiella Canter	179
Aphanomycopsis punctata Karling	179
Aphanodictyon papillatum Huneycutt *ex* M. W. Dick	170
Aphanomycopsis sexualis W. W. Martin	150
Brevilegniella keratinophila M. W. Dick	143
Leptolegniella keratinophila Huneycutt	130
Aphanomycopsis entophyta (Pringsh.) M. W. Dick	78
Aphanomycopsis saprophytica Karling	74

(Table I: 1, continued)

lagenidiaceous, hyphochytrid and plasmodiophorid species

Cystochytrium radicale Ivimey Cook	1288
Salilagenidium chthamalophilum (T. W. Johnson) M. W. Dick	573
Salilagenidium scyllae (Bian *et al.*) M. W. Dick	523
Cornumyces destruens (Sparrow) M. W. Dick	402
Cystosiphon pythioides Roze & Cornu	382
Salilagenidium callinectes (Couch) M. W. Dick	382
Haptoglossa heterospora Drechsler	382 and 75
Lagenidium giganteum Couch	350
Haliphthorus milfordensis Vishniac	283
Myzocytiopsis lenticularis (G. L. Barron) M. W. Dick	260
Lagena radicicola Vanterp. & Ledingham	256
Crypticola entomophaga (W. W. Martin) M. W. Dick	243
Atkinsiella dubia Vishniac	212
Pleocystidium parasiticum C. Fisch	205
Ducellieria chodatii (F. Ducell.) Teiling	180
Myzocytium proliferum Schenk	150
Crypticola clavulifera Frances *et al.*	143
Myzocytiopsis intermedia (G. L. Barron) M. W. Dick	143
Sorosphaera veronicae (J. Schröt.) J. Schröt. (2° zoospore)	130
Lagenisma coscinodisci Drebes	125
Cornumyces pygmaeus (Zopf) M. W. Dick	104
Halodaphnea parasitica (K. Nakamura & Hatai) M. W. Dick	87
Rozella septigena Cornu	83
Rozellopsis septigena Karling *ex* Cejp	83
Pleocystidium lundiae (Karling) M. W. Dick	78
Cornumyces muenscheri (Cutter) M. W. Dick	71
Haptoglossa mirabilis G. L. Barron	71
Halodaphnea hamanaensis (Bian & Egusa) M. W. Dick	65
Hyphochytrium saprobium (Karling) M. W. Dick	60
Polymyxa betae Woronin (1° zoospore)	56
Sorosphaera veronicae (J. Schröt.) J. Schröt. (1° zoospore)	44
Gracea gracilis (E. J. Butler) M. W. Dick	33
Plasmophagus deformans (Serbinow) M. W. Dick	33
Pleocystidium pygmaeoides (Karling) M. W. Dick	33
Rhizidiomyces apophysatus Zopf	33
Pythiella vernalis Couch	29
Olpidiopsis brevispinosa Whiffen	29
Ligniera junci (Schwartz) Maire & A. Tison (2° zoospore)	28
Endemosarca ubatubensis Erdos & L. S. Olive	24
Eurychasmidium tumefaciens (Magnus) Sparrow	24
Pseudosphaerita euglenae P. A. Dang.	23
Olpidiopsis achlyae D. A. McLarty	21
Endemosarca anomala Erdos	19
Phagomyxa algarum Karling	19
Rozellopsis simulans (A. Fisch.) Karling	18
Plasmophagus coleochaetis (Sparrow *et al.*) M. W. Dick	17
Rozella longicollis Karling	17
Sirolpidium bryopsidis (de Bruyne) H. E. Petersen	16
Dictyomorpha pythiensis (N. Sarkar & R. Dayal) M. W. Dick	15
Olpidiopsis saprolegniae (A. Braun) Cornu	14
Plasmodiophora brassicae Woronin (1° zoospore - Karling, 1968*e*)	14!
Sorodiscus radicicola (Cornu) A. Fisch. (2° zoospore)	14
Dictyomorpha dioica (Couch) Mullins	10
Pleotrachelus fulgens Zopf	9
Ectrogella bacillariacearum Scherff.	8
Plasmodiophora brassicae Woronin (2° zoospore - Karling, 1968*e*)	8!
Olpidiomorpha pseudosporae Scherff.	7
Hyphochytrium catenoides Karling	6
Woronina glomerata (Cornu) A. Fisch. (2° zoospore)	6
Ligniera plantaginis (Němec) Karling (2° zoospore)	2
Pseudosphaerita drylii Pérez Reyes	2

TABLE I: 2. *Variation in dimensions of tubular tripartite hairs and their density along the axoneme for various peronosporomycete zoospores (Hallett, 1975) and for other straminipiles (selected from Leadbeater, 1989 - density measurements were not given, base was included in shaft measurement, but given separately in parentheses).*

+ Present, but in insufficient numbers for accurate mean measurements to be obtained.*

PERONOSPOROMYCETES	Density No.μm^{-1}	Length of tubular tripartite hairs (μm) Shaft + base	long hair	short hair	Ratio of long hair to shaft
Saprolegniaceae					
Achlya bisexualis Coker & Couch	6·8	2·10	0·69	0·16	1:3·0
Achlya heterosexualis Whiffen	13·0	1·50	0·50	+*	1:3·0
Achlya colorata Pringsh.	12·7	1·80	0·60	0·10	1:3·0
Dictyuchus monosporus Leitg.	22·9	2·00	0·50	0·20	1:4·0
Protoachlya paradoxa Coker	19·2	1·80	0·55	0·14	1:3·3
Pythiopsis cymosa de Bary	18·0	2·10	0·50	0·10	1:4·2
Saprolegnia parasitica Coker	14·6	1·80	0·40	+*	1:4·5
Saprolegnia ferax (Gruith.) Thur.		1·80 (0·30)	1·10		1:1·6
Leptolegniaceae					
Aphanomyces stellatus de Bary	10·1	1·70	0·62	0·20	1:2·7
Leptolegnia caudata de Bary	13·3	2·00	0·50	+*	1:3·9
Leptomitaceae					
Leptomitus lacteus Roth	18·8	1·30	0·75	+*	1:1·7
Apodachlya minima Cejp	13·6	1·90	0·50	0·19	1:3·8
Pythiaceae					
Pythium mamillatum Meurs	11·2	2·10	0·70	0·16	1:3·0
Pythium middletonii Sparrow	14·2	1·90	0·70	0·15	1:2·7
Pythium monospermum Pringsh.	8·9	1·60	0·46	+*	1:3·5
Pythium torulosum Coker & P. Patt.	16·3	1·90	0·45	0·16	1:4·2
Phytophthora gonapodyides (H. E. Petersen) Buisman	15·3	1·70	0·40	0·20	1:4·1
Phytophthora cryptogea Pethybr. & Laff.	6·6	1·30	0·40	0·15	1:3·3
Lagenidium giganteum Couch		1·16 (0·16)	0·27		1:4·3
Albuginaceae					
Albugo candida (Pers.) Kunze	24·0	0·60	0·40	0·06	1:1·8
Hyphochytriaceae					
Rhizidiomyces apophysatus Zopf		1·30 (0·20)	0·37		1:3·5
Hyphochytrium sp.	13·5	0·75	0·50	0·10	1:1·5
PHOTOSYNTHETIC STRAMINIPILES					
Bacillariophyceae					
Lithodesmium undulatum Ehrenb.		1·73 (0·23)	0·50		1:3·5
Chrysophyceae					
Ochromonas danica Pringsh.		1·25 (0·25)	0·50		1:2·5
Synura petersenii Korshikov		1·60 (0·20)	0·25		1:6·4
Eustigmatophyceae					
Polyedriella helvetica Vischer & Pascher		1·00	0·75		1:1·3
Fucophyceae					
Dictyota dichotoma (Huds.) J. V. Lamour		1·10	0·70		1:1·6
Raphidophyceae					
Heterosigma luteus		1·75 (0·25)	0·50		1:3·5
Tribophyceae					
Bumilleria sicula Borzi		2·00	0·50		1:4·0

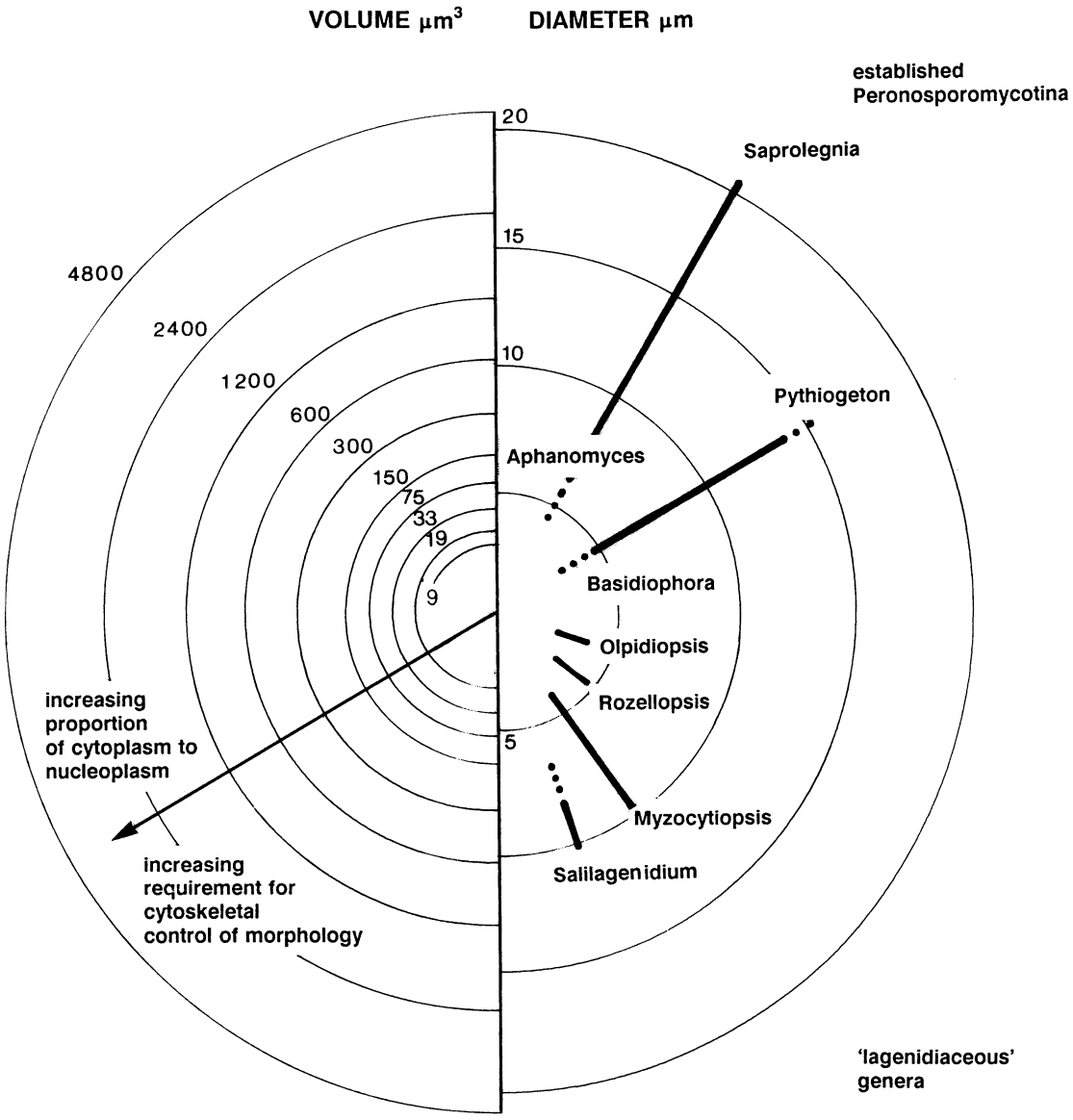

FIGURE I: 3. Inter- and intra-taxon variation in zoospore/zoospore cyst size; comparison of cyst diameters (right) with a geometric progression of volume equivalents (left) and ranges for a few selected taxa (see also Table I: 1).

Flagella, flagellar length and ornamentation, the straminipilous flagellum

Flagella are bounded by a flagellar plasmamembrane which is continuous with the cell plasmamembrane. The flagellar matrix (cytosol) is often devoid of structural detail except for the microtubules. There are normally two central and nine peripheral microtubules. The central pair are single microtubules, but the peripheral microtubules are usually doublets, composed of a complete microtubule of 13 subunits (A tubule) and an incomplete tubule of 11 subunits (B tubule), to which dynein arms may be attached (Smith & Sale, 1992). There may be ultrastructural diversity in both numbers and ultrastructural details of these microtubules.

Although Lange & Olson (1983) have stated that absolute estimates of flagellar length can be unreliable, relative lengths of flagella of zoospores fixed immediately after actively swimming are probably more reliable.

Sparrow (1976; cf. Sparrow, 1960: footnote, p. 913) and Karling (1981*a*) use the terms 'isokont' and 'heterokont' as antonyms, solely for differential length ratios, but there are different opinions for the terms used to describe differences in length and differences in morphology. In this text flagella of different lengths, whether of identical morphology or with ***non***-heterokont differences in morphology, are termed **anisokont** (in some algal groups, such as the *Pavlovales*, this term is used when there is non-heterokont difference in morphology and equal or unequal flagellar lengths). Most phycologists (e.g., Leedale, 1974: 269, the "Heterokont Kingdom"; Leadbeater, 1989) now restrict the term **heterokont** to the possession of two different kinds of flagella: the one ornamented with stiff **tubular tripartite (flagellar) hairs** (**TTHs**), usually in two rows (the tinsel flagellum, flimmergeissel), the other unornamented or with a fibrillar surface coat (peitschengeissel). This restricted definition of the term **heterokont** is adopted here. The term 'mastigoneme' for this distinctive appendage is not now approved (Andersen *et al.*, 1991); a new adjective **straminipilous** is introduced for the possession of these tubular tripartite hairs, whether borne on flagella, naked cells or as cyst-wall ornaments (see *Glossary* for derivation) (the retroneme - Cavalier-Smith, 1989, is a tubular **bi**-partite hair).

The tubular tripartite hair (**TTH**) is composed of a cone-like base, a tubular shaft and usually two unequal, diverging terminal fibres. The number of the terminal fibres is not easy to determine; only one terminal fibre has been found in *Labyrinthula* (Kazama, 1974*a*), *Crypticola* (Frances *et al.*, 1989) and *Labyrinthuloides* (Bower 1987*a*). The most detailed description, for ***any*** member of the *Straminipila*, of the spiral organization of the tubular shaft of the **TTH** is given by Domnas *et al.* (1986) and in Figure I: 4 for *Lagenidium giganteum** (see also Fuller & Reichle, 1965); the relative densities per unit flagellum length along the axoneme, and the dimensions of **TTHs** differ within peronosporomycetes, but most have a shaft length of 1·0-2·0 μm (Table I: 1, from Hallett, 1975; Figs 3 & 4) (see Leadbeater, 1989: table 8.1 for equivalent algal data); their effect on motility has been discussed by Jahn, Landman & Fonseca (1964) using the theory of Taylor (1952). See Sleigh (1991) and Holwill (1982) for a more recent accounts of morphology and dynamics. An hypothesis on their arrangement is given in Figure I: 4 (after Dick, 1990*a*).

The **TTHs** are assembled in antiparallel fascicles enclosed in a vesicle, and released as a group (Heath, Greenwood & Griffiths, 1970; Bowerman & Domnas, 1997). In chromistan algae this assembly takes place in the chloroplast endoplasmic reticulum or the nuclear envelope (Andersen *et al.*, 1991). Bouck (1971), for *Ochromonas*, suggested a somewhat different morphogenesis, whereby a similarly antiparallel arrangement was initiated in perinuclear cisternae, but with the individual **TTHs** subsequently receiving further ornamentation from dictyosome-derived vesicles, before being released at the base of the flagellum as the axoneme elongated. In the fungi (and *Synura*) the origin of the **TTH** is a dictyosomal vesicle (Heath *et al.*, 1970). After release, the **TTHs** normally become attached to opposite sides of the flagellum approximately parallel to the plane of the two central microtubules (cf. Manton, 1956), each row being radially aligned with a peripheral microtubule. Manton (1956) suggested an alignment with microtubules 3 and 8 (see Figure I: 3), but whether the alignment is with microtubules 3 and 8 or microtubules 4 and 7 there will be a 20° divergence from a true plane, thereby producing a shallow trough along the axis of the

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*FOOTNOTE: These data can be interpreted as a coil or stiff rope of 1 + 2 ply: in which case *Ochromonas* could be regarded as 1 + 3 ply. If one, two or all three of the more slender elements were spliced, the free ends along the length of the shaft could account for at least one of the two kinds of lateral hairs in *Ochromonas*, but compare accounts in Bouck (1971) and Deason (1971).

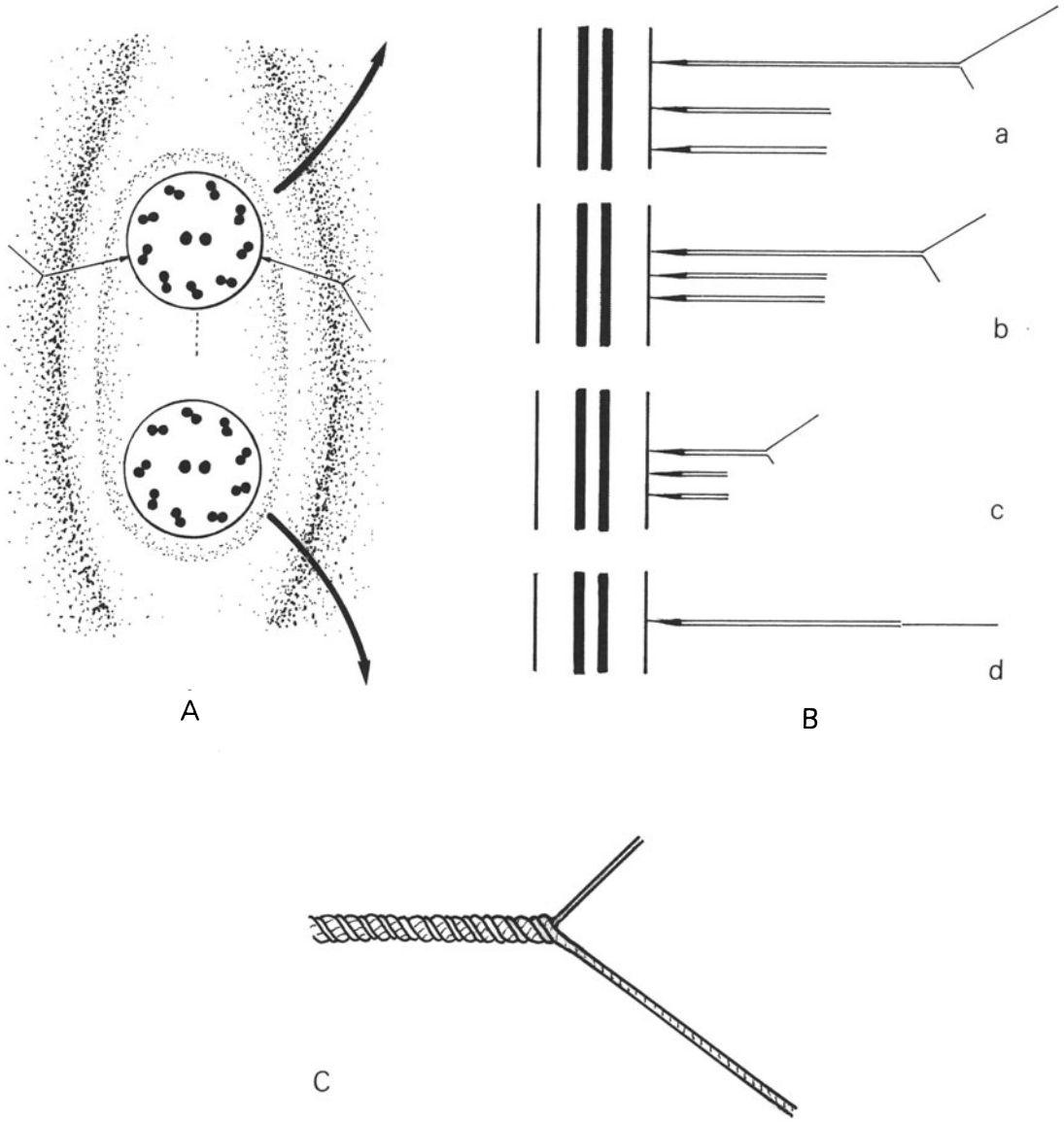

FIGURE I: 4. Flagellar structure and symmetry (after Manton, 1956; Dick, 1990*a*).
Fig. A: Hypothetical arrangement (freely adapted from Manton, 1956; Dick, 1990*a*) of straminipilous and whiplash flagella on the kinetosome boss (lightly stippled) within the longitudinal groove (heavily stippled). Note orientation of the two central microtubules and attachment of the tripartite tubular hairs to the plasmamembrane opposite peripheral doublet microtubules 3 and 8. The dotted line opposite doublet 1 represents the axis of the paraxonemal spine(s) or vane of fucophytes. The large arrows indicate the directions of the each flagellum; thus the peripheral doublet 1 is outward-facing or abaxial to the groove. The two ranks of tripartite tubular hairs are directed laterally so that the plane of the sine wave of the straminipilous flagellum is perpendicular to the dorsi-ventral axis.
Fig. B: generic variation in dimensions and densities of the tubular tripartite hairs: a: *Achlya bisexualis*: shaft length 2·1 μm, density $6{\cdot}8^{-1}\,\underline{\mu m}$; b: *Pythium torulosum*: shaft length 1·9 μm, density $16{\cdot}3^{-1}\,\underline{\mu m}$; c: *Albugo candida*: shaft length 0·6 μm, density $24{\cdot}0^{-1}\,\underline{\mu m}$ (after Hallett, 1975; Dick, 1990*a*); d: *Crypticola clavulifera* single hair drawn to scale from Frances *et al.* (1989).
Fig. C: part of a tubular tripartite hair: tubular shaft composed of two spiral elements of different diameter, each terminating as one of the distal fibres (after Domnas *et al.*, 1986). Figure I: 4 reproduced from Dick (1999: fig. 2B-D) by permission of Academic Press.

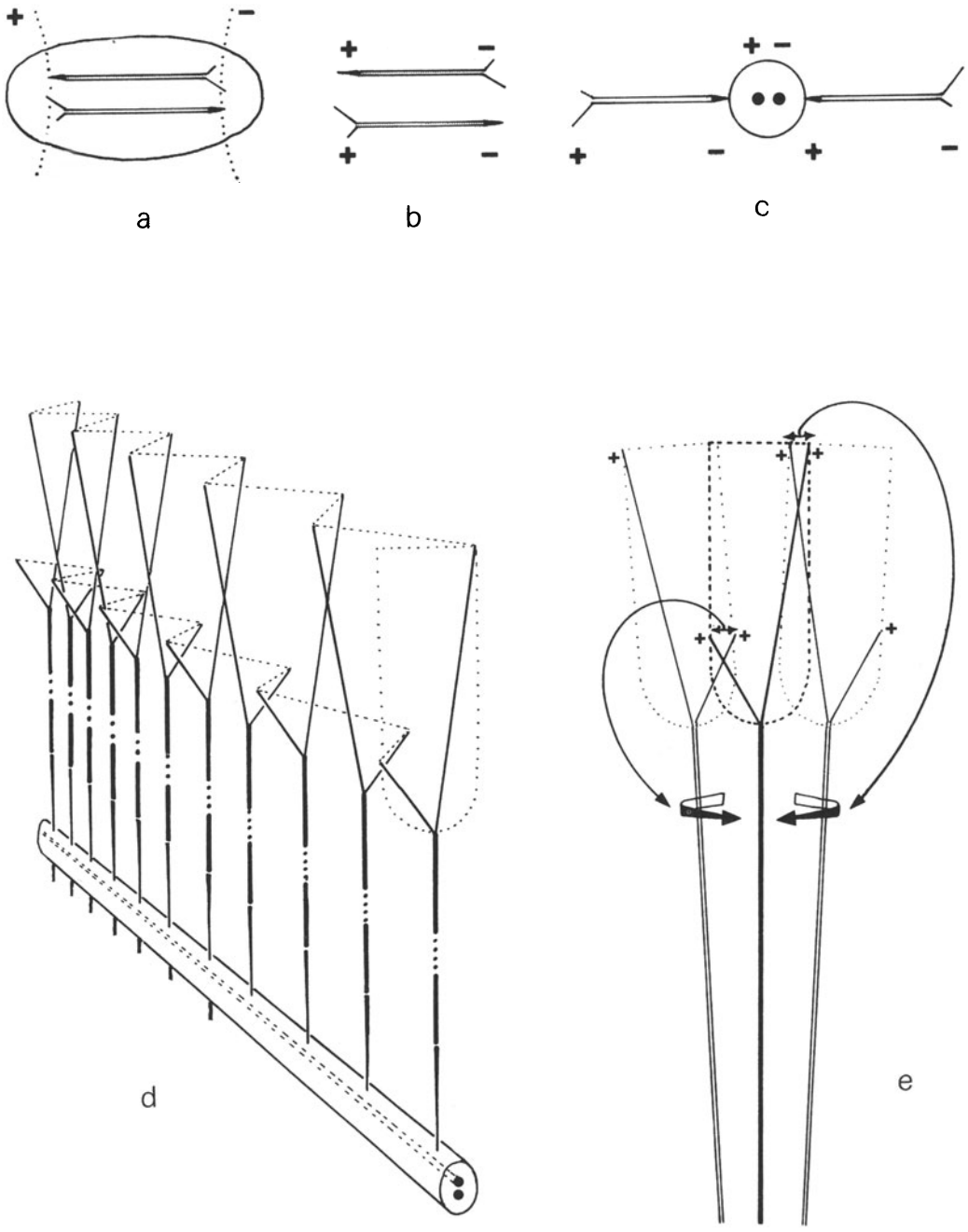

FIGURE I: 5. Hypothesis for the structural conservation and mode of function of the tubular tripartite hair (TTH). The theoretical problem is that, for the straminipilous flagellum to function in the way it has been observed to do, the TTHs must be held stiffly in the plane of the quasi-sinusoidal wave, yet they do not appear to have any structural mechanism to achieve this, particularly in view of the hydrodynamic stresses to which they are exposed. This hypothesis (after Dick, 1990*a*) is based on the antiparallel development of the TTHs, and depends on a resultant biochemical/electron polarity of the TTHs and the two central microtubules of the flagellar shaft; '+' and '-' are used loosely to indicate different polarity and 'like-repulsion'.

a: dictyosome-derived vesicle with antiparallel formation and array of TTHs (only 2 shown); b: TTHs of different polarity (each vesicle will produce populations of both); c: attachment of the two polarity classes of TTHs on the axoneme (possibly dependent on directional polarity of the central microtubules); d: perspective arrangement of one row of the TTHs: a common polarity of the solid bases will

flagellum/**TTH** complex; such a trough may have fluid dynamic significance. In a few, non-fungal, straminipilous organisms (*Ochromonas danica* - Bouck, 1971; *Labyrinthuloides haliotidis* - Bower, 1988*a*) this pattern of alignment does not hold. The phylogenetic significance of straminipilous flagellar morphology and morphogenesis is discussed further on p. 104.

The range of variation in both anisokont and heterokont flagellation (Figures I: 1 & I: 5) is much greater than has been recognized by mycologists (e.g., Lange & Olson, 1983; Barr, 1983). Thus, descriptions must be recast using the terminology of Andersen *et al.* (1991); earlier transcripts and secondary sources cannot be relied upon.

There is no terminology in general mycological usage to distinguish an unornamented flagellum from one (e.g. the posterior flagellum of *Achlya*, *Dictyuchus*, *Pythiopsis*, *Saprolegnia*, *Pythium* and *Phytophthora* - Hallett, 1975; see Figure I: 5) clothed with fine flexuous hairs (the **fibrillar surface coat** as defined by Andersen *et al.*, 1991).

Flagellar shape is not usually mentioned, but sometimes the term 'whiplash' is used. Again this term has been used in morphological and functional contexts: it should be used to indicate a flagellum with a narrower extension to the flagellum tip (the **acronema**), due to the greater length of the central microtubules compared with the nine peripheral doublets (but cf. Manton, 1965). It has also been used as a general term for any unornamented flagellum, irrespective of whether it is abruptly truncate, sharply attenuated (acronemate) or with a gradually tapered tip due to progressive termination first of the central fibres, and secondly components of the longer peripheral doublets (Gibbons & Grimstone, 1960; Manton, 1965) (Figure I: 5). In the peronosporomycetes the straminipilous flagellum does not possess a whiplash extension, but in chrysophyte algae there may be a terminal tuft of non-tubular hairs on this flagellum (Bouck, 1971, 1972). In different fucophytes there may be a short (Manton & Clarke, 1951) or very long (Müller & Falk, 1973) acronema, while in *Gracea* the acronema varies in length from zoospore to zoospore (Dick, 1997*a*, 2000*d*). Additional flagellar features found in chromophyte algae, but not so far in straminipilous fungi, include paraxonemal bodies (PABs) such as the intra-plasmamembrane spines (*Himanthalia* - Manton, Clarke & Greenwood, 1953: *Xiphophora* - Manton, 1956; *Dictyota* - Manton, 1959, 1965) and the extra-plasmamembrane flagellar scales characteristic of certain prymnesiophytes. The orientation of the

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(Figure I: 5, legend, continued)

space the TTHs at approximately regular intervals along the axoneme, the common polarity of the distal hairs will provide a mechanism of recoil and counter entanglement in the compressed phase of the sinusoidal wave; the dotted outline represents the 'face-view' of the 'paddle' created by the diverging terminal hairs, the opposed zig-zag dotted lines connect the long and short hairs respectively; e: interaction between a TTH (solid shaft) and its adjacent TTH (open shaft): as the adjacent TTH (left-hand) drifts *out of alignment* with the TTH (centre) the increasing strength of repulsion of the like polarity of the tips of the short terminal hairs (double-headed arrow) will correct alignment as indicated by the folded arrow (lower left); as the adjacent TTH (right-hand) drifts *out of alignment* with the TTH (centre) similar repulsion due to the like polarity of the tips of the long terminal hairs (double-headed arrow) will correct alignment as indicated by the folded arrow (lower right); the dimensions are such that the individual TTHs would stray from the palisade plane by less than 10°. These suggestions do not detract from the possible role of the terminal hairs in increasing viscous drag as they pass over the peak of the sinusoidal wave, thereby augmenting the action of the TTHs in reversing the thrust of the straminipilous flagellum.

The straminipilous flagellum 'paddles' the organism through the water. Using the paddle analogy, a 'skeleton blade' is represented by the long and short distal hairs. These hairs could be arranged in a plane perpendicular to the palisade of TTHs and also be perpendicular to the flagellar axis. Alternate TTHs are figured (d) with reciprocal arrangements of short and long hairs (possibly involving rotation at the point attachment of the TTH to the axoneme plasmamembrane).

The action of the straminipilous flagellum can be regarded as analogous to a rowing eight with fixed oars and a flexible keel (cf. the swimming action of nereid worms (Gray, 1939), and contrast both the 'sculls-action' of the pair of smooth flagella of *Chlamydomonas*, and the 'screw-action of a stern-rowlock single oar' of single, posteriorly directed flagella).

Fig. 5 d,e reproduced from Dick (1990*a*: fig. 3) by permission of Jones & Bartlett.

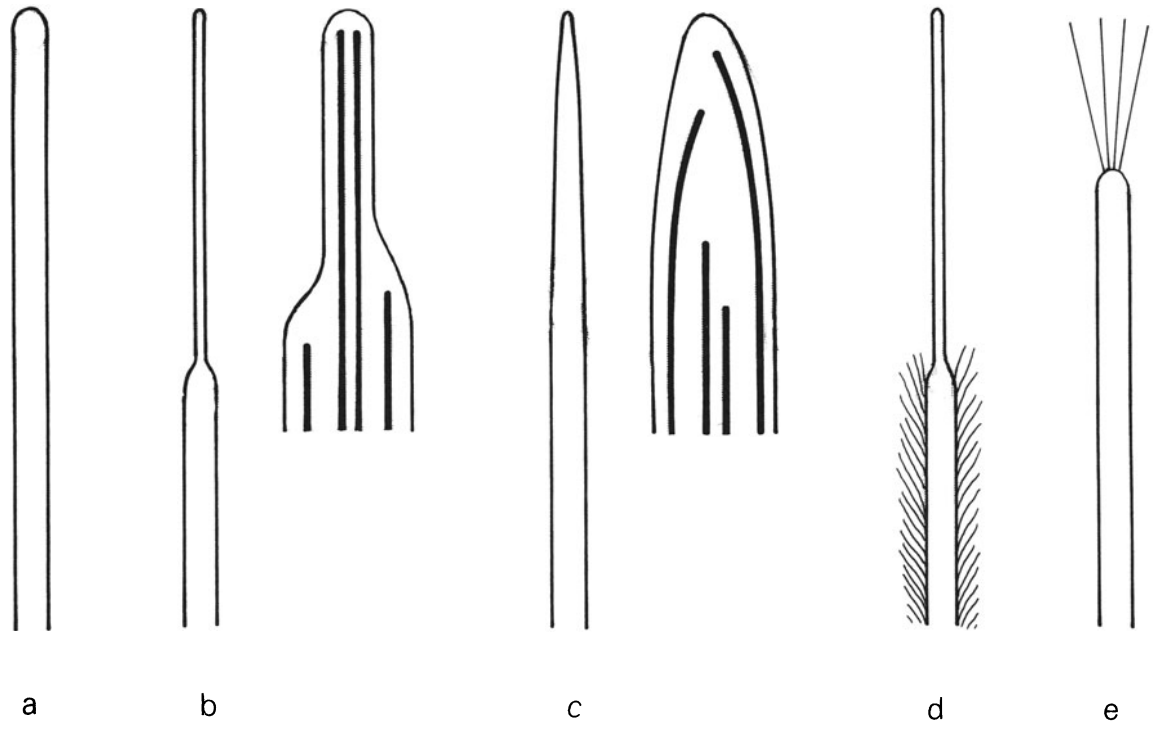


FIGURE I: 6. Diversity in flagellar tip morphology: a: truncate (central and peripheral microtubules approximately the same length); b: whiplash (central microtubules much longer than the peripheral microtubules); c: pseudo-whiplash (central microtubules shorter than the peripheral microtubules, which are also of different lengths); d: whiplash, with axoneme with a fibrillar surface coat; e: truncate with a terminal tuft of fine hairs (characteristic of some chrysophytes).

straminipilous flagellum relative to the whiplash flagellum and the spermatozoid body of fucophytes has been described by Manton (1956) and is adapted here in Figure I: 3, except that I am not adopting her numeration of the nine peripheral doublets as 0 (opposite the paraxonemal body) to 8 (with the tubular tripartite hairs associated with doublets 2 and 7), preferring to number the doublets 1 to 9. However, ***it must be emphasized that the orientation depicted in Figure I: 4A requires confirmation for straminipilous fungi*** with respect to the plane of the straminipilous flagellar beat (perpendicular to both the posterior- anterior and the dorsiventral axes); the direction of the arms on the peripheral doublets with respect to the transitional plate (dextrorotational or levorotational); whether this rotation on the whiplash flagellum is a mirror image or not, and whether the **TTH**s are associated with doublets 3 and 8 or 4 and 7.In the life-histories of the plasmodiophorids there are two zoosporic phases. The secondary zoospores, produced in the zoosporangia, possess two unornamented flagella of markedly different lengths, often in a ratio of 1:3 or 2:3. On this basis the flagellar morphology of the plasmodiophorids has been accepted as anisokont but not heterokont. However, there is still a very slight possibility of doubt. Kole & Gielink (1962) acknowledge that preparations of suspended cysts from soils, necessary for the production of primary zoospores, cannot be absolutely pure, but they provide quite convincing evidence (confirmed without presented data, by Aist & Williams, 1971) that the *Plasmodiophora* cyst produces a primary zoospore with a long whiplash flagellum and a short unornamented, often abruptly truncate flagellum. *Spongospora subterranea* (Wallr.) Lagerh. has two anisokont whiplash flagella (Kole & Gielink, 1961: fig. 3) and Glockling & Beakes (2000*a*, *b*) have shown that the two flagella of *Haptoglossa* are both acronemate. However, Kole & Gielink (1962) also reported that two other possibly contaminant zoospore morphologies occurred, albeit in very small numbers

in their more highly purified preparations of *Plasmodiophora*: one was sub-anisokont with two unornamented whiplash flagella, the other was "entirely identical with" that figured for *Plasmodiophora* by Seaman, Larson & Walker (1961) from Wisconsin soils (Dick, 1998*a*, 2000*d*). Merz (1992) has also reported that heterokont and uniflagellate zoospore contaminants were "always" present in preparations of secondary zoospores of *Spongospora*, but no evidence of tubular tripartite hairs was presented, only SEM data of different flagellar insertion and flagellar base morphology. Merz (1992) also distinguished these contaminants by their different swimming characteristics as revealed by video recording (he also reported differences in swimming characteristics between the primary and secondary zoospores of *Spongospora*). Clay & Walsh (1997) have provided the most recent ultrastructural data for the flagella of *Spongospora*. The zoospore described by Seaman *et al.* (1961) had flagella of obviously different lengths, and the hairs on the tinsel flagellum were almost certainly tripartite, indicating a heterokont state. The straminipilous flagellum was the longer. Neither Kole & Gielink (1962) nor Merz (1992) gave measurements for the three kinds of zoospores or their flagella, but Seaman *et al.* (1961) gave dimensions of living zoospores as 3·0 × 3·0 × 3·9-4·8 μm with flagellar lengths of 6·0-8·0 μm and 2·0 μm respectively. It is remarkable that all three preparations from Wisconsin (Seaman *et al.*, 1961) and that from the Netherlands (Kole & Gielink, 1962) for *Plasmodiophora* (and perhaps the study by Merz (1992) on *Spongospora*) should yield a contaminant fungal zoospore type, with a longer straminipilous flagellum and a shorter whiplash flagellum on a broadly pyriform zoospore, that is otherwise confined, outside marine environments, to *Gracea gracilis* (E. J. Butler) M. W. Dick (Dick, 1997*b*, 2000*d*). (Note that *Tribonema* is a non-marine alga with a longer straminipilous flagellum - Massalski & Leedale, 1969.) It is regrettable, and in view of the interest in these fungi as possible virus vectors, surprising, that these 'contaminant' zoospores have not been subject to more thorough comparative video and ultrastructural study.

A much more controversial alternative explanation would be to suggest that the heterokont zoospore type is part of the plasmodiophoraceous life-history, in a way analogous to the different kinds of flagellation found in *Vaucheria*, in which the spermatozoids are heterokont and markedly anisokont while the coenocytic polyflagellate zoospores are not heterokont and only slightly anisokont (Koch, 1951; Møestrup, 1970; Ott & Brown, 1974).

In the *Peronosporomycotina* (Dick, 1976, the non-photosynthetic, fungal, part of the *Straminipila*) the heterokont flagellation may or may not also be anisokont. In most peronosporomycetes flagella are sub-equal with the straminipilous flagellum slightly shorter (in *Phytophthora* (Cho & Fuller, 1989: fig. 1) and *Plasmopara* (Beakes, 1989: fig. 17.2) this anisokont condition is very marked), while in the thraustochytrids the anterior straminipilous flagellum is the longer one and in the hyphochytrids it is the only extant flagellum. Vishniac (1955*d*) reported that the marine *Sirolpidium* was anisokont and heterokont, with the anterior straminipilous flagellum the shorter. Neither Martin & Miller (1986*b*) nor Bortnick *et al.* (1985) precisely described the anisokont condition in *Olpidiopsis,* but the straminipilous flagellum is presumed to be the shorter (*contra Gracea gracilis* studied by Pemberton *et al.* (1990) and Dick, 2000*d*). Similar variation between the relative lengths of the straminipilous anterior flagellum and the smooth posterior flagellum is found in the *Fucophyceae* and *Tribophyceae*.

For the biflagellate fungi under discussion, positively identified straminipilous flagellation (that is, with **TTH**s figured, or with antiparallel packets in the cytoplasm clearly identified in TEM) is limited to *Lagenidium giganteum* (Domnas *et al.*, 1986), *Salilagenidium callinectes* (Bland & Amerson, 1973*a*; Gotelli, 1974*b*, Fuller & Jaworski, 1987), *Haliphthoros milfordensis* (Overton *et al.*, 1983), *Lagenisma coscinodisci* Drebes (Schnepf *et al.*, 1978*d*), *Ectrogella perforans* H. E. Petersen (S. Raghu Kumar, 1980*b*), *Olpidiopsis saprolegniae* var. *saprolegniae* (Bortnick *et al.*, 1985), *Olpidiopsis varians* Shanor (Martin & Miller, 1986*b*), *Gracea gracilis* (Dick, 2000*d*) and *Lagena radicicola* Vanterp. & Ledingham (Barr & Désaulniers, 1987*b*). It is probable that *Ducellieria chodatii* (F. Ducell.) Teiling is also straminipilous (Hesse *et al.*, 1989), although the tripartite nature of the flagellar hair is not clear. *Ciliatomyces spectabilis* I. Foissner & W.

Foissner (Foissner & Foissner, 1986*b*, 1995) is stated to be heterokont, but without documentary illustrations, except for the packets of **TTHs**. Manier (1976: fig. 15) has described "corps fibreux" in *Blastulidium* which are probably packets of tubular tripartite hairs, but she did not give information on flagellar ornamentation.

The fungus described by Martin (1977) as *Atkinsiella entomophaga* W. W. Martin (≡ *Crypticola entomophaga* (W. W. Martin) M. W. Dick - Dick, 1998*a*), though stated to be heterokont, is not heterokont within the definitions used here, and the same may apply to *Syzygangia nodosa* (P. A. Dang.) M. W. Dick (≡ *Lagenidium nodosum* (P. A. Dang.) Ingold, Ingold, 1949; Dick, 1997*b*). The reference to heterokont flagellation for this taxon in Karling (1981*a*) is based on the work of Couch (1941) and the redisposition of *Resticularia* to *Lagenidium* by Ingold (1949). Couch (1941) did not identify the species, nor state the origin of his *Resticularia*. He wrote that this, and his likewise unidentified *Myzocytium* species, had a similar swimming motion. His figure is a drawing of two (*contra* Karling, 1981*a*: fig. 103) stained zoospores. From this pair of drawings it is probable that the anterior flagellum had only a single row of hairs but these hairs may not have been tubular or tripartite. Most recently, Frances *et al.* (1989) have provided an electron micrograph which suggests that *Crypticola* also has **TTHs** in a single row, the hairs are tripartite and tubular but with only a single fine distal fibre (Dr S. P. Frances, *in litt.* 29 Jun 1992). In other words, there may be a group of fungi which has a non-peronosporomycetous but straminipilous flagellar ornamentation comparable to the flagellum with uniseriate hairs in the cryptophytes. (Perkins & Menzel (1967) provided another report of a fungus, *Perkinsus* (≡ *Dermocystidium*) with an anterior flagellum possessing a single row of (tripartite?) hairs.) Bower (1987*a*) has figured straminipilous hairs assembled in an arc around the flagellar cross-section for *Labyrinthuloides haliotidis* S. M. Bower, while Tong (1995) has described, in *Developayella*, a straminipilous flagellum which is not directed anteriorly. It must be anticipated that further variants of the basic pattern may exist, but the extents to which phylogenetic significance is accorded to flagellar ornamentation are still likely to be reflected at appropriately high taxonomic levels.

The descriptions of flagellation by Barron must be reassessed, since both *Myzocytiopsis lenticularis* and *Pythium caudatum* (G. L. Barron) M. W. Dick *loc. cit.* (≡ *Lagenidium caudatum* G. L. Barron) are said to have anterior whiplash flagella (Barron, 1976*b*). If the anterior flagellum has **TTHs** it is unlikely to have a whiplash extension.

Flagellar base: kinetosome, its root organization and transitional zone

The flagellar bases are of phylogenetic importance in any discussion of the interrelationships within and between chromistans and other major taxa. The terminology of Andersen *et al.* (1991) is used in the following general account, but ultrastructural information is not available for most of the lagenidiaceous fungi. The flagellar base is composed of the kinetosome and the attached roots of microtubules. Between the kinetosome and the axoneme there is a transitional zone (for reviews see Grain, Mignot & Puytorac, 1988; Preisig, 1989, 1999; Barr, 1992; there is a useful summary diagram in Sleigh, 1989: fig. 5.33), and further structural features may be discernable at the base of the axoneme and in the core of the kinetosome.

The peronosporomycete root system is composed of six parts (Barr & Désaulniers, 1987*b*, 1989*a*) (Figure I: 6). Attached to the kinetosome of the anterior straminipilous flagellum there are two roots - **R1**: a triplet with ribbed microtubules directed anteriorly (three main microtubules with secondary microtubules extending laterally), with a posteriorly directed associated cord (**CD**) in *Phytophthora* and *Pythium* (Barr & Désaulniers, 1992), and **R2**: a doublet usually comprised of two simple microtubules. The ribbed triplet for *Phytophthora* is right-handed (Barr & Désaulniers, 1989*b*) not left-handed as presented in Barr & Allen (1985). I have followed the right-handed convention (Barr & Désaulniers, 1992) in Figure I: 6. Two parts

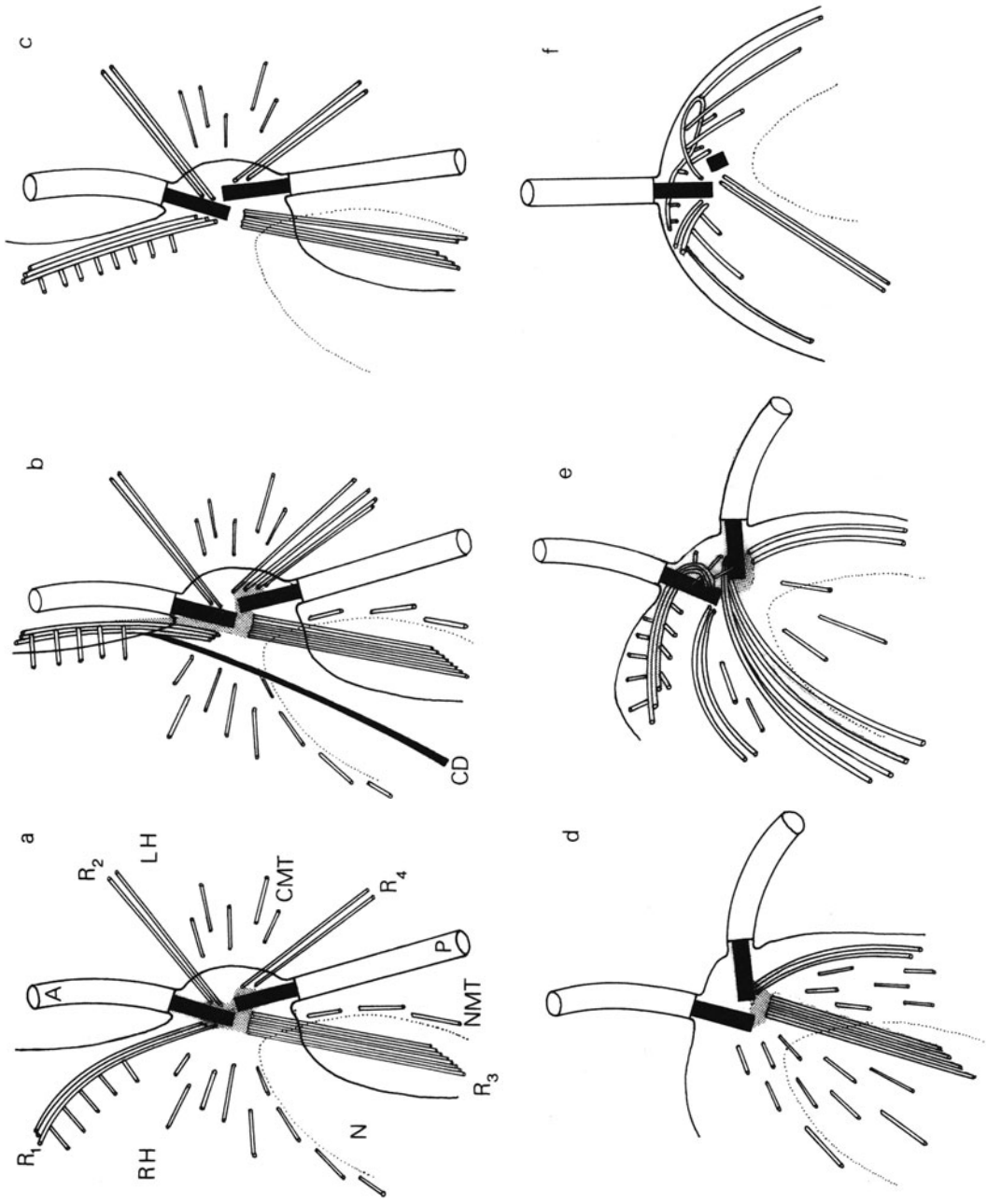


FIGURE I: 7. Simplified diagrams of kinetosome flagellar roots of zoospores, drawn from a slightly oblique ventral view: RH right-hand side of zoospore; LH left-hand side of zoospore; R1, R2 roots to kinetosome of the anterior flagellum; R3 (MS), R4 roots to kinetosome of the posterior flagellum; A anterior flagellum; P posterior flagellum; N nucleus; CMT cytoplasmic microtubules; NMT nuclear-associated microtubules; CD R1 cord. Kinetosomes shown as solid cylinders; electron-opaque microtubular organizing material stippled (after Barr, 1981, 1992; Barr & Allan, 1985; Barr & Désaulniers, 1987, 1989, 1992).
a: *Saprolegnia* principal-form zoospore; b: *Pythium*, note the R1 cord and the number of microtubules to R4; c: *Thraustochytrium*, note overlapping kinetosomes, divided R3 and sparce CMT; d: *Saprolegnia* auxiliary form, note the absence of R1 and R2, and density of Cmt; e: *Lagena*, note apparent rotation of each flagellum 'winding up' R1 and R3 and free R3 microtubules; f: *Rhizidiomyces*, note vestigial posterior kinetosome in same orientation as principal form zoospores, ribs on R2 and absence of R4.

Fig. 7 a,b reproduced from Dick (1999: fig. 3) by permission of Academic Press.

of the root complex are attached to the kinetosome of the posterior flagellum - **R3**: a posteriorly directed multistranded ('octet') band-shaped root, and **R4**: a counterbalancing posterior root of 3-6 microtubules; between the two kinetosomes is a microtubule organizing centre with microtubules radiating into the cytoplasm (**CMT**) and a group of specifically nucleus-associated microtubules (**NMT**). Fibrillar material connects the two kinetosomes, and in *Salilagenidium callinectes* (Bland & Amerson, 1973*a*) and *Haliphthoros milfordensis* (Overton *et al.*, 1983) this appears prominently striated. Although the overall pattern of roots is similar in the *Peronosporomycetes*, each root shows independent variation between taxa, and sometimes within a species (Dick, 1997*a*). **R1** possesses an electron-opaque (microtubule organizing ?) longitudinal rod or 'spine' giving attachment to the length of the kinetosome and which is extended posteriorly as a prominent cord in most of the *Pythiaceae* (all *Peronosporomycetidae* ?); it is confined to two microtubules in *Rhizidiomyces* and has two free ribbed microtubules in *Lagena*; it is absent (as is **R2**) from the auxiliary zoospore of *Saprolegnia*, suggesting that the straminipilous flagellum of this zoospore may have impaired function. **R2** is most constant in ultrastructure, but is reduced to a single ribbed microtubule in *Rhizidiomyces*. **R3** (multistranded root) varies in the number of microtubules involved, and the extent to which they are free (two groups in *Thraustochytrium*; reduced to two in *Rhizidiomyces* (Cooney *et al.*, 1985); absent in *Phytophthora infestans* (Mont.) de Bary (Barr & Désaulniers, 1992)). **R4** is also more conserved, although frequently of a pair of microtubules, it has more numerous components in the *Peronosporomycetidae*, with the greatest number of microtubules (5-6) in *Phytophthora infestans*. Variation in the frequencies of cytoplasmic and nuclear-associated microtubules is also reported. In *Lagena* each flagellum appears to be rotated clockwise between 90-180°, 'winding up' the R1 and R3 (MS) roots.

By way of contrast, a striated, fibrous root (sometimes termed a rhizoplast), is a prominent feature in *Rozella* (Held, 1975), and this structure is also found in *Chytridiomycetes* and the choanoflagellates. The structure and mechanism is distinct from the ultrastructural morphology described above.

Barr (1981) has stated that the primary function of the root system is to dissipate the forces of flagellar motion: it is reasonable to postulate that the powerful anterior flagellum with tripartite tubular hairs would require a stronger root system, and this may be manifest by the **R1** ribbed triplets (i.e., three microtubules with secondary microtubules extending laterally). It has also been suggested (M. J. Powell, pers. comm.) that the **R3** multistranded root (of two to ten parallel microtubules in the oomycetes) may be reduced in smaller zoospores. If there is homology, then *Olpidiopsis* has a quintet multistrand root, and *Lagena* 4-10 microtubules in its multistranded root. Zoospore volumes vary widely (Figure I: 1): the ***volume*** of the zoospore (or cyst) of *Saprolegnia* may be ***100 times*** greater than that of *Olpidiopsis* or *Haptoglossa*. It is conceivable that zoospores may show size-related differences in the degree of ultrastructural differentiation. It is difficult to determine whether reduction or elaboration from a primitive state is involved (Dick, 1997*a*).

The root complex must be assessed as a unit of several independent variables, and thus the data base is still much too small to make phylogenetic generalizations across the full range of straminipilous fungi. As with the diversity of flagellar construction, the extent of the diversity of root systems may be even greater than that already described. However, similarities between the *Saprolegniomycetidae* and the *Peronosporomycetidae*, and the differences ***within*** the *Peronosporomycetidae*, suggest that root organization and structure will be of taxonomic value within families and genera. There is at present no simple correlation or explanation for the variety of patterns between root organization and lateral or sub-apical flagellar insertion.

Barr & Désaulniers (1987*b*) distinguished four types of transitional zones (Figure I: 7). In each case there is a transitional plate, attached to the kinetosome at the end distal to the nucleus, which transects the axoneme core in a plane above that of the cell plasmamembrane and which extends to the flagellar plasmamembrane. At the centre of the transitional plate there is a bead-like projection. Towards the base

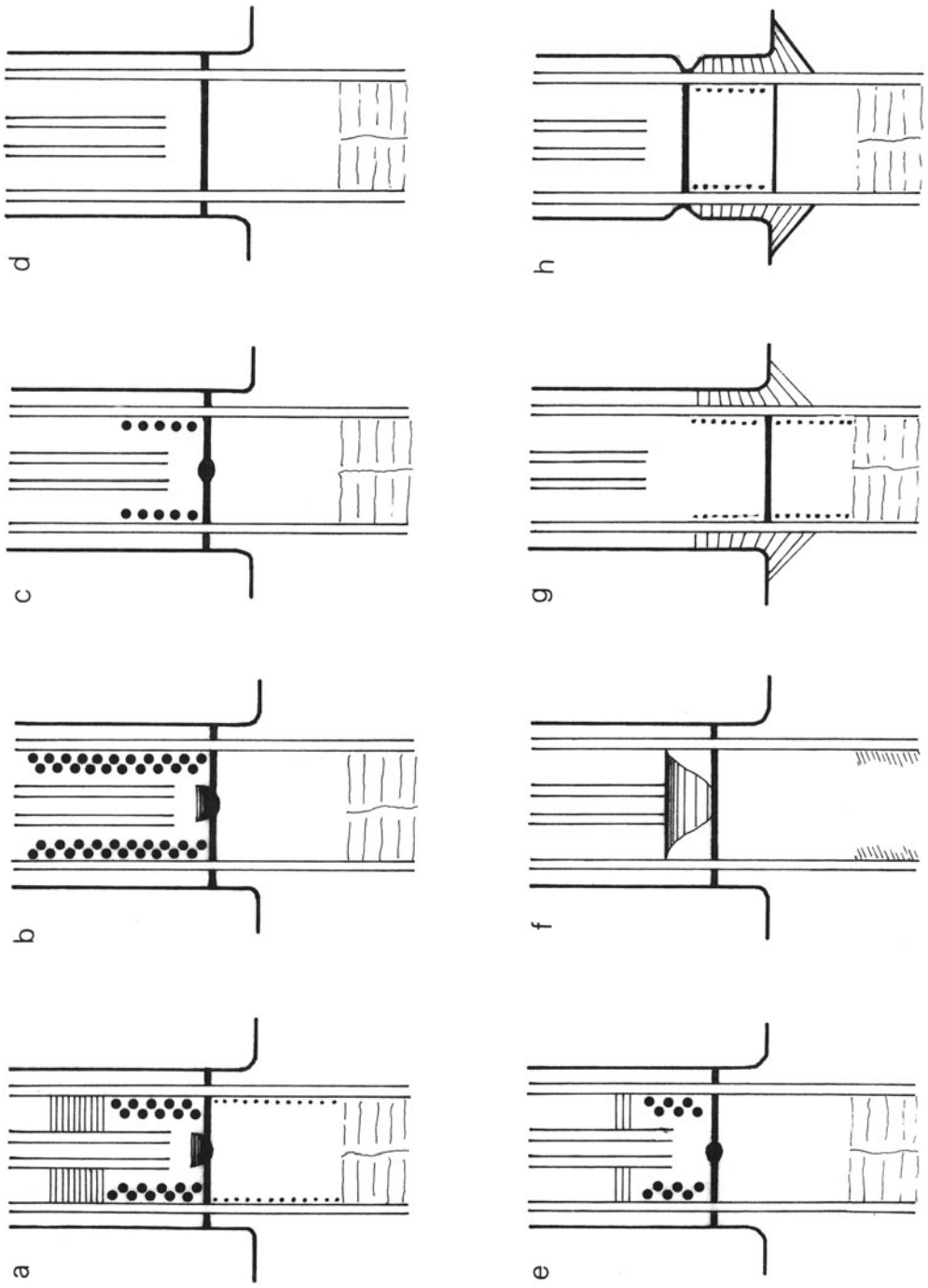


FIGURE I: 8. Simplified diagram of flagellar bases and transitional zones, see text for detailed descriptions (after Barr & Allen, 1985; Barr & Désaulniers, 1987, and Barr, 1992):
a: *Saprolegnia*
(and all *Saprolegniomycetidae* ?)
b: *Pythium*
(and all *Peronosporomycetidae* ?)
c: *Olpidiopsis*
d: *Lagena*
e: *Rhizidiomyces*
(and all *Hyphochtriomycetidae* ?)
f: *Thraustochytrium*
(and all *Labyrinthista* ?)
g: *Polymyxa*
(and all *Plasmodiophorales* ?)
h: a choanoflagellate.

Fig. I: 8 a,b reproduced from Dick (1999: fig. 5) by permission of Academic Press.

of the kinetosome, proximal to the nucleus, some indications of the 'cartwheel' of alternate triplet cross-linking (Manton, 1964, 1965) can usually be seen (see Glockling, 1994). However, the detailed serial transverse sections of this region necessary to describe its structure have not yet been made for flagellate fungi. It is possible that the detailed structure of this zone may differ between representatives of the straminipilous fungi.

Within the axoneme core (i.e., inside the cylinder of the nine doublets) and distal to the transitional plate there is an electron-opaque structure appearing in longitudinal section like a concertina or double helix of repeating units. In the *Pythiaceae* there may be up to 20 such units (Barr & Allen, 1985), but in the *Saprolegniaceae* (Heath & Greenwood, 1971; Holloway & Heath, 1977*a*, *b*; Hoch & Mitchell, 1972*b*), *Leptomitaceae* (Randolph & Powell, 1992), *Rhipidiaceae* (Gotelli & Hanson, 1987) and *Hyphochytriaceae* (Cooney *et al.*, 1985) and in *Petersenia palmariae* Van Der Meer & Pueschel (Pueschel & van der Meer, 1985), there is a short concertina (*ca* 6 units) with struts (struts unresolved for *Petersenia*) to the central doublet distally. Lange, Olson & Safeeulla (1984) described the flagellar base of *Sclerospora*, and Olson subsequently stated (L. W. Olson, pers. comm.) that the concertina of this material was also short and resembled that of *Saprolegnia*. *Olpidiopsis* (Bortnick *et al.*, 1985) also has a group of five or six rings arranged as a single helix. In *Salilagenidium callinectes* (Bland & Amerson, 1973*a*), *Haliphthoros milfordensis* (Overton *et al.*, 1983), *Ectrogella perforans* (Raghu Kumar, 1980) and *Crypticola clavulifera* Humber *et al.* (Frances *et al.*, 1989) the transitional plate is also above the plane of the plasmamembrane, but only in *H. milfordensis* has a concertina or short stack of about eight rings been described. *Lagena* lacks these electron-opaque units, and is unique in lacking a central bead to the transitional plate.

In the labyrinthulids the transitional zone is more complex. The flagellar base has been illustrated for *Thraustochytrium* (Kazama, 1972*a*) and *Schizochytrium* (Kazama, 1980), revealing a transitional plate situated above the plane of the plasmamembrane. Distal to this plate and dilating towards, and connecting to the peripheral doublets of the flagellum, was a cone-like structure, which might be homologous to a short concertina. The flagellar diameter was smaller proximal to the transitional plate. The middle third of longitudinal profiles of the kinetosome core also contained an ill-defined electron-opaque cylinder. Some of these details have been confirmed by Raghu Kumar (1982*b*) for *Ulkenia*.

Aist & Williams (1971), Barr & Allen (1982), Clay & Walsh (1990, 1997), Miller, Martin & Dylewski (1985) and Talley, Miller & Braselton (1978) have described the ultrastructure of the plasmodiophoraceous zoospore, and it is clear that the flagellar apparatus differs considerably from that of the peronosporomycetes. A fine transitional plate extends across the axoneme core at a point level with the cell plasmamembrane, but it does not extend to this membrane nor does it have a bead-like projection at its centre. Merz (1992) has used SEM to reveal ring-like swollen bases to the flagella at the junction with the plasmamembrane in *Spongospora*. The transitional zone also differs from that of the peronosporomycetes in lacking a concertina.

Schnepf (1994) did not compare his material of a *Phagomyxa*-like endoparasite with the *Thraustochytrium* of Kazama (1992*a*): there is some resemblance between the distally dilated cone on the transitional plate distal to the kinetosome (Schnepf, 1994: figs 14, 27; Kazama 1972*a*: figs 2, 4,,5). The absence of the dynein arms and the inner ring of electron-opaque material at the base of the axoneme are also similar. A somewhat similar construction, though less resolved, was noted by Barr & Allen (1982: fig. 1, 12) for *Polymyxa graminis*.

In common with many protists (cf. Hibberd, 1975), the kinetosome core of the plasmodiophorids contains two (?) rows of diffuse, slightly more electron-opaque spheres. Just within the triplets of the kinetosome there is a series of concentric fibres (visible in Barr & Allen, 1982: fig. 25 - D. J. S. Barr, pers. comm.) and this series of fibres continues throughout the transitional zone; in the peronosporomycetes a similar

series of concentric fibres is also present in the kinetosome, but these fibres do not extend into the transitional zone (Dr D. J. S. Barr, pers. comm.). Evidence of this series of concentric fibres can be deduced for the kinetosomes of *Myzocytiopsis lenticularis* (Glockling, 1994). The kinetosome root system in the plasmodiophorids is simple and similar for each flagellum, each kinetosome having two sets of microtubules; a triplet and a doublet. Barr & Allen (1982) concluded that this root morphology is more similar to that for certain protists than to that of any of the described flagellate fungi or algae. (Could the triplet perhaps be equivalent to the **R1** ribbed triplet or **R3** octet of the peronosporomycete apparatus which are more prominent in these much larger zoospores?)

In the choanoflagellates (e.g., Leadbeater & Morton, 1974) and in the monoblepharids (Fuller, 1966) the transitional plate is complex with two electron-opaque zones, a very faint one level with the cell plasmamembrane, as in *Polymyxa* and many protists, and a second, more distinct zone a short way into the flagellum (Figure I: 7). The relationship of this construction with that of the thraustochytrids should be assessed. Cavalier-Smith (1987) uses kinetosome and rootlet structure, among other characters, to derive the *Chytridiomycetes* from a choanomastigote-like progenitor, but it should be noted that the range of morphological variation in the limited number of species studied makes relationships conjectural although they are supported, in general, by molecular analyses (Tehler *et al.*, 2000).

The kinetosome of the posterior flagellum butts on to the shaft of the kinetosome to the straminipilous flagellum in the oomycetes, *Olpidiopsis* (Bortnick *et al.*, 1985; Martin & Miller, 1986*b*) and the labyrinthulids (Kazama, 1972*a*, 1980; Porter, 1974, 1990). The abuttment may be less obvious when the base of the posterior kinetosome is approximately level with, but offset from, that of the anterior flagellum as in *Phytophthora* (Barr & Désaulniers, 1989*a*, *b*, 1990*b*) (Figure I: 8). (A similar kinetosome arrangement occurs in the *Pedinomonadales* -Melkonian, 1990.) In the plasmodiophorids the same base-butting-to-shaft arrangement is found, although the two flagella cannot be uneqivocally identified (*Sorosphaera*, Talley *et al.*, 1978; *Polymyxa*, Barr & Allen, 1982; *Ligniera*, Miller *et al.*, 1985; *Spongospora*, Clay & Walsh, 1990). The same appears to be true for *Blastulidium* (Manier, 1976). The abuttment may be related to the replication sequence of the centriole-kinetosome complex, the posterior kinetosome being derived from the 'older' centriole in motile photosynthetic unicells (cf. Beech *et al.*, 1991), irrespective of whether it has subtended a straminipilous or a smooth flagellum: it is inferred that centriole replication is semi-conservative, in which the maturation of the new centriole is not complete until the second mitosis. The angle between the kinetosomes of the plasmodiophorids is variable: within the zoosporangium prior to flagellar activity it approximates to 30° (Clay & Walsh, 1990), but in active, free-swimming zoospores the angle is 150° (Barr & Allen, 1982) or 180° (Merz, 1992).

In the uniflagellate fungi the non-functional centriole is located in different positions. In *Hyphochytrium* the non-functional centriole is offset, and at a different level from the functional kinetosome (Cooney *et al.*, 1985) but in the *Chytridiales* and *Monoblepharidales* it is approximately parallel to the kinetosome and their bases are at the same level (Lange & Olson, 1979; Montecillo, Bracker & Powell, 1980; Barr & Désaulniers, 1987*a*). In the *Spizellomycetales* the position of the non-functional centriole is variable, and in the *Blastocladiales* it is structurally diminished or absent, but if present it is at right angles to the functional kinetosome.

The correlations between the transitional zones and the root systems in non-hairy anisokont, and heterokont organisms have not been fully evaluated.

While the ultrastructural morphology of the flagella, the kinetosomes and root arrangement may show a certain amount of variation from organism to organism, there is a group of core characters that is common to the straminipilous fungi (Barr, 1981*c*; Barr & Allen, 1985; Barr & Désaulniers, 1987*b*, 1989*a*; Barr, 1992). Nevertheless, it should be restated that because flagellation is heterokont it is not necessarily

peronosporomycetous; likewise, molecular sequences have shown that some flagellates without straminipilous ornamentation are related to the straminipiles (Silberman *et al.*, 1996). The flagellar ultrastructural characters that are ***diagnostic*** for the ***class Peronosporomycetes*** have ***not*** been established; it may be that no one character, on its own, will prove to be diagnostic. For the great majority of the lagenidiaceous organisms discussed here the information is either nonexistent, or far too fragmentary, to contribute to their classification.

ZOOSPOROGENESIS AND ZOOSPORE CYST GERMINATION

Asexual reproductive morphology

Most *Peronosporomycetes* have asexual reproduction but a few taxa are apparently incapable of producing any asexual stage. The diversity of asexual reproduction is considerable and can be summarized by noting the following points:

1. production of zoospores, aplanospores, or conidiosporangia
2. shapes and dimensions of the sporangia or conidiosporangia
3. morphogenesis and morphology of distinct sporangiophores or conidiosporangiophores
4. deciduous or caducous nature of the sporangia
5. ability of any sporangia to germinate indirectly by zoospores or directly by germ tubes
6. sporangial renewal: determinate or indeterminate (i.e., internal, cymose, basipetal, or percurrent) and/or accompanied by simultaneous or sequential sporangiogenesis
7. site and mechanism of protoplasmic cleavage and zoospore discharge including:
   - presence of a papilla or an operculum
   - presence of a discharge vesicle
8. site of encystment
9. ornamentations of the zoospore cyst wall

Asexual reproductive units may develop in terminal, lateral or intercalary positions on the assimilative thallus. The vegetative system then shows **eucarpic development**. Alternatively, the entire thallus may assume the role of a sporangium or, if septate, a series of sporangia (**holocarpic development**, irrespective of whether the sporangia mature simultaneously or sequentially). This transformation from an assimilative thallus to a sporangium (or sporangia) is frequently without morphological modification other than the formation of an exit tube and dehiscence papilla.

Most sporangia are of regular shape, frequently globose or fusiform, but *Pythium*, in particular, shows a wide intergrading range of sporangial shapes, from spherical, globose-clustered, through toruloid and digitate forms to otherwise-undifferentiated lengths of hyphae. Examples can be found of every one of these forms in terminal or intercalary positions. In the myceliar *Peronosporomycetes* the sporangium is usually terminal on a hypha and separated from the assimilative thallus by a **septum** (a wall deposit indistinguishable from and continuous with the thallus wall). In the *Rhipidiales* the sporangium is abstricted by a constriction and the remaining canal is blocked by a **plug** (material not confluent with the thallus wall) while in the *Leptomitales* the **cellulin granule** separates the sporangial protoplast from that of the other loments.

A zoosporangium usually has one or more apical papillae. The papilla normally protrudes from the general sporangial outline due to its thicker, often more hyaline wall. At maturity the papilla deliquesces to form the exit tube through which the planonts are extruded. In a few genera (e.g., *Calyptralegnia*, *Rhipidium*) the deliquescence is confined to a circumscissile ring to form a calyptra.

In the *Saprolegniales* the nuclei of the sporangial protoplast do not undergo further division, but in *Phytophthora* (Graham, 1954; Laviola, 1975; Maltese, Conigliaro & Shaw, 1995) and the *Peronosporaceae* (*Peronospora*, Trigiano & Spurr, 1987; *Bremia*, Tommerup, 1989; *Plasmopara*, Burruano *et al.*, 1992) the nuclei of the sporangioplasm undergo further mitoses. Khan (1976) described both nuclear degeneration and mitosis in the morphogenesis of sporangia of *Albugo*: in this genus the sporangia are formed in percurrent chains and so progressive morphogenetic stages could readily be traced. In taxa where the thallus becomes converted into the sporangium there cannot be different developmental pathways involving nuclear migration

or nuclear mitosis, such as occur in the eucarpic *Peronosporomycetes*. At maturity the lagenidiaceous sporangia are multinucleate, but neither the sequence of mitoses leading up to and following thallus septation nor the pro-sporangial morphogenesis has been documented.

Descriptions of zoospore discharge by lagenidiaceous fungi are often difficult to interpret because they refer to a pattern typical for another genus without precisely conforming to every feature in that genus. This is true for references to 'achlyoid' discharge (see Dick, Wong & Clark, 1984) and for '*Pythium*' discharge, with which discharge by lagenidiaceous fungi is compared. In the descriptions for many species assigned in the past to the *Lagenidiales*, sporogenesis does not correspond strictly to that outlined above for *Pythium*. In the lagenidiaceous fungi there are no exact equivalents to the zoosporogenesis of *Pythiogeton* or *Phytophthora*.

There are three stages in zoosporogenesis that need to be assessed critically:

1 the process of cleavage into spore initials, its site, timing and ultrastructural morphogenesis
2 the nature of the vesicle
3 the behaviour of the expelled spore - as a naked protoplast; in relation to flagellar development; in encystment, and in possible re-emergence

Cleavage

Cleavage of zoospore initials occurs either within the zoosporangium (**intrasporangial zoosporogenesis**) or after discharge of the sporangial protoplasm (**extrasporangial zoosporogenesis**). Zoosporogenesis is intrasporangial and cleavage furrows developed from dictyosome-derived cisternae first become confluent with the prominent central tonoplast vacuole and eventually breach the plasmamembrane in *Achlya* and in *Saprolegnia* (Gay & Greenwood, 1966; Money *et al.*, 1987). There is a consequent ***loss of volume*** (ca. 10%) of the zoosporangium as turgor is lost. Zoospore discharge is achieved by imbibition of water through the sporangial cell wall in response to the release, within the confines of the zoosporangial wall, of water-soluble (osmotically active) ß-1,3-glucans from the zoospore initials and residues from the cleavage cisternae and central vacuole (Money & Webster, 1985, 1988; Money *et al.*, 1988). Intrasporangial cleavage may be followed by planont encystment before discharge (*Aphanodictyon, Calyptralegnia*), extrusion with subsequent encystment (*Achlya, Verrucalvus*), or the zoospore initials may develop flagella prior to extrusion (*Saprolegnia, Phytophthora*). In *Blastulidium*, *Eurychasma* and *Eurychasmopsis* planonts become parietally rearranged prior to intrasporangial encystment.

Pythium is characterized by the extrusion of uncleaved multinucleate protoplasm into an extraplasmamembranic, membranous, glucan-polymer vesicle which is formed simultaneously with discharge and is therefore initially in close contact with the sporangial protoplasm and confluent with the sporangium wall (extrasporangial zoosporogenesis). In *Phytophthora* (and *Pseudoperonospora* of the *Peronosporaceae*) cleavage takes place ***within*** a persistent zoosporangial plasmamembrane. Such a zoosporangial membrane, could account for the 'evanescent' vesicles also reported for the *Rhipidiomycetidae* if the entire plasmamembrane bound packet of zoospores were emergent.

Whereas in the *Saprolegniaceae* there is a prominent central vacuole up to zoospore delimitation, in *Phytophthora* the immature sporangium loses the central vesicle before the cleavage cisternae are in place and only small remnants of the initial vacuole occur among the cleavage cisternae (Hohl & Hammamoto, 1967; Williams & Webster, 1970). Since these earlier studies, Hardham & Mitchell (1998); Harold & Harold (1992); Heath & Harold (1992); Hyde, Gubler & Hardham (1991); Hyde & Hardham (1992, 1993) and Jackson & Hardham (1998) have shown that the actin fibres of the cytoskeleton play a vital role in the

topographic organization of the nuclear spacing, cleavage cisternae and zoosporogenesis in *Phytophthora* and *Achlya*. Work of comparable detail is not available for other straminipilous fungi.

In the *Salilagenidiales* most of the cytoplasm is peripheral at the mid-cleavage stage, prior to discharge, and large vacuoles probably merge with the cleavage cisternae, so that the development is similar to, but not identical with, that of the *Saprolegniaceae*. Coincident with this zoosporogenesis is the presence or absence of a gelatinous matrix surrounding the planonts as they are discharged. A 'vesicle' develops from the exit tube apex as a gelatinous matrix ***prior to*** the extrusion of the protoplasm (see also Glockling & Dick, 1997: some *Myzocytiopsidales*). The protoplasm ***does not fill*** the clearly-defined 'vesicle'. At maturity this 'vesicle' becomes partially inverted and collapses down the outside of the exit tube during zoospore maturation, often remaining as a sleeve after discharge (Alderman, 1976). The boundary of a gelatinous matrix can often be distinct and it may be difficult to distinguish between such a boundary and the presence of a membranous vesicle. A fibrillar or amorphous vesicular 'membrane' could thus be formed as a precipitation reaction between such a colloidal matrix and the environment.

Studies of *Myzocytiopsis lenticularis* (Glockling, 1994) indicated that the vegetative thalloid segments do not possess a tonoplast, but that a few tonoplast vacuoles first develop early in the presporangial stage and persist while the cleavage cisternae reach an advanced stage of orientation. This developmental pattern is not saprolegniaceous (G. W. Beakes, pers. comm.) but neither can it be clearly equated with those of *Salilagenidium callinectes* or *Phytophthora*. Sparrow (1939*b*) also described a more or less *Saprolegnia*-like zoosporogenesis with a central vacuole for *Myzocytiopsis zoophthora* (Sparrow) M. W. Dick *sensu lato* (see Glockling & Dick, 1997; ≡ *Lagenidium oophilum* Sparrow). Photographs of living material (Canter-Lund & Lund, 1995: fig. 621) show that discharge in *Myzocytium megastomum* De Wild. and *Cystosiphon canterae* (Karling) M. W. Dick *loc. cit.* (≡ *Lagenidium canterae* Karling) is '*Pythium*'-like in every morphological detail. For many taxa assigned to the *Lagenidiales* there are clear statements that spore initials are fully preformed within the sporangium (but with extrusion of quiescent zoospores in *Myzocytiopsis lenticularis*, Glockling, 1994), or that the initials are to a greater or lesser extent delimited (*S. callinectes*, Bland & Amerson, 1973*a*), but with protoplasmic links similar to those described for *Aphanomyces* (Hoch & Mitchell, 1972*a*) and *Verrucalvus* (Dick *et al.*, 1984). In other species there are suggestions that both maturation and cleavage may be sequential, like *Schizochytrium*, with successive rather than simultaneous cleavage planes: for example the bipartitioning of the vegetative thallus of *Gonimochaete* (Drechsler, 1940) and chlamydospore formation in *Chlamydomyzium septatum* (Karling) M. W. Dick (≡ *Lagenidium septatum* Karling, Karling, 1969, Dick, 1997*b*). Cleavage sometimes occurs either within or outside the sporangium (*S. oedogonii*, *Cornumyces pygmaeus* (Zopf) M. W. Dick (≡ *L. pygmaeum* Zopf), Karling, 1981*a*: 118 and 126 respectively, Dick, 1998*b*). Another developmental pattern described for *Crypticola entomophaga* (≡ *Atkinsiella entomophaga*) (Martin, 1977), *Eurychasma dicksonii* (E. P. Wright) Magnus (Aleem, 1950*b*) and *Eurychasmopsis multisecunda* Canter (Canter & Dick, 1994 - the *Eurychasma* species described by Canter *et al.*, 1990)) is with the cleavage taking place within the sporangium but followed by sequential release of spores. Kinetosomes with their transitional plates are present in the uncleaved and as yet non-flagellate zoospore initials of *Myzocytiopsis* (Glockling, 1994).

How much variation in the morphogenesis of cleavage can be accepted within a single genus? Are these mechanisms of sporogenesis compatible with concepts for *Pythium*; the *Pythiaceae*, the *Pythiales* or the *Peronosporomycetes*? If these diverse patterns of sporogenesis are correlatable with other differences, there may be grounds for separation at a high taxonomic level.

Evidence for a vesicle

Pythium is characterized by the extrusion of uncleaved multinucleate protoplasm into an extra-plasmamembrane, membranous, glucan-polymer vesicle which is formed simultaneously with discharge. The vesicle membrane probably has a fibrillar nature since it has sufficient tensile strength to prevent escape of the vigorously motile zoospores for a perceptible length of time. Lunney & Bland (1976) have shown that the vesicle membrane is continuous with, and attached to the exit tube wall (≡ modified papilla) (cf. Hallett & Dick, 1986: fig. 11 for a *Pythium* 'microsporangium'). The dynamics of sporangial discharge are discussed by Money, Webster & Ennos (1988) for *Achlya* and Gisi & Zentmyer (1980) for *Pythium* and *Phytophthora*.

Within the *Pythiaceae* there are two other sequences which are derived conditions. In *Pythiogeton* the discharged and encapsulated sporangial protoplast becomes detached from the exit tube, but the membranous nature of the vesicle is preserved so that the vigorously motile zoospores are contained or restrained for a short time before rupture of the vesicle (M. W. Dick, unpublished obs.). In *Phytophthora* the inner wall layers of the discharge papilla extend down the inside of the sporangial walls for a considerable distance, rather as if a 'vesicle' had been retained within the sporangial cavity (Chapman & Vujičić, 1965; Gisi, Hemmes & Zentmyer, 1979).

Lange, Edén & Olson (1988) and Hyde, Gubler & Hardham (1991) have shown that cleavage takes place ***within*** a persistent zoosporangial plasmamembrane in *Pseudoperonospora* and *Phytophthora* respectively. Such a membrane, if emergent as it sometimes is, would be fragile and could account for the 'evanescent' vesicles described, for example, in the *Rhipidiomycetidae* (Sparrow, 1960). Hyde & Hardham (1992) and Jackson & Hardham (1998) have shown that the morphogenesis of cleavage is governed by the development and array of cytoskeletal filaments which, in turn, determine the nuclear spacing and cleavage planes. Inflation of the cleavage cisternae into vesicles may be an artefact.

Clay, Benhamou & Fuller (1991) have shown that the sporangial contents of *Rhizidiomyces apophysatus* Zopf are discharged as a single protoplast bounded by a thin, ephemeral, chitinous extension of the inner sporangial wall. The fate of this wall during cleavage is not established.

The range of patterns of discharge of 'lagenidiaceous' zoospores includes:

1 individual emergence of active, flagellate zoospores
2 extrusion of fully formed aplanospores
3 extrusion of fully formed quiescent (incompletely flagellate) zoospores
4 extrusion of partially delimited planonts
5 extrusion of a naked coenocytic protoplast

Coincident with this range is the presence or absence of a gelatinous matrix (Couch, 1942). The boundary of such a matrix will often be evident against the water of the environment (see Canter, 1979: plate 4A) and it may be difficult to distinguish between such a boundary and the presence of a membranous vesicle. Fibrillar or amorphous vesicular 'membranes' could also be formed as a precipitation reaction between a colloidal matrix and the environment (cf. Bland & Amerson, 1973*a*). An analogous situation is the mucilaginous condensation to form a wall-like membrane outside the oogonium in *Aplanopsis terrestris* Höhnk (Dick, 1969: plate 2, fig. 7). Two observations are important: the visible and ultrastructural nature of the junction of the vesicle with the discharge tube, and the sequence of cleavage and flagellar activity in relation to vesicle formation.

The developmental sequence for marine '*Lagenidium*' species has two features not found in the *Pythiaceae* and which may indicate a different vesicle morphogenesis. The vesicle develops from the exit tube apex

as a gelatinous matrix ***prior to*** the extrusion of the protoplasm. The protoplasm ***does not*** fill the clearly-defined vesicle, nor is it initially spherical; also the vesicle becomes partially inverted and collapses down the outside of the discharge tube during zoospore maturation, often remaining as a sleeve after discharge (see Alderman, 1976). The zoosporogenesis of the *Myzocytiopsis* sp. described by Glockling & Dick (1997) is initially similar.

In contrast, *Lagenidium rabenhorstii* was described by Zopf (1884) as possessing a vesicle, but both Atkinson (1909) and Ivimey Cook (1935) state that the sporangial protoplast emerges naked and that cleavage takes place in the absence of a vesicle. There are several other instances (e.g., *Myzocytiopsis zoophthora* (Sparrow) M. W. Dick (≡ *Myzocytium zoophthorum* Sparrow, Sparrow, 1936*b*; Dick, 1997*b*; Glockling & Dick, 1997) in which cleavage and incipient flagellation appear to take place in the naked protoplast, so that the 'vesicle' is said to "deliquesce completely". Such 'vesicles' are probably not homologous with those of *Pythium*. Likewise, suggestions that the vesicle is developed ***subsequently*** to cleavage (*Myzocytiopsis humana* (Karling) M. W. Dick (≡ *Lagenidium humanum* Karling, Karling, 1947*b*; Dick, 1997*b*; the description for [*Sali*]*lagenidium* sp. (marine): Lightner & Fontaine, 1973) may also relate to an extra-spore and intra-sporangial secretion rather than to an essentially wall-like extra-plasmamembrane. Hesse *et al.* (1989) observed an initial interface between the protoplasm and the environment which developed into a "mucilaginous court" in *Ducellieria*.

A further possibility is that the vesicle may be derived from a persistent plasmamembrane as in *Phytophthora* and *Pseudoperonospora* (Hyde *et al.*, 1991; Lange *et al.*, 1988).

It thus appears that three major categories of 'vesicle' occur in the *Peronosporomycetes* and *Hyphochytriomycetes*:

Homohylic vesicle

An extra-plasmamembrane wall of fibrillar polyglucan or chitin continuous with one of the sporangial wall layers (e.g., *Pythium* and *Hyphochytrium* respectively) (ὁμο-homo - same ὑηλ-hyle - material).

Plasmamembranic vesicle

An evanescent plasmamembrane sac which was the sporangial plasmamembrane (e.g., *Phytophthora*, *Pseudoperonospora*).

Precipitative vesicle

A non-membranous (or initially gelatinous, non-membranous and non-fibrillar), fluid boundary between the peri-zoosporic oligoglucan colloid and the water of the environment (e.g., *Saprolegnia* and possibly *Salilagenidium callinectes*).

Data distinguishing this degree of precision are often totally lacking.

Zoospore taxis

Zoospores would inevitably have developed a recognition system and a mechanism for locating a suitable substratum over evolutionary time. The recognition system is not well understood, but the response has been documented more thoroughly. Tropic and taxic responses have been reviewed by Wynn (1981). Zoospore attraction to roots was first reported by Goode (1956). Directional movement, or **taxis** may have different stimuli, and chemotaxis (Khew & Zentmyer, 1973; Rai & Strobel, 1966; Zentmyer, 1979); electrotaxis (Khew & Zentmyer, 1974; Morris & Gow, 1993; Morris *et al.*, 1992; Troutman & Wills, 1964); negative geotaxis (Cameron & Carlile, 1977), and phototaxis (Muehlstein & Amon, 1987*b*) have all been described, usually for *Phytophthora*, *Aphanomyces* and other plant pathogenic fungi or *Peronosporomycetes* used for physiological and biochemical studies. Zoospore taxis has been recorded for

parasites of plants (Mitchell & Deacon, 1986*a*); algae (Kerwin *et al.*, 1992); fish (Willoughby & Pickering, 1977) and nematodes (by *Catenaria*, see Jannson & Thiman, 1992).

The pattern of zoospore aggregation along roots has been reported by Dale & Erwin (1991), Halsall & Williams (1984), Hinch & Weste (1979), Ho & Hickman (1967*b*), Irwin (1976), Jones *et al.* (1991), Kraft *et al.* (1967), Kuan & Erwin (1980), Milholland (1975), Palloix *et al.* (1988*a*), Shishkoff (1989), Spencer & Cooper (1967), Tippett *et al.* (1976) and Zou & Paulitz (1993). Royle & Hickman (1964*a*, *b*) made a thorough study of aggregation to roots by *Pythium aphanidermatum*. Root surface topography has been implicated for zoospore aggregation by Deacon & Donaldson (1993).

In dense spore suspensions the phenomenon of autoaggregation may occur. The phenomenon was first noted by Lounsbury (1927) for *Isoachlya* and has occasionally been noted since then; Porter & Shaw (1978), working with *Phytophthora drechsleri*, viewed the autoaggregation as a taxis. Jones *et al.* (1991) have suggested that autoaggregation may be instigated soon after a few zoospores have clumped together. Reid, Morris & Gow (1995) have found that autoaggregation of zoospores is 'genus specific'. If the degree of specialization is confirmed for more genera, then this attribute could add to the criteria for diagnoses.

Flagellar retraction

The shedding or retraction of flagella at encystment and flagellar transformation (Beech *et al.*, 1991) are important, but rarely observed or recorded, criteria. For the chytrids, Koch (1968) has provided the most orderly account, defining five patterns of flagellar loss:

1. total detachment and loss
2. 'vesicular coiling', either from the flagellar tip or part-way along the flagellum, with eventual absorption of the vesicle into the zoospore body
3. 'straight-in', direct retraction
4. 'body-twisting', a rotation of the zoospore, acting as a spool for the axoneme
5. 'lash-around', an exceptional movement of the flagellum towards the zoospore body

The two last patterns have close similarities in that they result in the flagellar plasmamembrane making longitudinal contact with the zoospore body so that the flagellar axoneme temporarily resides tangentially within the encysting zoospore. There is no comparable review for straminipiles, although all of these patterns may occur.

Crump & Branton (1966) described a combination of vesicular coiling and body-twist for the auxiliary zoospore of *Saprolegnia*, and Holloway & Heath (1974) subsequently provided a more detailed account. There is non-synchronous shortening (presumed depolymerization) of the axoneme lengths of the two flagella, apparently unaccompanied by any change in kinetosome/nucleus orientation. When very short (<3 μm) the axoneme wraps against the zoospore plasmamembrane and becomes incorporated within the cell. A wrapping around of the straminipilous flagellum appears to be a feature of *Gracea gracilis* (Dick, 1998*a*). In the chrysophyte *Hibberdia*, Andersen (1989) also concluded that flagellar retraction was achieved by the flagellum wrapping round the body of the cell. Holloway & Heath (1974) were unable to distinguish between the straminipilous and smooth flagella, but it should be noted that a wrapping of the straminipilous axoneme around the body of the zoospore would be inconsistent with the presence of a tuft of tubular tripartite hairs on the resultant cyst wall (cf. Dick, 1990*a*). (Such tufts may be found during flagellar transformation and development in motile chrysophytes - R. A. Andersen, pers. comm., but this is not a strictly comparable situation.) Schnepf, Deichgräber & Drebes (1978*d*) reported that the flagella of the first-formed zoospores of *Lagenisma* were retracted by a movement of the whole kinetosome-axoneme unit

towards the centre of the cell, which ought to account for a tuft of tubular tripartite hairs, but unfortunately these authors did not report the presence of any such tuft on the cysts of *Lagenisma*.

Whittick & South (1972) stated that, for *Pontisma antithamnionis* (Whittick & South) M. W. Dick *loc. cit.* (≡ *Olpidiopsis antithamnionis* Whittick & South), contact with the host is established by the longer posterior flagellum, followed by rapid activity (flagellar retraction?) resulting in the zoospore encysting at the host surface. Held (1973*a*) gave TEM evidence that the single smooth flagellum of *Rozella allomycis* F. K. Foust is retracted after making contact with the host. Equivalent information for *Rozellopsis* is more circumstantial: Prowse (1951) confirmed the observation by Fischer (1882) that attachment to the host is via the tip of the longer posterior whiplash flagellum, which then becomes retracted, drawing the encysting zoospore body to the host surface.

Ivimey Cook (1935: plate 4, fig. 1) photographed the attachment of a *Syzygangia zygnematicola* zoospore to a *Spirogyra* filament by the tip of the anterior flagellum. Similarly, Waterhouse (1940) described the attachment of *Gracea waterhouseae* (Karling) M. W. Dick (≡ *Rozellopsis waterhouseae* Karling, Karling, 1942*c*; Dick, 1997*b*) as by the longer anterior (straminipilous ?) flagellum. This would be the normal mode of attachment for cells with chemotactile-sensitive flagella, as has been demonstrated for fucophyte antherozoids (Müller & Falk, 1973).

For the plasmodiophorids, Fuchs (1966) has shown that the *Polymyxa* zoospore appears to make a "probe contact" with potential substrates by the tip of the shorter anterior flagellum, which is presumably chemotactile-sensitive. After contact, the anterior flagellum is retracted first. The suggestion has been made that virus particles, transmitted by plasmodiophorids, become bound to the flagellar membrane and become internalized following flagellar retraction (Temmink, Campbell & Smith, 1970; Stobbs, Cross & Monocha, 1982). Internalization of that part of the parasite plasmamembrane would not be necessary when the entire protoplast becomes ***inserted*** within the host cytoplasm. Presumably the mechanism would be different when *Lagena* is implicated as a vector.

Flagellar retraction has been presumed for hyphochytrids (Fuller & Reichle, 1965). Porter (1990) stated that it was not known whether the flagella of labyrinthulid (or thraustochytrid) zoospores are retracted or shed, but Bower (1987*a*) stated that the flagella of *Labyrinthuloides haliotidis* were shed. The planonts of species currently placed in *Olpidiopsis* appear to combine some characters of both the principal and the auxiliary zoospore types of *Saprolegnia*, but with additional, non-peronosporomycete features.

Flagellar detachment is characteristic for principal form zoospores of the *Peronosporomycetes* (Crump & Branton, 1966). The distinction between flagellar retraction or the shedding of flagella at encystment may be a more fundamental criterion than the point of flagellar insertion (cf. auxiliary and principal form zoospores).

Recognition of the substratum by the zoospore, or perhaps its straminipilous flagellum, is a prior requisite for encystment. Bimpong (1975) and Bimpong & Hickman (1975) described the metabolic reserves, enzyme activities and the time scales for encystment and germination. Paktitis, Grant & Lawrie (1986) noted surface changes which were consistent with stimulus-mediated secretion and there has been considerable work carried out on the calcium metabolism during these processes (see Broembsen & Deacon, 1996, 1997; Deacon & Donaldson, 1993; Donaldson & Deacon, 1992, 1993; Griffith, Iser & Grant, 1998; Grant, Griffith & Irving, 1986; Hill, Grayson & Deacon, 1998; Irving & Grant, 1984; Irving, Griffith & Grant, 1984; Iser *et al.*, 1989; Jackson & Hardham, 1996; Jackson & Heath, 1993; Reid, Morris & Gow, 1995; Warburton & Deacon, 1998 and references therein).

Encystment

The processes involved in the production and encystment of zoospores have been studied most intensively for *Phytophthora*, and to a lesser extent, *Achlya* (Hardham, 1987*b*, 1989; Hardham, Gubler & Duniek, 1990; Hardham & Mitchell, 1998; Hardham & Susaki, 1986; Hardham, Susaki & Perkin, 1985, 1986; Harold & Harold, 1992; Heath & Harold, 1992; Hyde, Gubler & Hardham, 1991; Hyde & Hardham, 1992, 1993, and Jackson & Hardham, 1998).

The zoosporangial and zoospore dictyosomes secrete three kinds of vesicles which are peripheral at the time of encystment. Two of these kinds of vesicle are located in the ventral groove of the swimming zoospore while the other is located under the remaining plasmamembrane. In *Phytophthora* the zoospore comes to rest on the substratum with the ventral groove against the substratum. The flagella are shed and the ventral vesicles attach the encysting zoospore to the substratum. The remaining vesicles secrete the cyst wall precursors which rapidly polymerize to form the cyst wall.

Cyst morphology

Cyst morphology has been described by Beakes (1983, 1987) and Dick (1990*a*) (Figure I: 9). For *Peronosporomycetes*, it is generally accepted that flagella of the auxiliary zoospore are retracted, except for the tubular tripartite hairs which are left in a tuft at the point of resorption (Dick, 1990*a*), while the flagella of the principal zoospore are shed (the membranes of these flagella may round up and bear scattered tubular tripartite hairs). Subsequent ornamentation of the cyst wall is derived from preformed structures in cytoplasmic vesicles (Beakes, 1983, 1987). References to cyst wall ornamentation are lacking in all but three of the lagenidiaceous taxa. In two of the exceptions spines are formed (*Haliphthoros milfordensis,* Overton *et al.*, 1983; and *Lagenisma coscinodisci*, Schnepf *et al.*, 1978*c*, *e*). In *Lagenisma*, as in *Leptomitus* (Hallett, 1975; Hallett in Dick, 1990*a*) and *Hyphochytrium* (Hallett, 1975) the spines are formed as finger-like protrusions, initially filled with cytoplasm. In *Gracea gracilis* the straminipilous flagellum is retracted by the 'lash-around' mechanism, leaving an equatorial ring of tubular tripartite hairs over the cyst surface (Dick, 2000*d*). The only other fungus known to have such hairs distributed over the cyst surface is the hyphochytrid, *Rhizidiomyces apophysatus* (Fuller & Reichle, 1965); in which the spiral banding of the tubular shafts was particularly prominent (cf. Domnas *et al.*, 1986, *contra* Beakes, 1987) (comparisons should also be made with the slopolinids - Patterson, 1989).

For a few lagenidiaceous species (see PART VI) the zoospore is reported to come to rest on a host, not by forming a spherical cyst as would be expected in any peronosporomycetous fungus, but by forming a hemispherical structure attached to the host over a large surface area. This again suggests that there is a greater tendency for the planont to revert to an amoeboid form than is normal for *Peronosporomycetes*. *Gracea*, *Rozella* and *Lagena* cysts undergo changes of shape during encystment but are spherical when attached to their hosts (Pemberton *et al.*, 1990; Held, 1973*a*; Barr & Désaulniers, 1987*b*).

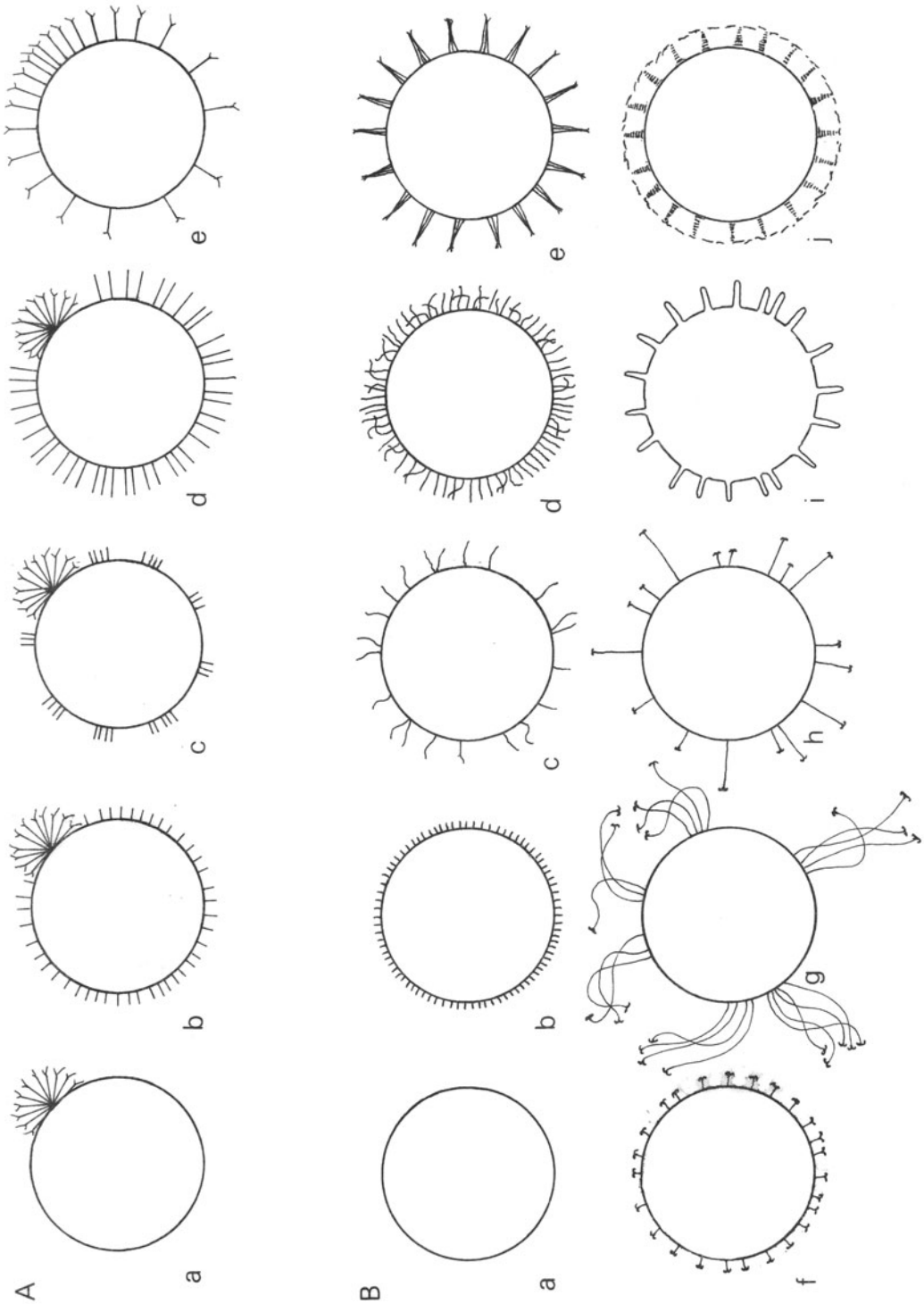


FIGURE I: 9. Zoospore cyst morphology (after Beakes, 1987; Dick, 1990*a*; Hallett, 1975).
Fig. A: cysts of auxiliary-form or pyriform zoospores (*Saprolegniomycetidae*) with a tuft of sloughed tripartite tubular hairs as straminipilous flagellum is resorbed: a: *Saprolegnia ferax*; b: *S. diclina*; c: *S. parasitica*; d: *S. megasperma*; e: *Rhizidiomyces apophysatus*; *Gracea gracilis* (this configuration is also found on membranes from shed straminipilous flagella).
Fig. B: cysts of principal-form zoospores (straminipilous flagellum shed): a: unornamented, *Saprolegnia turfosa*; *Pythium middletonii*; b: investment of short hairs - *Phytophthora cryptogea*; c: sparce investment of long hairs - *Pythium monospermum*; d: dense investment of long hairs - *Apodachlya minima*; e: regular distribution of tight fascicles of hairs, fimbriated at tips - *Dictyuchus sterile*; f: regularly spaced bifurcated hairs ('boat-hook' hairs - *Saprolegnia diclina*; g: tufts of long 'boat-hook' hairs - *S. parasitica*; h: 'boat-hook' hairs of varying lengths - *S. hypogyna*; i: hollow papillae - *Leptomitus lacteus*; *Hyphochytrium* sp.; *Lagenisma coscinodisci*; j: striated spines under a fibrillar layer - *Haliphthoros milfordensis*.
Fig. 9 Aa, Bb, Bh, Bi, reproduced from Dick (1999: fig. 4) by permission of Academic Press.

Germination

Cyst germination in the *Peronosporomycetes* can follow one of three basically different patterns of development, further elaborated in Figure I: 10):

1 in the non-filamentous *Rhipidiomycetidae* the germination is polar, with the production of a rhizoidal system followed by the enlargement of the original cyst to form an isodiametric or lobed basal cell, which subtends the sporulating structures. The 'mycelial' development of *Sapromyces* probably represents proliferations of potential reproductive units rather than a vegetative axis bearing sporangia

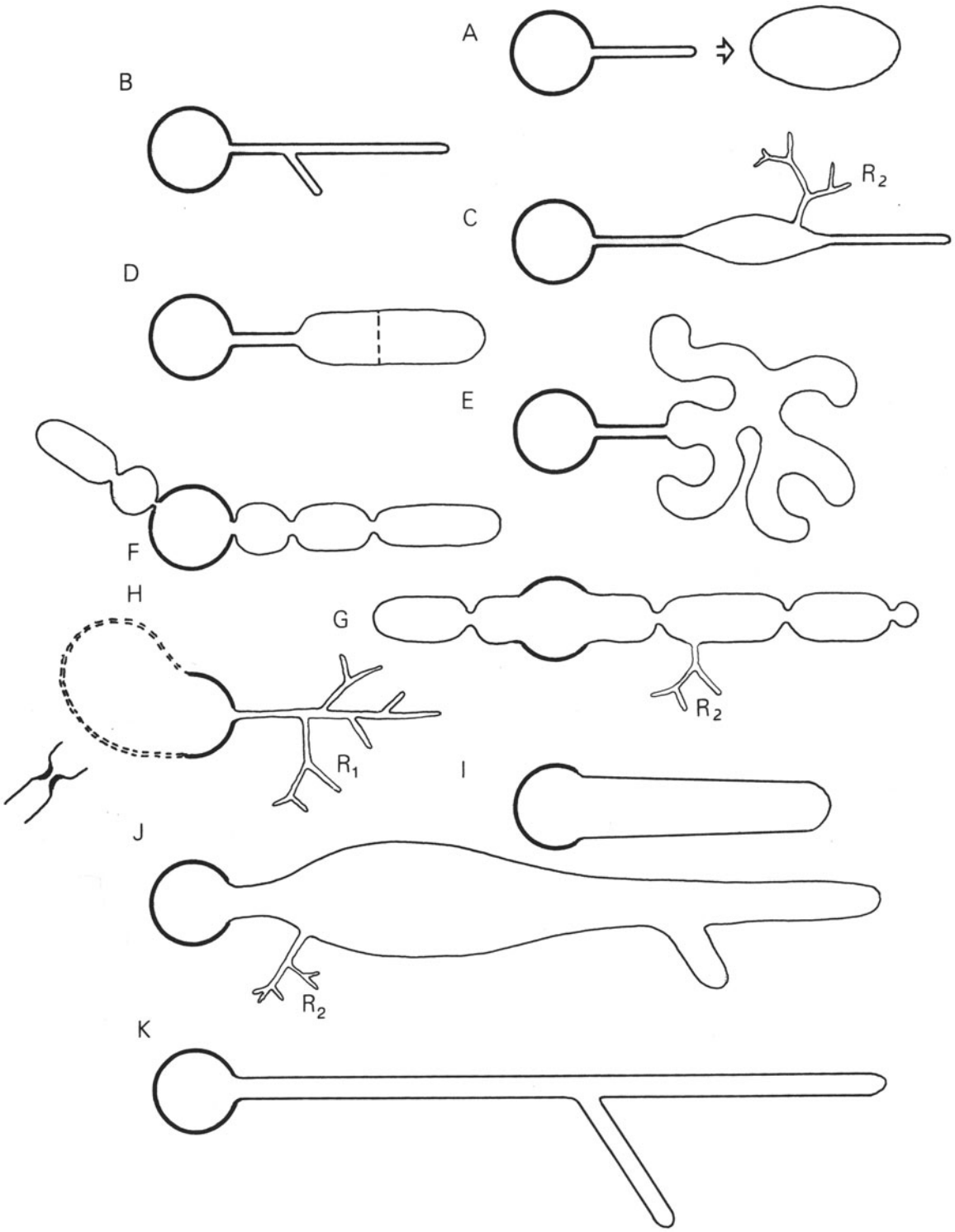
2 in the *Peronosporomycetidae* and the *Saprolegniomycetidae* (with the exception of the *Leptomitales*) two partially interchangeable patterns are known: either a very narrow (*ca* 1-3 μm diam.) germ-tube is produced which may maintain a branched vegetative existence as in the *Sclerosporales* (Dick *et al.*, 1984, 1989) or subsequently undergo rapid transition to a hyphal diameter of 5 μm or more; or a large diameter germ tube may be produced directly so that the spore cyst is scarcely discernible

3 in the *Leptomitales* the development is blastic and each segment or bud so produced is capable of further budding, or in *Leptomitus*, rhizoids may be produced from these secondary buds

The wide-diameter hyphal systems of the *Saprolegniales* may represent modifications from a basal-cell -type of organization, with isodiametric growth being replaced by tip growth accompanied by a shift of assimilatory function from a well-defined rhizoidal system to these broader hyphae (which, in *Achlya* particularly, may show continued growth in diameter). The 'vegetative' system of the *Saprolegniales* may thus be considered as an assimilative, growth-unlimited sporangio-gametangiophore.

There is probably a developmental relationship between the fine germ tube and the rhizoid. The development of the coralloid thallus of certain *Leptolegniellaceae* from an initial narrow diameter germ tube could be envisaged as an extension, with less coordinating control, of the blastic pattern (**3**). This same developmental pattern is found in *Haliphthoros* and in a number of other species which have been referred to the *Lagenidiales*. In most of the endoparasitic fungi which have been studied by TEM, the development of the fine penetration tube is accompanied by the expansion of a prominent distal vacuole in the zoospore cyst (Held, 1973*b*).

FIGURE I: 10. Diagrams of peronosporomycete thallus forms from zoospore cyst germination patterns to secondary developments: independent origins of the mycelial habit may be discerned. **Fig. A.** Tip growth of a narrow germ tube of limited length, with injection of protoplasmic contents to form a detached intra-host-protoplasmic non-mycelial thallus: *Olpidiopsis*, *Gracea* (*Olpidiopsidales*). **Fig. B.** Indeterminate tip growth and branching from a narrow germ tube (no vegetative expansion or intussusception) dilating only for reproductive function: *Verrucalvus*, *Sclerospora* (*Sclerosporales*), hyphae of *ca* 5 μm diam. **Fig. C.** Indeterminate tip growth and branching, presumably from a narrow germ tube, dilating periodically and producing secondary rhizoids (R_2), also dilating for reproductive function: *Medusoides* (*Pythiogetonaceae*), hyphae of *ca* 5 μm diam. **Fig. D.** Broad elongate growth from an expanded germ tube apex (narrow germ tube), allantoid or branched: *Myzocytiopsis* (*Myzocytiopsidaceae*). **Fig. E.** Broad elongate growth from an expanded germ tube apex (narrow germ tube), coralloid or branched: *Leptolegniella* (*Leptolegniellaceae*). **Fig. F.** Blastic with elongate segments (non-polar): *Apodachlya* (*Leptomitaceae*). **Fig. G.** Parablastic with elongate segments (non-polar), with or without secondary rhizoids (R_2): *Leptomitus* (*Leptomitaceae*). **Fig. H.** Cyst polar with primary rhizoids (R_1), cyst becoming swollen (forming the basal 'cell' by generalized intussusception) with proliferous tubular or clavate elements separated by thick-walled narrow isthmuses, eventually terminating in reproductive structures: *Sapromyces* (*Rhipidiaceae*). **Figs I, J.** Indeterminate tip growth and branching from a broad or very broad germ tube, with considerable later near-basal intussusception, with or without secondary rhizoids (R_2): *Achlya* (*Saprolegniaceae*), hyphae of 40-140 μm diam. **Fig. K.** Indeterminate tip growth and branching from a moderately broad germ tube, without intussusception, not dilating for reproduction, sometimes with retraction septa: *Pythium*, *Peronospora* (*Peronosporales*), hyphae of *ca* 10 μm diam.


A
B
C
R2
D
E
F
H
G
R2
R1
I
J
R2
K

An additional feature of some of the lagenidiaceous fungi is the production of an ellipsoidal swelling part-way along the thin germ tube (*Cystosiphon canterae* (≡ *Lagenidium canterae* - Canter & Lund, 1969; *Pleocystidium lundiae* (Karling) M. W. Dick *loc. cit.* (≡ *Lagenidium lundiae* Karling) - Canter & Lund, 1969; *Lagenidium* sp. on *Oocystis* - Masters, 1971, and *Crypticola entomophaga* (≡ *Atkinsiella entomophaga*) - Martin, 1977). In *Gonimochaete* the cyst germinates on the surface of the nematode, producing an extremely fine penetration tube of *ca* 0·1-0·2 μm diameter (Saikawa & Anazawa, 1985). This tube subsequently enlarges to form the first unit of the disarticulating thallus. The discharged intermediary infection cell of *Haptoglossa mirabilis* (Barron, 1980) also has an ellipsoidal swelling in its tube-like extension analogous to those mentioned above.The *Peronosporomycetes* have a highly developed complex of extrusomes associated with encystment and germination. Exemplary studies by Hardham, Suzaki & Perkin (1985, 1986) and the overview by Hardham (1998) indicate that extra-plasmamembrane secretions in the region of the flagellar insertion are responsible for cyst attachments in *Phytophthora*. Donaldson & Deacon (1992) give further information on cyst adhesion for *Pythium* and Deacon & Saxena (1998) have extended the information to cover *Aphanomyces* and *Phytophthora*. In *Achlya* the encysting aplanospores become cemented together to form a hollow sphere of cysts. Secretory vesicles (mucosomes?, K-bodies - kinetosome-associated bodies - Lehnen & Powell, 1991) associated with this process may have a dictyosome origin. Further information on the distribution and ultrastructural differences of K_2 bodies, peripheral vesicles and encystment vesicles within the *Peronosporomycetes* (*Pythiaceae*, *Saprolegniaceae*, *Leptomitaceae* and *Rhipidiaceae*) is available in Sadowski & Powell (1990) and Randolph & Powell (1992). Held (1973*b*) has reviewed the more physiological aspects of germination in the endobiotic parasitic fungi. Further studies of 'lagenidiaceous' fungi since then have revealed a secretion/cementation of a different magnitude. An adhesive pad is apparently secreted from the cyst, whether that cyst has been formed from an aplanospore (*Myzocytiopsis subuliformis* M. W. Dick, Dick, 1997*a*) or from a zoospore (*Myzocytiopsis glutinospora* (G. L. Barron) M. W. Dick [≡ *Myzocytium glutinosporum* G. L. Barron], Dick, 1997*a*). Newell *et al.* (1977: 189) have postulated that the apparatus developed in *Haptoglossa* may merely be a further elaboration of such a secretion, but it is more likely that an intermediary infection cell, with its own adhesive pad (Barron, 1980) is developed from the cyst.

Extrusomes

Many protists possess organelles which extrude or evert their contents to the exterior, but which are not associated with generalized excretion or the development of extra-plasmamembrane investments such as walls or scales (Hausmann, 1978). Sleigh (1989: table 5:1) gives an indication of the distribution of extrusomes in flagellate protoctists; homologies have not been fully explored, but extrusomes are reported from diverse groups of chromistan algae - chrysophytes, raphidophytes, cryptophytes and other anisokont flagellates such as euglenoids and glaucocystophytes, and some green flagellates.

Extrusomes include vesicles with different levels of complexity, ranging from mucosomes to nematocysts. In the *Peronosporomycetes* mucosomes, or K_2-bodies, may be associated with specific secretions for wall modification (Lehnen & Powell, 1991). Hardham and coworkers (Gubler & Hardham, 1988, 1990; Gubler, Hardham & Duniec, 1989; Hardham, 1989, 1998; Hardham & Gubler, 1990; Hardham, Gubler, & Duniec, 1990; Hardham & Suzaki, 1986; Hardham *et al.*, 1985, 1986) have provided a detailed description of the three kinds of vesicle in *Phytophthora*. All are involved in encystment and attachment by cementation to the substrate. Prominent peripheral vesicles are figured for *Myzocytiopsis* (Glockling, 1994) and by Canter & Dick (1994: fig. 27) for zoospores of *Eurychasmopsis*.

The K_2-bodies, which have a tubular or structured matrix lumen, are regarded as having phylogenetic significance within the heterokont fungi and *Peronosporomycetes* in particular (Powell *et al.*, 1985; Powell & Blackwell, 1995; Randolph & Powell, 1992). They have been identified in several genera of the

Saprolegniaceae, where the ultrastructure is similar; in *Aphanomyces* there are some differences and Hoch & Mitchell (1972*b*) called them U-bodies. *Apodachlya* (*Leptomitaceae*) has similar K_2-bodies to the *Saprolegniaceae*. No comparable structures have been reported for the *Pythiaceae* or other *Peronosporomycetidae*. *Olpidiopsis saprolegniae* var. *saprolegniae* also has K_2-bodies, and these are compared with those of a tribophyte alga, as well as with other heterokont fungi (Powell *et al.*, 1985). These authors concluded that K_2-bodies were not discernable in micrographs published for *Ectrogella perforans*, *Salilagenidium callinectes*, *Lagenisma coscinodisci* or *Haliphthoros milfordensis*. The descriptions of *Ciliatomyces spectabilis* (Foissner & Foissner, 1986*a*, *b*) and *Ducellieria chodatii* (Hesse *et al.*, 1989) do not mention structures equatable with K_2-bodies.

Much more elaborate ultrastructure is possessed by the nematocysts of the dinoflagellates, the gullet extrusomes of the cryptophytes and the rohr/stachel/schlauch (glossoid cell/gun cell) complex of the plasmodiophorids and *Haptoglossa*. The relationship between the complex penetration apparatus of the plasmodiophorids and of *Haptoglossa* is reviewed in more detail in PART IV, pp. 202-213.

No comparable information is available for other lagenidiaceous fungi, either with respect to the simpler mucosomes or the more complex gun cells.

Dense-body vesicles

Dense-Body Vesicles (DBVs, also known as Finger-Print Vacuoles - FPVs - Hemmes, 1983) are often prominent in the reproductive structures of the *Peronosporomycetes*, although they can be found at all developmental stages. They are implicated in the production of the inter-protoplasmic colloids responsible for zoosporangial evacuation in the *Saprolegniaceae* (Money & Webster, 1985, 1988); in cleavage of ooplasm in oosporogenesis (Gay, Greenwood & Heath, 1971; Fletcher, 1979; Beakes, 1981*b*); in wall formation in oogonia of *Achlya radiosa* Maurizio (but not *Saprolegnia*) (Al-Rekabi, 1979), and are known to coalesce to form the ooplast in the mature oospores of all *Peronosporomycetes* (Howard & Moore, 1970; Fukutomi, Aki & Shiraishi, 1971; Hemmes & Bartnicki-Garcia, 1975; McKeen, 1975; Sargent, Ingram & Tommerup, 1977; Beakes & Gay, 1978*a*; Beakes, 1981*b*; Dick, 1990*a*, 1995). At different phases of the life-history and organ development they may show different and developmentally intergrading morphology, as revealed by TEM. At one extreme there is either a single central or one or more peripheral, more or less sharply defined electron opaque core or cores in an electron-translucent matrix. At the other extreme the matrix becomes almost completely filled with myelin-like configurations (hence the FPV connotation - Hemmes, 1983: figs 5 & 6) which initially take the form of layers joining the electron-opaque core to several parietal electron-translucent aggregates within the vesicle membrane (Al-Rekabi,1979; Al-Rekabi in Dick, 1990*a*: fig. 2). These myelin configurations can also appear as 'exfoliations' from the electron-opaque core, and strata-like nonconformity between blocks of myelin configurations can also be found within the same vesicle. Wang & Bartnicki-Garcia (1973, 1974), Powell & Bracker (1977) and Bartnicki-Garcia & Wang (1983) have shown that the DBVs contain phosphorylated mycolaminarans, and such ionized glucans may be responsible for the myelin-like appearence in TEM.

The prominence and presumed importance of DBVs, and their involvement in glucan and phosphate metabolism and storage, might suggest an explanation for the phosphate/polyphosphate storage differences between *Eumycota* and oomycetes (absence of metachromatic granules as determined by toluidine blue O staining in the latter) that have been noted by Dietrich (1976) and Chilvers, Lapeyrie & Douglass (1985), and discussed by Niere, Griffith & Grant (1990) and Grant, Grant & Harris (1992).

The occurrence of DBVs in the plasmodiophorids has not been reported.

For the lagenidiaceous fungi there are no clear figures of the distinctive myelin-like form of DBVs, although TEMs may show possible cored DBVs, as in *Salilagenidium callinectes* (Gotelli, 1974*b*: fig. 9) and *Blastulidium* (Manier, 1976); the ooplast of *Ciliatomyces* (Foissner, 1987) also appears to have a DBV origin, although the electron-opaque regions are isolated and peripheral. Ultrastructural work (Glockling, 1994) has revealed the coexistence of two different sizes of DBVs with electron-opaque cores, and with possibly different functions, in species of *Myzocytiopsis* parasitic in nematodes. The larger class of DBVs appears to become terminalized and perhaps have a role in disarticulation and in providing templates for the restricted central swelling of the oogonial segments in *M. osiris* (Dick, 1995). Comparisons should be made between the large DBVs of *M. osiris*, and an illustration of a similar feature in *Crypticola clavulifera* (Frances *et al.*, 1989: fig. 3).

A careful search by electron microscopists for homologous DBVs in possibly related *Protoctista* and *Straminipila* (*Cafeteria*, *Developayella*, *Labyrinthuloides* spp.) may help to strengthen phylogenetic relationships indicated by molecular biological analyses (Leipe *et al.*, 1994, 1996; Tong, 1995; Silberman *et al.*, 1996). TEMs of *Haptoglossa* thalli (Glockling, 1994) reveal the presence of electron-opaque vesicles with peripheral electron-lucent 'bubbles' reminiscent of the coalescing DBVs of the pythiaceous ooplasts. However, fixation of *Haptoglossa* was consistently less satisfactory than for *Myzocytiopsis* (itself an interesting pointer to relatedness, but perhaps the thicker cell wall may have been a factor) although procedures have now been improved (S. L. Glockling, pers. comm.).

SEXUAL REPRODUCTION

Oogamy

The process of oogamous sexual reproduction is normally regarded as involving the production of an egg (oosphere or female gamete) in an oogonium (female gametangium) and an antherozoid in an antheridium (male gametangium) (see Kniep, 1928; Moewus, 1943). Different kinds of oogamy are known in lower plants as well as in animals, characterized for example, by life-history, or ploidy cycle (see Dick, 1987; GLOSSARY entries). In the *Peronosporomycetes* there are female gametes in the oogonia but the differentiation of antherozoids (discrete male gametes) does not occur. The antheridium merely contains donor gametangial nuclei. Gametangia of the *Peronosporomycetes* are **meiogametangia**, that is, diploid cells in which meiosis takes place. For instance, in some of the *Chytridiomycetes* the gametangia are haploid and the male gamete is motile. Gametangia may be developed in different positions on the thallus:

1 terminally, subterminally, or in an intercalary position on main or branch hyphae
2 as terminal or lateral appendages to a nonmycelial thallus
3 from the entire thallus

In the *Peronosporomycetes* synchronous meioses occur in the coenocytic gametangia. After meiosis, the contents of the receptor gametangium (oogonium) become separated as one or several uninucleate and initially unwalled gametes. For *Apodachlyella* Dick (1986) presented a deliberate ambiguity in descriptions of the sexual morphology. Preliminary work, which requires further research, indicated that the additional wall developed after meiosis; suggesting that in this genus walled female and male gametes are formed. The septate antheridia of *Eurychasmopsis* appear to be similar (Canter & Dick, 1994).

In the *Myzocytiopsidales* and a few *Pythiales* the thallus becomes septate and adjacent segments assume the function of gametangia. In such cases the thallus segment is the site of meiosis (**thalloid meiosis**), but the relationship with the eucarpic *Peronosporomycetes* is evident. Data are generally lacking on nuclear behaviour and ploidy cycles in *Lagenidium* and *Myzocytium*, so that the kind of oogamy cannot be determined. However, TEM profiles of *Myzocytiopsis* species (Glockling, 1994) suggest that meiosis precedes oosphere formation. Martin & Miller (1986*c*) have confirmed that the ploidy cycle of *Olpidiopsis varians* is haplomitotic B (gametangial meiosis).

The range of sexual morphology within a family such as the *Leptolegniellaceae* is also wide: from the eucarpic *Aphanodictyon*, which is homothallic with differentiated gametangia, through the intercalary gametangial development in *Brevilegniella*, to holocarpic genera such as *Leptolegniella* (coralloid thallus) and *Nematophthora* (ellipsoid thallus in which the whole thallus functions as an automictic gametangium, eventually containing multiple oospores).

The occurrence of multiple synchronous meioses in a gametangium makes it possible for karyogamy to take place between two haploid nuclei from adjacent meioses in the same gametangium (**automictic sexual reproduction**). Sexual reproduction can thus occur without a separate male gametangium or antheridium (Dick, 1995: fig. 2). The morphogenesis of the two kinds of gametangia and the sexual process (meiosis and karyogamy) should therefore be considered as separate criteria. The absence of a male gametangium does not necessarily indicate parthenogenetic (i.e., no meiosis, no karyogamy) development, therefore automictic sexual reproduction and parthenogenesis ***cannot be distinguished without cytological evidence*** (Dick, 1972, 1987*a*). There are few reports of the actual site of karyogamy; karyogamy is presumed to occur either in the haploid coenocyte of the oogonium after meiosis, or in the oosphere after fertilization. The distinctive cytoplasmic reorganization of the oospore, compared with that of the chlamydospore or

resting sporangium, is indicative that functional or nonfunctional meioses precede oospore formation, as in *Aplanopsis* (Win-Tin & Dick, 1975).

The oospore protoplast (ooplast, lipid globules and nuclear spot) and oospore wall of the common *Peronosporomycetes* were clearly figured by de Bary (1887), and wall structure was already in use for taxonomic purposes by Schröter (1897) and Wilson (1907*b*) for the *Peronosporales*. The taxonomy of the *Peronosporomycetes* is based on oosporogenesis (vacuolar or periplasmic), oospore wall structure and oospore protoplasmic reorganization.

Sexuality and life-histories

The evolution of the *Peronosporomycetes* differs from that of eumycote fungi in that it is based on vegetative diploidy, not haploidy. The ploidy cycle is **haplomitotic B** (Dick, 1987), in which mitosis is confined to the diploid phase; haploid mitosis does not occur. Haplomitotic A or diplomitotic ploidy cycles are absent from this class, although they occur in the phylum and kingdom. For example, the life-history of certain labyrinthulids may involve pre-meiotic and post-meiotic mitosis (Perkins & Amon, 1969) so that the ploidy cycle would then be diplomitotic. Loss of sexuality has apparently occurred in several genera, leaving anamorphic diploids either with or without morphological structures homologous to oogonia and oospores. Diploidy has resulted in the ***oospore*** population functioning in ecological or population genetics in a way analogous to that of heterokaryotic ***anamorph spore*** populations in eumycote fungi. The functions of the sexual cycle may, in part, be for adverse condition survival and in part for control of genetic variability; the relative importance of these functions may differ between closely related taxa. Dick (1990*c*) has pointed out that in genera such as *Pythium* there may be congenitally foreshortened life-histories (Figure I: 11), in some of which sexuality has been lost.

Life-history studies in the *Peronosporomycetes* are relatively few. Dick (1970) showed that saprotrophic *Aphanomyces* species probably went through many asexual cycles in a summer season before a generation produced oogonia. A difference of emphasis is shown by *Plasmopara viticola* (Burruano *et al.*, 1994, 1995, 1997, 1999) which depends on the production, survival and germination of oospores for the annual production of conidiosporangia to initiate the new disease cycle in the spring.

Sexual systems have not been unequivocally described for the hyphochytrids and are unknown in thraustochytrids.

Homothallism and heterothallism: sex hormones and sterols

The majority of species of most of the genera of the *Peronosporomycetes* are homothallic; heterothallism may be secondarily derived. When present, the antheridial branch grows, under hormonal attraction, to the oogonium; the antheridium (donor or male gametangium) is differentiated after contact with the oogonium (the exception is the amphigynous antheridium of certain species of *Phytophthora* which is formed ***before*** the oogonium). The classic studies of heterothallism in *Achlya* initiated by Raper (1936, 1939*a*, *b*) have led to some understanding of the mating systems, morphogenesis of directional growth and penetration, and the identification of a C_{29} steroid sex hormone, antheridiol (Arsenault *et al.*, 1968; Barksdale, 1960, 1962*a*, *b*, 1963*a*, *b*, 1966, 1970; Barksdale & Lasure, 1973; Barksdale, Carlile & Machlis, 1965; McMorris, 1978; McMorris & Barksdale, 1967; Mullins (1968); Mullins & Raper (1965); Raper, 1951). This work was followed by research into heterothallism in *Phytophthora* (Galindo & Gallegly, 1960) and *Pythium* (Hendrix & Campbell, 1968; Hendrix, 1970). Leonian (1931) and De Bruyn (1935) reported heterothallism in *Peronospora*; Gustafsson & Arhammer (1983), Michelmore & Ingram (1980, 1981) and Michelmore &

Sansome (1982) have given accounts for *Bremia*, and Bishop (1940) described heterothallism in *Sapromyces*. Interpretation of the genetics underlying the differential sexuality in *Achlya* [six states, probably involving two independent genes, each with two alleles: homothallic (AABB); congenitally sterile (aabb); oogonial (AAbb); antheridial (aaBB); predominantly oogonial (#A>#B); predominantly antheridial (#A<#B)] was not possible until the kind of ploidy cycle had been resolved. Relative sexuality is also known in the *Peronosporales* but whether the mechanism depends on trisomy or lethals is disputed (Judelson et al. 1995; Van der Lee et al. 1997).

Various possible modes of action of sterols during sexual reproduction have been suggested, including induction (or suppression, Thomas & McMorris, 1987) of sexuality (with the indication that there may be substitution or analogue induction), directional growth of the gametangial axes (McMorris, 1978), localized stimulation of wall softening at the point of contact between gametangia (Gow & Gooday, 1987), and their effect on meiosis (Elliott & Sansome, 1977). Intertaxon induction of oosporogenesis (Brasier, 1975) and synergism (Sansome & Sansome, 1974) have also been suggested.

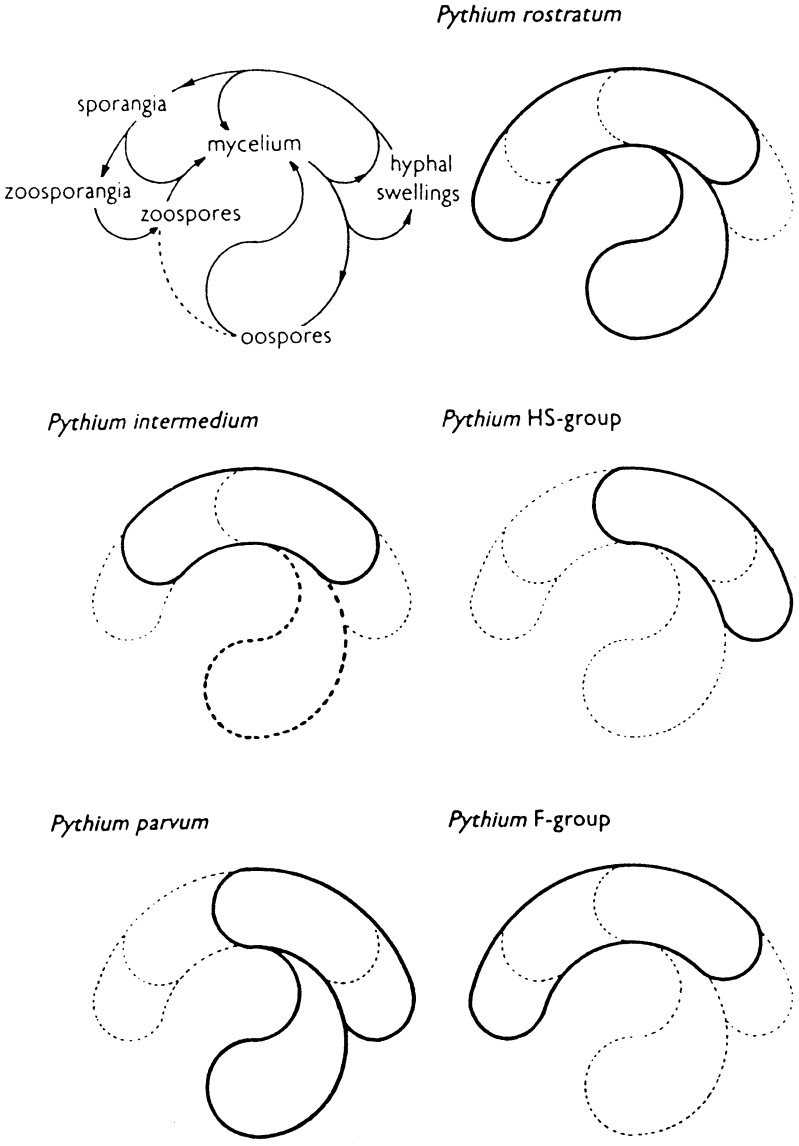


FIGURE I: 11. Diagrams of constitutionally foreshortened life-histories within the genus *Pythium* (from Dick, 1990*c*).

The oogonium

The oogonial initial develops as a swelling without septation or by a transformation of a gametangial segment. The first trigger for this morphogenetic change must be endogenous, from either a nutritional or biochemical stimulus.

The importance of intercalary initiation has not been sufficiently stressed (Dick, 1995). Intercalary differentiation is common in the *Pythiales* and the *Saprolegniales* and can occur in the *Leptomitales*. The oogonial initial is frequently subapical and terminal oogonia on main or lateral branch hyphae may have evolved by terminalization of the initial. Near-terminal oogonial differentiation leaving an apiculus is often seen in the *Saprolegniaceae* and *Pythiaceae*.

No single group within the *Peronosporomycetes* displays all the morphological diversity, morphogenetic patterns and wall layering that can be found in the class. Many species have oogonia with a smooth, more or less spherical outline, but many others are ornamented. The pattern of development of wall ornamentation depends on three criteria: initial expansion, secondary primordial initiation and wall deposition. Six examples of the sequential or simultaneous expression of these criteria will (Dick, 1995, 2000*c*) illustrate the diversity of form:

1 initial swelling accompanied by the generation of numerous new growing tips results in the formation of hollow papillae; further swelling and wall deposition result in the formation of a spherical oogonium with dense or scattered papillae (*Scoliolegnia subeccentrica*)

2 as above, but with the attenuation of these growing points before final swelling and wall deposition to form spines, the tips of which are sometimes completely occluded (*Achlya radiosa*, *Pythium echinulatum*)

3 as **1**, but with the apices of the papillae remaining as thin stretched membranes after secondary wall deposition to form truncate papillae (*Aphanomyces stellatus*, *Achlya recurva*)

4 initial swelling followed by the development of small circular areas under which there is no further wall deposition, resulting in the formation of simple pits (*Isoachlya toruloides*, *Saprolegnia ferax*)

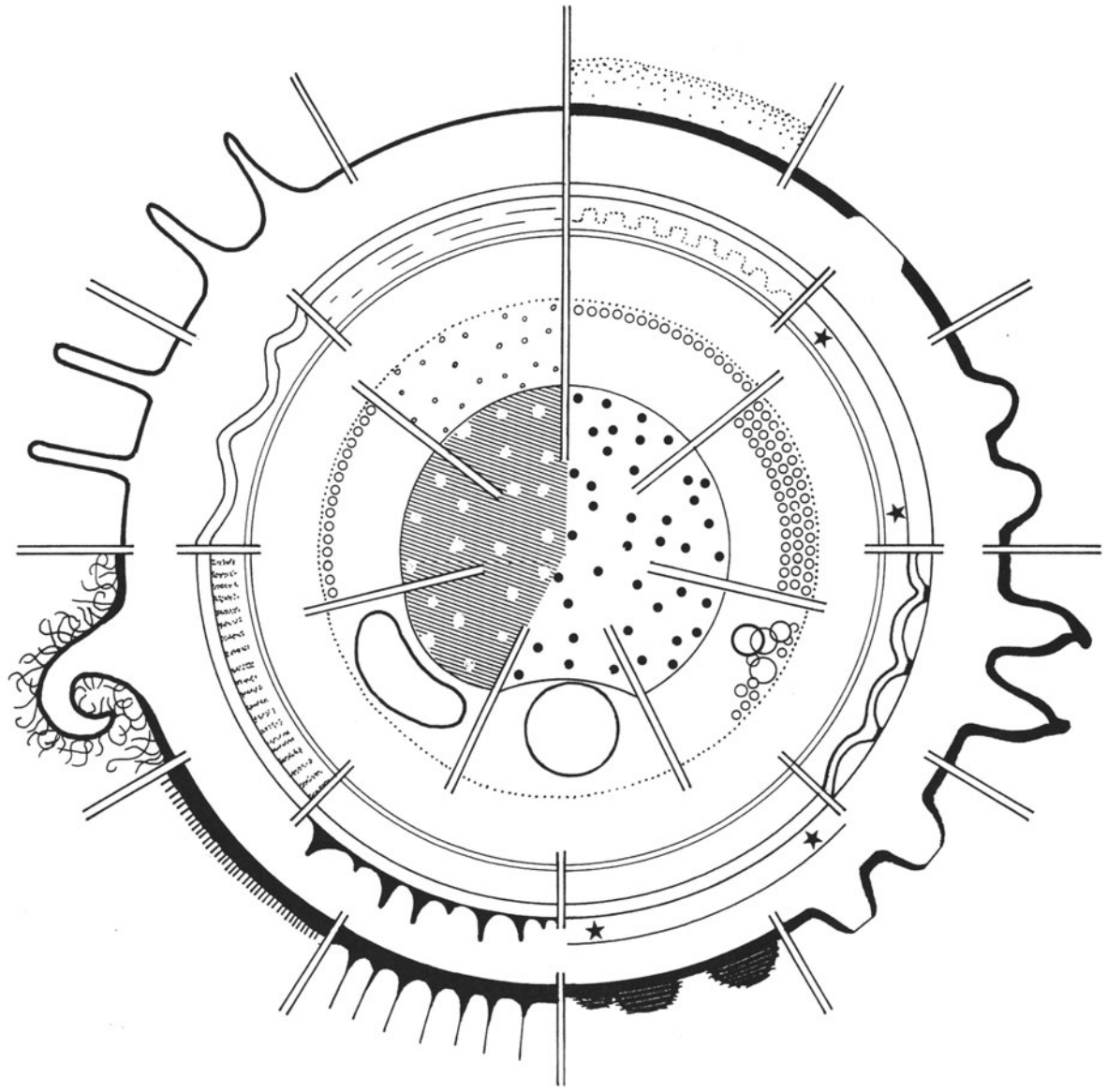
5 secondary wall deposition proceeding simultaneously with swelling, resulting in plaques of thickened wall areas to form solid, U-shaped, vermiform or arched verrucae (*Pachymetra chaunorhiza*)

6 long outgrowths from the oogonial initial becoming almost completely occluded with oospore wall development extending into the outgrowths (*Medusoides argyrocodium*)

FIGURE I: 12. The biodiversity of oogonial and oosporic wall structure and oosporic cytoplasmic organization.

The diagram is arranged as three partially exploded wheels of sectors: the outermost sector is of oogonial wall structure; the middle sector is of oospore wall structure; the innermost, with the dotted line indicating the plasmamembrane of the zygote or oospore displays the protoplasmic reorganization in the resting spore. The diagram ignores the plerotic/aplerotic states of the oogonial/oospore relationship; it does not indicate the nuclear spot(s) and cannot show the fact that the ooplast is often not centric with respect to other organelles. The asterisk indicates a fluid layer. The three wheels of sectors may be partially, but not totally, independent of each other. The following notes relate to each wheel in turn, starting at the top and working anticlockwise.

Outermost wheel (oogonial wall structure): smooth, thin wall (*Pythium*); conic-papillate thin wall (*Pythium*); cylindric-papillate thin wall (*Pythium spinosum*); recurved-papillate, lanose wall (*Medusoides*); thick, acicular wall (*Aqualinderella*); thick spiny wall (*Olpidiopsis*); laminate veruccose wall (*Pachymetra*); thick wall with truncate papillae (apices thin) (*Achlya recurva*); thick wall with mamillate papillae (apices occluded) (*Achlya radiosa*); thick wall with hemispherical papillae (*Scoliolegnia*); smooth, pitted wall (*Saprolegnia*); smooth, mucilaginous wall (*Aplanopsis*).



(Figure I: 12, legend, continued)

Middle wheel (oospore wall structure): outer epispore wall, thick, zoned endospore wall, inner boundary membrane; as before, but with a collapsed endospore wall giving rise to ridges/reticulations (*Pythium*, *Cystosiphon*); endospore formed of radial rods (*Medusoides*); as *Pythium*, but with a condensed outer exospore layer derived from oogonial periplasm (*Albugo*, *Peronospora*); female gamete membrane outside the epispore and endospore walls (*Apodachlyella*); reticulately ornamented inner wall layers between the smooth, outermost membrane and the smooth membrane bounding the protoplast (*Myzocytiopsis*); endospore with a fluid layer between the epispore and the boundary membrane (*Leptolegniella*); smooth epispore and endospore wall layers with some differentiation within the latter.

Innermost wheel (protoplasmic organization): solid ooplast with translucent zones, cytoplasm with little coalescence of lipids (Peronosporomycetidae); solid ooplast with translucent zones, cytoplasm with coalescence of lipids to form an outer shell (Leptolegniaceae); solid ooplast with translucent zones, cytoplasm with coalescence of lipids to form a single globule deformed by the ooplast (Leptolegniaceae); fluid ooplast with granules in Brownian motion, cytoplasm with coalescence of lipids to form a single globule deforming the ooplast (*Achlya*); fluid ooplast with granules in Brownian motion, cytoplasm with coalescence of lipids to form a single shell of uniformly sized globules (Saprolegniaceae); as previously, but with multiple shells of lipid globules (*Saprolegnia*); as previously, but with coalescence of lipid globules into globules of various diameters (*Scoliolegnia*).

The oogonial wall may be composed of up to three major layers, some of which can themselves be multilayered. The deposition of an internal layer or layers results in the thick oogonial walls characteristic of the *Saprolegniales*. The pits are not predetermined sites of antheridial attachment, but they are places through which the oospore germinates, either as a hypha or a zoosporangium. Some orders, such as the *Pythiales*, are normally characterized by having thin oogonial walls which can be contrasted with the thick oogonial walls of the *Saprolegniales*. Apart from the 'primary' and 'secondary' walls, there may be additional layers, sometimes as mucilage secretion through the oogonial wall (*Aplanopsis terrestris*). The mucilage may have an ecological function, cementing the oogonium to organic detritus. The size ranges of oogonia and oospores can be enormous: there is a 300-fold difference in oospore volume between *Calyptralegnia achlyoides* and *Pythium parvum* although both species have uniovulate oogonia and the oospores are presumably uninucleate. Oogonial morphology is thus useful for species identification, and some patterns of morphogenesis (e.g., pits) may have phylogenetic value.

The antheridium and the fertilization process

The morphology and morphogenesis of the antheridium are simpler than those of the oogonium, and the antheridial wall is seldom much thicker than that of the subtending hypha. A septum normally delimits the antheridium. When the antheridium is of regular occurrence, the shape, origin and position of the antheridium can be useful taxonomic criteria. The mode of application of the antheridium to the oogonium is distinctive for species and sometimes groups of species, but the continuous interspecific cline of variation between fully apical and completely lateral attachments makes accurate illustration essential. Antheridial origin can either be characteristic for a species, or highly variable within a species. Antheridia may arise from just below the oogonium, from the hypha subtending the oogonial branch or from a different hyphal system. These states are termed **closely monoclinous**, **monoclinous** and **diclinous** respectively. In a few species the antheridium may be delimited from a branch arising from the swollen oogonial initial itself (an **epigynous** antheridium). Where the oogonial initial grows ***through*** the ***preformed*** antheridial initial, a characteristic of some species of *Phytophthora*, the antheridial development is **amphigynous**. It is possible that the amphigynous condition of *Phytophthora capsici* (Stephenson & Erwin, 1972) may not be homologous with that for *Phytophthora megasperma* (Hemmes & Bartnicki-Garcia, 1975; Hemmes & Ribeiro, 1977).

The morphogenesis of the process of fertilization shows differences with respect to penetration and protoplast fusion (Dick 1995). When an antheridium is present, and fertilization occurs through **gametangial contact**, there is the injection of a small part of the antheridial protoplasm into the oogonium through a **fertilization tube**. This tube can be recognized as a walled structure within the oogonium ***after*** fertilization. In the polyoosporous *Saprolegniomycetidae* the fertilization tube may be branched, although there is no evidence that the number of branches ever equates with the number of apparently mature oospores, it is usually much less. In all ultrastructural studies of oogonial wall penetration by a fertilization tube there is a characteristic concentration of small vesicles on both sides of the penetration zone. Marchant (1968) claimed that there was no fertilization tube in *Pythium ultimum* Trow, and Win-Tin & Dick (1975) figured apparently naked antheridial protoplasm within the oogonium of *Achlya racemosa* Hildebr., but in both species walled fertilization tubes can be seen in mature oogonia. Laviola (1974*a*) has described the fertilization tube of *Phytophthora infestans*. It is significant that in the *Peronosporomycetes* the presence of this fertilization tube can be deduced even in strictly plerotic oospore/oogonium arrangements because of a deformation of the oospore wall (e.g., *Verrucalvus*, Dick *et al.*, 1984). In nearly plerotic arrangements, as in several *Pythium* species, the presence of the fertilization tube tends to push the oosphere to the opposite side of the oogonium (e.g., *P. lutarium* Ali-Shtayeh, Ali-Shtayeh & Dick, 1985: figs 41-45). In *Apodachlyella* and *Eurychasmopsis* a *fertilization hypha* develops when each cell of a chain of endogenous cells within the antheridium 'germinates' to cross the void between non-contiguous gametangia.

In *Lagenidium sensu stricto* and *Myzocytium sensu stricto* the thallus becomes septate and adjacent segments assume the function of gametangia. Thus, although the thallus is eventually holocarpic, the relationship with the eucarpic *Peronosporomycetes* is readily made. The range of morphology in the *Leptolegniellaceae* is greater, from the eucarpic *Aphanodictyon*, which is homothallic with differentiated gametangia, and *Brevilegniella*, in which swollen segments function as automictic gametangia, to holocarpic genera such as *Leptolegniella* and *Nematophthora*, in which the whole thallus functions as an automictic gametangium containing multiple oospores. In none of these taxa has the cytology been investigated, but it is presumed that meiosis occurs in the thallus/gametangium.

In *Lagenidium giganteum* the morphology of sexual reproduction has been illustrated by Willoughby (1971), Umphlett (1973), Kerwin & Washino (1983) and Brey (1985). These authors describe an extra-oogonial 'fertilization tube' which is not merely a fine tubular extension from an antheridial cell as in *Apodachlyella* or *Eurychasmopsis*, rather it resembles the structure originally figured for *Lagena*, and which Barr & Désaulniers (1990*a*) dismissed as an artifact. Although it would be possible to postulate that the antheridium is equivalent to a single antheridial cell of the kind found in *Apodachlyella* or *Eurychasmopsis*, the absence of an equivalent oosphere wall, such as occurs in these two genera, makes this unlikely. Also, given the many biochemical and morphological similarities between this particular species of *Lagenidium* and *Pythium*, I think it inconceivable that such a morphologically peculiar interpretation can be maintained. It is much more likely that the 'fertilization tube' is in fact the antheridium, which would then be epigynous (cf. *Achlya colorata* Pringsh.). A true fertilization tube may exist within the oogonium. The problem will only be resolved by a nuclear cytological investigation to reveal the site of meiosis. The proximity of the oospore to the antheridium may be due to the irregular shape of the oogonium which has its maximum diameter close to the point of antheridial contact.

Thalloid meiosis is also presumed in *Lagena*, *Ciliatomyces* and *Eurychasmopsis*; in *Lagena* no evidence for meiosis has been presented, but the organism is presumably automictic, given the significant protoplasmic reorganization. *Ciliatomyces* and *Eurychasmopsis* are heterothallic although it is not possible to decide whether heterothallism is genotypically or environmentally determined in these hologametangial fungi. Foissner (1987: fig. 9) figured a fertilization pore for *Ciliatomyces*, but in this species oosphere formation is periplasmic.

Thalloid meiosis has been demonstrated in *Lagenisma* (Schnepf *et al.*, 1978*b*, Schnepf & Heinzmann, 1980). Sexual reproduction (meiosis) appears to be induced in stressed environments (Schnepf & Drebes, 1977; Schnepf *et al.*, 1978*b*, *e*). The subsequent development, taken as a whole, is unique. However, it is possible to put parts of this development into a comparative sequence. Unlike any other peronosporomycete (with the possible exception of *Apodachlyella* and *Eurychasmopsis*, see previous page) the meiotic products of *Lagenisma* become discrete uninucleate cells. These flagellate meiospores are discharged like the zoospores. Aggregations of encysted meiospores occur and a functional 'male' forms a fertilization hypha which grows towards and then penetrates a functional 'female'. This growth could be compared with the fertilization hyphae of *Apodachlyella* and *Eurychasmopsis*. Plasmogamy results in the growth of a short zygotial hypha from the 'female' cyst. The binucleate protoplast then contracts to form a non-periplasmic zygote which secretes a wall. Karyogamy occurs in the developing zygospore. There is no evidence of haploid mitosis, so the ploidy cycle is still haplomitotic B. The morphological sequence opens up the possibility of deriving a diplomitotic cycle (cf. the *Blastocladiales*, which are freshwater not marine) from the haplomitotic B ploidy cycle.

In the *Olpidiopsidales* the donor gametangium is transformed from a contiguous smaller thallus, the **companion cell**. This smaller thallus (invariably of less than 10% of the volume of the receptor gametangium) is presumed to be derived from an independent infection, thus making the sexual union heterothallic. For *Olpidiopsis varians*, Martin & Miller (1986*c*) have figured a penetration tube from the

male gametangium that differs little from the fertilization tube of a strictly plerotic oomycete, although their other figures relate to a narrow pore *ca* 0·4 μm in diameter.

In the *Myzocytiopsidales* the two gametangial initials are from contiguous thallus segments (homothallic) and of approximately the same size. The two gametic protoplasts condense towards a common pore in the contiguous walls of the gametangia and **gametangial copulation** occurs. This system should be contrasted with the above account of gametangial contact. The condensation is dependent on vesicle expansion distal to the point of union in both gametangia. The morphogenetic distinction between a fertilization tube which forces the oosphere away from the point of union and a conjugation pore, towards which both gametangial protoplasts converge, appear to be diametrically opposite systems. Although Zopf (1884) in his figures for *Lagenidium rabenhorstii* and *Myzocytium proliferum* Schenk, depicts a typical peronosporomycetous fertilization tube and describes the oogonial contents as aggregating at the tip of this tube, his account for *Myzocytiopsis vermicola* (Zopf) M. W. Dick (≡ *Myzocytium vermicola* Zopf, Dick, 1997*a*) is different. For *M. vermicola* the fertilization tube is shown as trumpet-shaped, dilating on to the sphere of the maturing resting spore: it is thus likely that the structure is cytoplasmic rather than extra-cytoplasmic. Newell *et al.* (1977) have provided a more detailed description of a marine parasite which they equate with this species. For their isolate they describe a septal papilla (deformed pore ?) which is not attached to the oosphere, but there is a tubular remnant cytoplasmic membrane which passes from the ***distal*** septum of the donor gametangium through the papilla to the developing zygote. Dangeard (1906), also working with *M. vermicola*, described the copulation as occurring between different thalli. All the protoplasm of both gametangia is involved (Barron & Percy, 1975). The aggregation of the protoplasm of both protoplasts against the common wall has been figured by many authors and is particularly well illustrated by Barron (1985: fig 12). Light micrographs and TEMs of the pore in the common wall for *Myzocytiopsis* have been published in Dick (1995). The question remains as to whether copulation involves wall dissolution or gametangial penetration. Some drawings (e.g., Karling 1981*a*: plate 25, fig. 61; plate 27, fig. 93) indicate that the pore is of sufficient width that wall dissolution is the more probable alternative. Gametangial copulation accompanied by aggregation and contraction of the combined protoplast in one of the gametangia is followed by the formation of a resting spore wall around that protoplast. Given the proximity of the gametangial protoplasts, it is a small step to the formation of that spore in the pore of the common gametangial wall. Such a position is described for *Myzocytiopsis subuliformis*, *Syzygangia sacculoides* (Karling, 1942*a*, 1981*a*; Dick 1997*b*) and possibly for *Syzygangia oedogonii* (Scherffel, 1925*a*; Dick, 1997*b*).

Drawings (Karling, 1942*a*, 1981*a*) of the morphogenesis and structure of the mature resting spore indicate that there is a contraction of the protoplasts away from the distal gametangial walls and descriptions sometimes indicate that there is no periplasm "as in *Pythium*". *Pythium* has minimal periplasm and the real distinction may concern the outline of the contracting protoplast. In *Pythium* the developing oosphere is always more or less spherical with a smooth plasmamembrane whereas the contracting protoplast of these lagenidiaceous fungi is more often figured as irregular, amoeboid, or with point attachments to the gametangial wall (e.g., *Myzocytiopsis humana* - Karling, 1947*b*; *M. microspora* (Karling) M. W. Dick (≡ *Lagenidium microsporum* Karling, Dick, 1997*b*) - Karling, 1944*c*; *Gonimochaete lignicola* G. L. Barron - Barron, 1985: fig. 12). This morphogenesis is confirmed by observations of living material (S. L. Glockling; M. W. Dick: Glockling, 1994): during mutual gametangial contraction one protoplast possesses a convex boundary to the intra-wall space while the other protoplast has a concave boundary to this space (which sometimes contains some large vesicular spheres). Ultrastructural studies (Glockling, 1994) have confirmed these observations but add a further complication. The 'pore' appears to be a deformed septum (or an incipient penetration tube?) formed from ***both*** protoplasts, protruding ***into*** the concave protoplast, which might have been presumed to be the 'antheridial' or donor protoplast because of the concavity. Furthermore, this 'tube' differs from that of *Olpidiopsis* in the involvement of both septal origins (cf. Martin

& Miller, 1986*c*) and in the apparent absence of the zone of small vesicles at the tip of the fertilization tube (Hemmes & Bartnicki-Garcia, 1975; Beakes & Gay, 1977; Al-Rekabi, 1979).

A further anomalous description of sexual development is provided by Ivimey Cook (1935) for *Syzygangia zygnematicola*, in which a walled oosphere is formed attached to the oogonium wall by a stalk. The stalk appears to be tubular (Ivimey Cook, 1935: plate 1, fig. 6) and although antheridial penetration is described as separate from this tube (Ivimey Cook, 1935: plate 1, figs 6, 8); there could have been confusion between a normal fertilization tube and supernumary antheridial cells.

Oosporogenesis: protoplasmic reorganization and oospore wall structure

The morphogenesis of the oosphere into the mature oospore is of major phylogenetic significance in the *Peronosporomycetes* in two ways:

1 the protoplasmic organization subsequent to meiosis follows one of two alternatives: **centripetal oosporogenesis** or **centrifugal oosporogenesis**

2 the extent to which there is exclusion of part of the nucleate or enucleate oogonioplasm from the oosphere(s) as **periplasm**

The kind of oosporogenesis is diagnostic at sub-class level. Cleavage in the polyoosporous taxa of the *Saprolegniales* is essentially centrifugal and similar to zoosporangial cleavage (Lohwag, 1926), with a tonoplast vacuole fusing with cleavage cisternae to form peripheral mounds of presumptive oosphere protoplasts. When the oogonial plasmamembrane is finally breached, the oospheres tumble to the centre of the oogonium. A similar development appears to take place in *Sclerospora* (McDonough, 1974*a*, *b*) although the single oosphere makes the interpretation more difficult. In the *Pythiales* the oogonioplasm does not contain a tonoplast vacuole, and the peripheral oogonioplasm gradually loses its organelles to define a centripetal oosphere. In the *Peronosporales* and *Rhipidiales* this peripheral protoplasm (periplasm) may be substantial, persistent and sometimes initially nucleate, eventually contributing the exospore layer (not a wall), often with distinctive reticulations. When the entire oogonial cavity is occupied by the oospore, the oospore is **plerotic**: very few taxa are truly plerotic: examples are confined to the *Saprolegniomycetidae*, *Olpidiopsidales* and perhaps the *Pythiogetonaceae* (Voglmayr *et al.*, 1999). In *Pythium* the concept of an **aplerotic index** has been proposed to aid taxonomic assessment of species differences (Dick, 1990b; Shahzad *et al.*, 1992).

The presence of nucleated periplasm has been demonstrated for *Ciliatomyces* (Foissner, 1987: fig. 5; Foissner & Foissner, 1986*a*) and *Lagena* (Barr & Désaulniers, 1990*a*). The full significance of this feature will not become apparent until the morphogenesis of the oosphere of the *Peronosporales* (nuclear persistence and the formation of the exospore?) and *Araiospora* (*Rhipidiales* - a possibly ***cellular*** periplasm?) has been reinvestigated.

Within the oospore, the protoplasmic contents become reorganized during maturation (Beakes, 1980*c*). Apart from the deposition, outside the plasmamembrane but inside the zygotic wall, of glucan polymers as the **endospore** (which is usually as concentric layers, Beakes, 1981; but rarely as radial rods, Voglmayr, *et al.*, 1999), there are two complementary processes of coalescence which proceed simultaneously:

1 the DBVs gradually coalesce to form a large single membrane-bound structure, the **ooplast** (Howard & Moore, 1970), which can be seen clearly in the oospores of almost all Peronosporomycetes

2 there may be a variable degree of coalescence of **lipid globules**

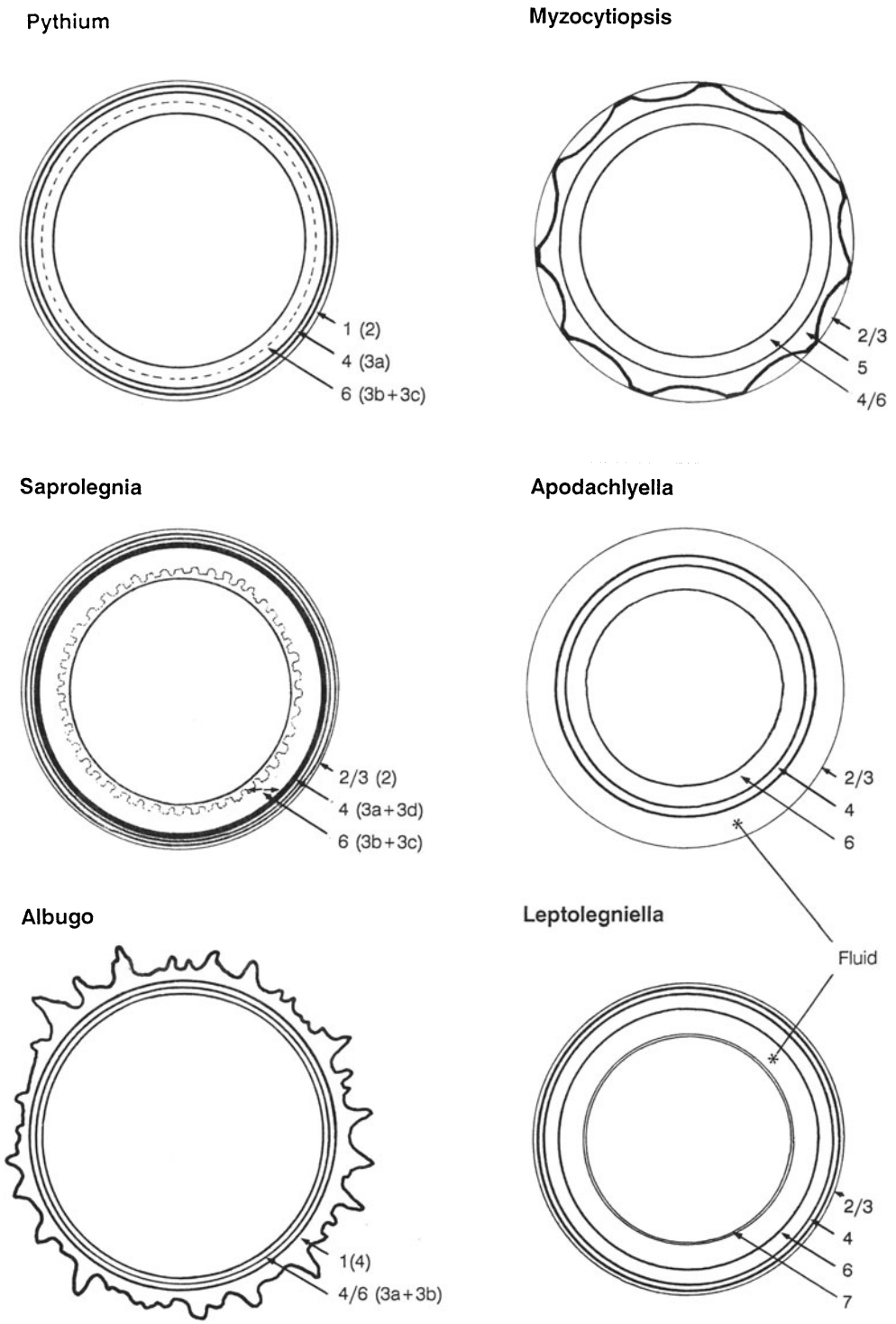


FIGURE I: 13. Oospore wall diversity: homologous and non-homologous wall layers as seen in light and TEM microscopy (oogonial wall not indicated). Hypothetical sequence of wall layers (arabic numerals) from the oogonial cavity to the oospore protoplast (after Beakes, 1981*b*; Dick, 1995): (1) periplasmically derived exospore deposit and ornamentation; (2) oosphere membrane (if present); (3) zygote membrane (continuous with (4)?); (4) smooth concentric epispore layer(s) (stratified ?); (5) reticulate epispore deposition with or without outer or inner smooth concentric layers; (6) endospore (resorbed on germination); (7) tertiary boundary membrane. Provisional identification of wall layers in different genera, Beakes' numbering in brackets. The asterisk marks a non-membranous discontinuity.

Both coalescence patterns contribute to the characteristic bubbly appearence of the partially mature oospore. One or more transparent ellipsoid zones in the mature oospore mark the position(s) of nuclei (**nuclear spots** or **pellucid spots**). At the most extreme state of coalescence, seen in some species of *Apodachlya* and *Achlya*, the mature oospore contains a single ooplast and a single lipid globule. The patterns of lipid coalescence and their distribution underneath the plasmamembrane (states described as **centric**; **subcentric**; **subeccentric**; and **eccentric**) have taxonomic value and possibly some ecological or phylogenetic significance.

Under both light and electron microscopy the ooplast has different characteristics within the class (Dick, 1971*a*, 1990*a*; Dick *et al.*, 1989; Howard, 1971). In the *Saprolegniaceae sensu stricto* the ooplast is fluid, with Brownian movement of granules (DBVs?), but in the *Leptolegniaceae* the granules are not in motion and are less numerous. In the *Pythiales* and *Leptomitales* the ooplast appears to be homogeneous under light microscopy. The *Pythiales* possess a distinctive ooplast (Dick, 1969; Howard & Moore, 1970; Dick *et al.*, 1984; Hemmes & Stasz, 1984) which is electron-opaque with a dispersed electron-lucent phase. The difference between a solid or fluid ooplast can be deduced from surface tension effects: in *Apodachlya* the ooplast bulges into the lipid globule; in *Achlya* the lipid globule causes deformation of the ooplast (Dick, 1969). *Ciliatomyces* (Foissner, 1987) again appears to be anomalous with respect to the discrete electron opaque cores. It is often difficult to interpret the oospore structure from the illustrations and light microscope descriptions of lagenidiaceous taxa; clear photographs, such as that for *Myzocytium megastomum* (Canter-Lund & Lund, 1995) or *Myzocytiopsis intermedia* (G. L. Barron) M. W. Dick (Dick, 1997*b*; Glockling, 1994), are rare. The information is usually limited to statements concerning the presence of vaguely defined fat globules. In a few cases (e.g., *Salilagenidium callinectes*, *S. chthamalophilum* (T. W. Johnson) M. W. Dick *loc. cit.* (≡ *Lagenidium chthamalophilum* T. W. Johnson) the descriptions are more detailed, and serve to emphasise a divergence from the typical morphology of the *Pythiales*, but with closer similarities to the *Saprolegniales*, rather than to other lagenidiaceous fungi. The ooplast was wrongly identified as the nucleus in *Pythium ultimum* by Marchant (1968).

The oospore wall itself is complex, possibly up to seven or more layers can be distinguished, but not all organisms have all layers (Dick, 1999, 2000*d*). It is not clear whether any exosporial deposit is universally present on aplerotic oospores, but it will be absent from ***strictly*** plerotic oospores. In the *Peronosporomycetes* as a whole, the wall layers of the resting spores are concentric and smooth. The wall layers as observed by light microscopy were redefined by Dick (1969) as:

1 **endospore**, which is resorbed on germination
2 **epispore**, representing the zygote wall (and oosphere wall if one is present)
3 **exospore**, which is deposited by periplasmic activity

Beakes (1981*b*) based his numbering system on ultrastructural and physiological criteria, while the numbers allocated to wall layers by Dick (1995) were topographic and morphogenetic. Oogonial wall layers were included by Beakes (1981*b*) as 1*a* and 1*b* (the ex-oogonial spines of *Olpidiopsis* were not considered), but I prefer to exclude oogonial wall layers from this account of the oospore walls (Figure I: 12). The sequence from the outside to the inside of the oospore, with Beakes' numbering in parentheses, is as follows:

1 periplasm-derived exospore (4*c*, 4*b*, 4*a* - numbered centrifugally)
2 primary oosphere wall (2 - not always present)
3 primary zygote wall; may not be ultrastructurally distinct from 2 (3*a* - 3*a*, 3*d*, 3*b*, 3*c* numbered centripetally (except for 3*d*)
4 epispore wall; not resorbed (3*d*?)
5 endospore/epispore wall; outer, collapsed layer, not resorbed (3*b*?, + 3*c*?)
6 endospore wall; resorbed (3*b*? + 3*c*?)
7 tertiary boundary membrane; resorbed? (3*c*?)

The ontogeny of these complex wall layers is not well understood (Beakes & Gay, 1978*a*, *b*: *Saprolegnia*; Haskins *et al.*, 1976: *Pythium*; Fukutomi *et al.* (1971) and McKeen (1975): *Phytophthora*; Lippman, Erwin & Bartnicki-Garcia (1974); Sargent *et al.* (1977): *Bremia*; Tewari & Skoropad, 1977: *Albugo*; and reviews by Beakes, 1981*b*; Dick, 1995), but seems to be evolutionally conserved and therefore of taxonomic value. It cannot be assumed that the component monomers or the degrees of hydration are the same between species, so numbering systems can only provide an approximate comparisons. Nevertheless, the degree(s) of condensation in the endospore and the ooplast are consistent for a species and will have a biological significance in maintaining the biodiversity in the community.

Foissner (1987) described the formation of an exospore in *Ciliatomyces* which was not periplasmic in origin and therefore not an exospore within the definition given above. Exceptional formations occur in other genera and these need to be studied in much more detail; the species concerned may need to be reclassified (note *Leptolegnia eccentrica* Coker & V. D. Matthews and *Cystosiphon dictyosporum* (Racib.) M. W. Dick *loc. cit.* (see pp. 189-190; PART V and PART VI). In the *Leptolegniellaceae* the endospore is also distinctive (Dick 1971*b*), although it is doubtful whether *Aphanomycopsis* (punctate?) has precisely the same structure as *Leptolegniella*, *Brevilegniella* and *Nematophthora*. There appear to be similarities between the wall ornamentation of the resting spores of *Aphanomycopsis* and *Latrostium* (Zopf, 1894), placed in the *Hyphochytriomycetes* by Karling (1977) and Fuller (1990).

Certain species of lagenidiaceous fungi have resting spores with tubercular or spiny ornamentation. Such ornamentation is only known elsewhere in the *Peronosporomycetes* in taxa whose taxonomic position is not well established. Ultrastructural work (Glockling & Beakes, 2000*b*) on *Myzocytiopsis* indicates that the prominently ridged oospore may develop within a thin endothallial membrane (layer 2 or 3?). Drawings and photographs of ornamented oospores of *Myzocytiopsis* (*Myzocytium*) (e.g., Barron, 1976*b*: figs 6b, 10, 11) would appear to support this morphology. Homology between the gametic (?) membranes of *Apodachlyella*, *Eurychasmopsis*, *Leptolegniella*, *Brevilegniella* and *Myzocytiopsis* is thus a possibility. Exo-oospore ornamentation (exospore wall), such as occurs in the *Peronosporales*, has not been suggested for *Lagenidium* or *Myzocytium*. Exo-oogonial ornamentation in *Olpidiopsis* (Martin & Miller, 1986*c*) and resting spore ornamentation in *Rozella* and *Rozellopsis* have not been examined in detail, and exo-oogonial ornamentation has no equivalent in the endobiotic lagenidiaceous fungi, although the *Aqualinderella* oogonium has a fibrillose outermost wall layer (Emerson & Weston, 1967).

Oospore germination

The oospores of many species of Peronosporomycetes are notoriously difficult to germinate, but success has been achieved for some species (see Dick, 1995; Lucas *et al.*, 1995). The method of germination may be either by zoospores, the oospore behaving as a zoosporangium, or by a germ tube. Constitutive dormancy may relate to wall composition or nuclear behaviour, such as delayed karyogamy. Germination may depend on the elution of various substances or layers of the oogonium and oospore wall, and light effects have also been implicated (for references see Dick 1995).

The germination of *Plasmopara viticola* and *Phytophthora* oospores have been studied most thoroughly from Gregory (1916) to Burruano, Ciofalo & Conigliaro (1995), Burruano, Conigliaro & Ciofalo (1997), Burruano, Conigliaro & Laviola (1999) and Burruano *et al.* (1992). In the spring the oospore of *Plasmopara* germinates, in the decayed detritus of its host, to produce a single conidiosporangiophore. The conidiosporangia are dispersed and set up the initial infection on young vine leaves. The germination of *Phytophthora* oospores has been studied primarily in relation to genetic studies (Banihashemi & Mitchell, 1976; Beakes, El-Hamalawi & Erwin, 1986; Blackwell (1943); Brasier & Brasier (1978); Brasier & Sansome (1975); El-Hamalawi & Erwin, 1986; Eriksson & Laane (1982); Erwin & McCormick, 1971;

Förster, Ribeiro & Erwin, 1981, 1983; Jiang *et al.*, 1989; Jimenez & Lockwood, 1982; Kaosiri, Zentmyer & Erwin, 1980; Klisiewicz, 1970; Meyer, 1975; Ribeiro, Erwin & Zentmyer, 1975; Salvatore, Gray & Hine, 1973; Shaw, 1967; Sney, Eye & Lockwood, 1981). One of the problems in genetic studies is the often poor percentage germination achieved. Jiang & Erwin (1990) have suggested the use of tetrazolium bromide as a criterion for determining percentage viability of *Phytophthora* oospores as an adjunct to the use of morphology and plasmolysis. *Albugo* oospore germination has been clearly illustrated by Verma & Petrie (1975) and *Pythium* oospore germination has been studied by Adams (1971) and Ruben & Stanghellini (1978). *Saprolegnia* oospore germination has been studied by Beakes (1980*a*, *b*, *c*, *d*, 1981*d*; Beakes *et al.*, 1986), that for *Aphanomyces* by Scharen (1960). Much more information will be needed before oospore germination can provide diagnostic criteria.

Oospore germination has only been reported once in the lagenidiaceous fungi, for *Syzygangia zygnematicola* by Ivimey Cook (1935). The oospore was reported to germinate to produce a single zoospore, slightly larger, but otherwise similar in morphology to the sporangial zoospores (see Figure I: 1 B m, n).

Morphometry of the oogonium and oospore

Descriptions of species have nearly always included measurements of reproductive structures. The science of measurement came much later and developed more gradually. Size ranges were included in descriptions, sometimes with extreme observed limits, sometimes with central tendencies, but no consensus regarding the presentation of these data was achieved. The number of measurements made for any one criterion was arbitrary. Latterly, statistics have usually been employed to assess central tendencies, but without critical evaluation of either the most appropriate statistic or the degree of accuracy that would be most useful for taxonomic purposes. **Continuous variation** should be expressed as a **mean** with standard variation; **discontinuous variation** should be expressed as a **modal value**. Shahzad, Coe & Dick (1992) were the first to consider the presentation of morphometric data for any straminipilous fungi. They found that measurements for 20 oogonia were sufficient to provide the central tendencies for oogonial and oospore parameters in *Pythium*.

Gäumann (1918*a*) was the first to assess morphometry of the conidia of *Peronospora* and applied his method in his monograph (Gäumann, 1923). However, his experimental approach was questioned by Gustavsson (1959*b*) because the field variation was as great as that found in experimental plots. Smith (1970) made further investigations into the effects of temperature and light on conidial size and concluded that the variation due to temperature was significant, but that variation between pathogenic strains under identical environmental conditions was also significant. Doubt was therefore cast on the value of dimensions for species determinations.

Hendrix & Campbell (1974) also dismissed the value of dimensions when assessing variation in *Pythium irregulare*. After assessing each parameter one by one, they concluded that the variation recorded was not of taxonomic use. Unfortunately they did not consider the total morphometric data. When Shahzad *et al.* (1992) reviewed the morphometric data obtained for a large number of species of *Pythium* they found that the size distributions for **single** characters within and between species formed a continuum. However, when these characters were combined to form derived character indices, there were distinct discontinuities between species (see also Dick, 1990*c*, 1992, 1995). Dick (1990*c*) used three indices:

1 **aplerotic index**, the ratio between oospore volume and oogonial volume
2 **wall index**, the volume of wall material as a function of the entire oospore volume
3 **ooplast index**, the ratio of ooplast volume to the entire oospore volume

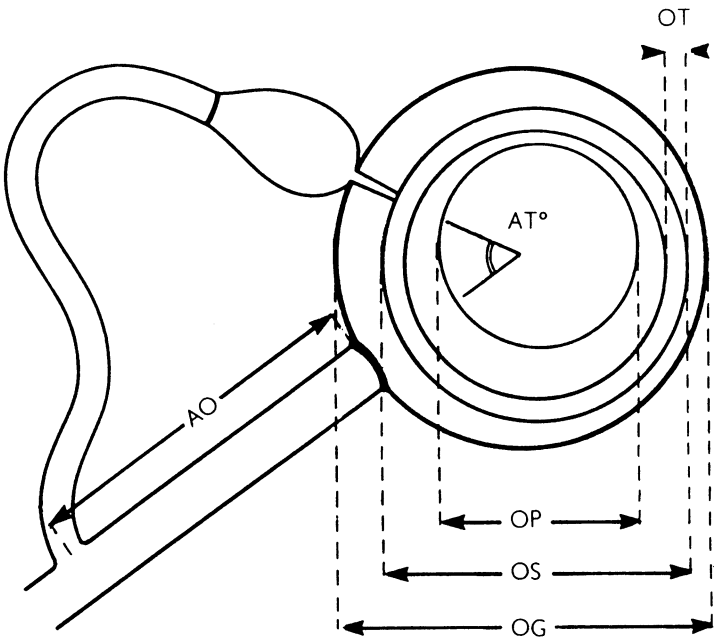


FIGURE I: 14. *Parameters for morphometry of* Pythium *oogonia and oospores.* OG *oogonial diameter;* OS *oospore diameter;* OP *ooplast diameter;* OT *oospore wall thickness;* AO *distance between antheridial origin and oogonial basal septum;* AT *angle of application of antheridial penetration. From Dick (1990c).*

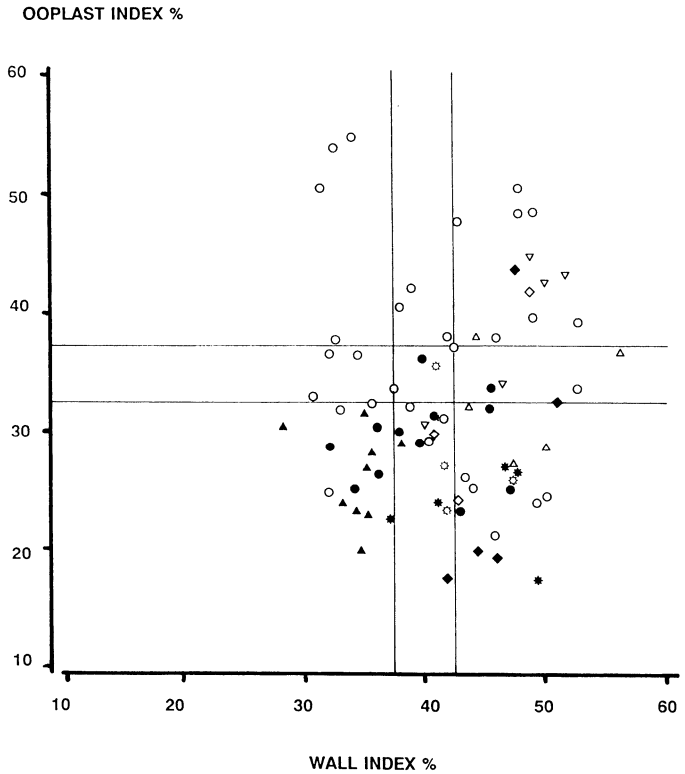


FIGURE I: 15. *The use of ooplast index and oospore wall index to demonstrate species discontinuities for 39 species of* Pythium, *following canonical variate analysis. 79 isolates (1 to 3 isolates per species) were plotted as follows:* ○ hypogynum, okanoganense, lucens, middletonii, multisporum, opalinum, salpingiphorum, rostratum, vexans *(spp. with spherical sporangia);* aquatile, coloratum, pachycaule, tenue *(spp. with filamentous sporangia);* australe, parvum, pleroticum *(spp. with sporangia unknown);* ● aphanidermatum, arrhenomanes, dissimile, dissotocum, ultimum *var.* ultimum; ◆ iwayami, ultimum *var.* sporangiiferum; ◇ deliense, graminicola, torulosum; ▲ echinulatum, irregulare, mamillatum; △ nagae, paddicum; ▽ anandrum, debaryanum, heterothallicum; ★ acanthicum, hydnosporum, sinense; ☆ acanthophoron, periplocum. *The lines parallel to the axes represent 5% tolerance limits. See Shahzad* et al. *(1992) for data base, calculations of indices and further diagrams of principal component analyses.*

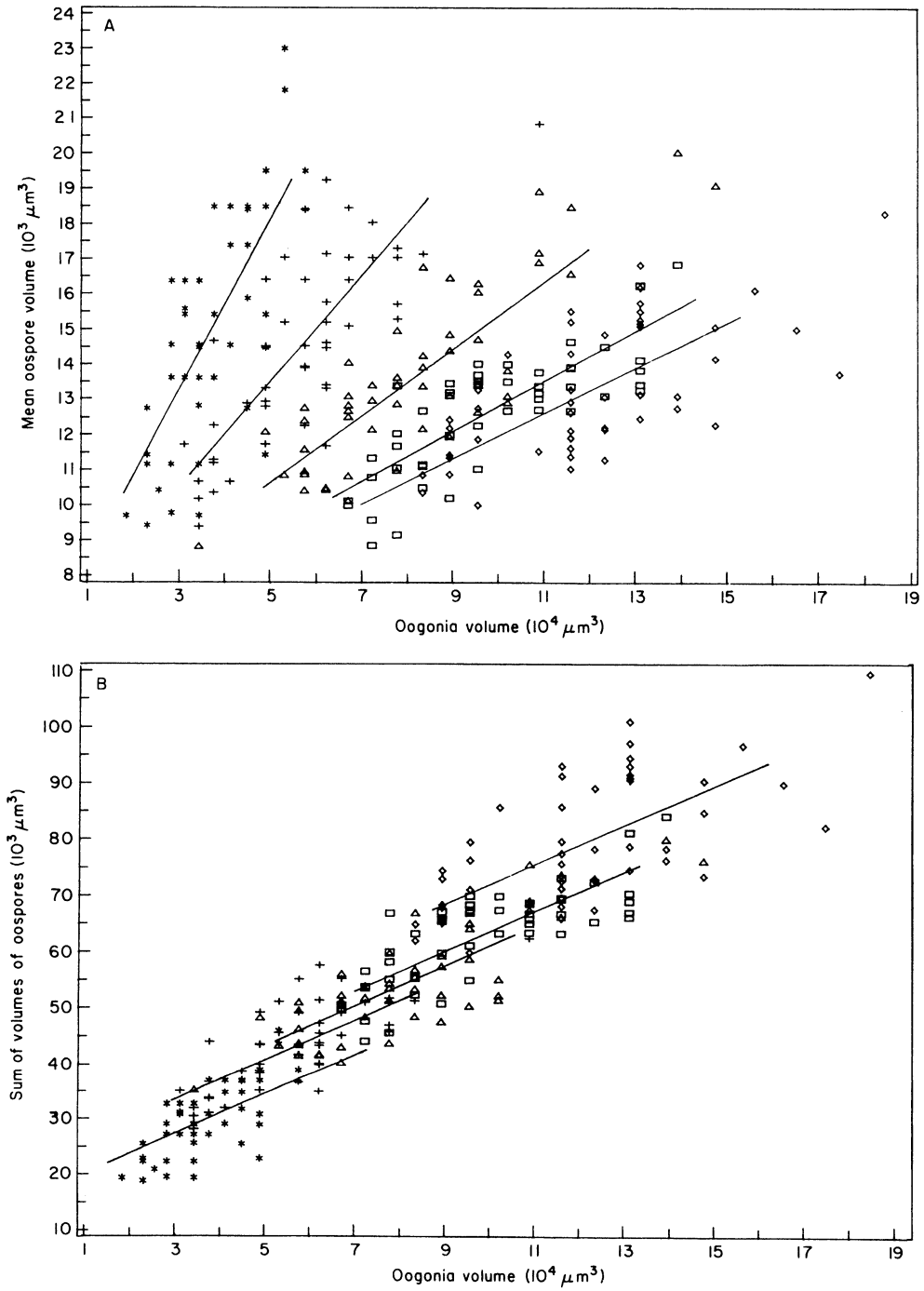


FIGURE I: 16 A,B. *Relationships between oospore size and oogonial size in pluriovulate oogonia of* Saprolegniaceae. Saprolegnia litoralis. *A. Mean oospore volume plotted against oospore volume for three modal classes of oospore number per oogonium. Computer-generated graph with common intercept lines fitted for each modal class. B. Sum oospore volume plotted against oogonial volume for the three modal classes of oospore number. Computer-generated graph with parallel lines fitted for each modal class (the slope of the parallel lines is distinctive for different species, see Table I: 3). Symbols for modal classes: * 2 oospores per oogonium; + 3 oospores per oogonium △4 oospores per oogonium; □5 oospores per oogonium; ◇6 oospores per oogonium. From Soumati & Dick (1989).*

The raw data of Shahzad *et al.* (1992) have been analysed by a completely independent statistic (statistical classification trees) which confirmed that of canonical variate analysis: the derived indices are of prime importance in determining discontinuities between species (P. A. Taylor & M. W. Dick, unpublished). With such a precise protocol for measurement it might be expected that variation would be introduced by different operators or different methods of presenting images for measurement. This has also been examined using Australian isolates of *Pythium*, although differences of means were recorded, the discontinuities beween isolates and species were unaffected (B. G. Hawke & M. W. Dick, unpublished data). The value of such indices has recently received independent confirmation from Møller & Hockenhull (2000), who used more isolates from fewer species. However, they preferred to use the linear measurements rather than calculated volumes. There will not be any difference in the relative differentials although the discontinuities will be less because the micrometer measure is raised to the third power in volumes. The purpose of using volumes is to express the actual endogenous reserves of the ooplast and endospore in oospores of one species compared with another.

Dick (1969) suggested that in the *Saprolegniaceae* and *Pythiaceae* there were 1:2:4 ratios of oospore volumes between different species of a genus. However, the relationship of such proportionality could not be based on any known factor such as polyploidy (Win-Tin & Dick, 1975).

In the multiovulate *Saprolegniaceae* Soumati & Dick (1989) investigated the morphometry of oogonia and oopores of 13 species. They showed that the number of oospores in an oogonium affects the mean oospore volume, but that the intercept slopes for each class of oospore number were the same for a species, although different species had different intercept slopes for the combined data (Figure I: 16; Table I: 3). The control of the range of oogonial size and shape is probably complex. Very careful selection of statistical treatments is required to handle the large intra-isolate variation that is concomitant with interspecific variation.

TABLE I: 3. *Ascending order of slopes of parallel lines regressions in 13 species of* Saprolegniaceae *(from Soumati & Dick, 1989)*

Species	**Slope of parallel lines regressions**
Thraustotheca clavata	0·1234
Achlya racemosa	0·1433
Achlya recurva	0·1746
Achlya hypogyna	0·1878
Achlya apiculata	0·1918
Achlya prolifera	0·1962
Isoachlya monilifera	0·2028
Achlya colorata	0·2085
Saprolegnia diclina	0·2116
Saprolegnia anisospora	0·2193
Saprolegnia furcata	0·2227
Saprolegnia terrestris	0·2796
Saprolegnia litoralis	0·3649

The higher the slope the more proportionally direct is the relationship between oogonial size and oospore size (i.e., the most proportionally direct relationship was for *S. litoralis*).

The functional significance of oospore morphology

The protoplasmic reorganization that takes place during the formation of the oospore is extensive and involves the redistribution of three kinds of reserve: the endospore (glucan polymers secreted outside the plasmamembrane), the ooplast (phosphate rich) and the lipid globules. The oospore is organized for the rapid mobilization of each of these reserves for specific aspects of growth and the proportions of each of these components is probably related to the function of the oospore in its life history. The endospore is mobilized rapidly at germination. Endospore mobilization may differ between taxa: extraplasmamembranic depolymerization may enable cell wall material to flow ***over*** the elongating germ tube plasmamembrane, while in other species pinocytotic ***resorption***, followed by secretion, may be involved. The endospore would thus be essentially potential wall material; the ooplast, membrane precursor; the lipid, an endogenous energy supply (Dick, 1995). Germ tube formation and rapid elongation will require relatively more wall glucan; zoospore production will require more plasmamembrane and DBVs for low polymer dehiscence mechanisms; zoospore production with extended periods of motility before any assimilative activity will require large lipid fuel reserves. These functional requirements are reflected in the proportionality of the reserves, which are specific rather than generic characteristics (Shahzad *et al.*, 1992).

The numerous (up to 40) oospores (*ca* 25 μm diam.) contained in single oogonia of aquatic species of *Saprolegnia* and the large populations of small (*ca* 12 μm diam.) unioosporus oogonia of some terrestrial species of *Pythium* reflect very different life-history strategies. The nutrient status of the pre-oogonial assimilative phase can affect the size of oogonia and number of oospores produced in multiovulate species. Depauperate oogonia can often be found in rough culture, leading to relatively large standard deviations about the mean but almost nothing is known about oogonial production in natural conditions (Dick, 1992).

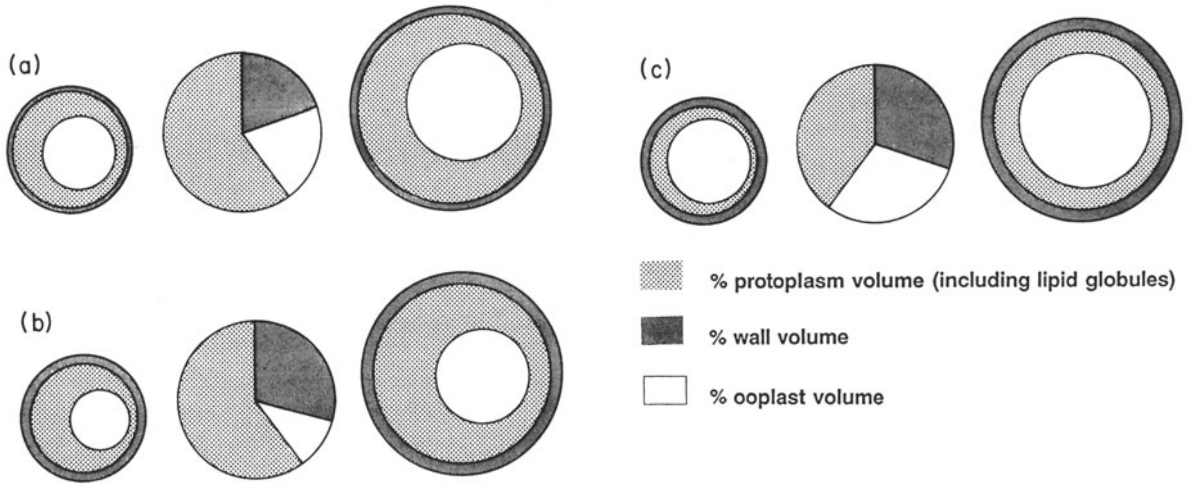


FIGURE I: 17 *Proportionality of the different oospore reserves in* Pythium. *Representative oospores with (1) different relative sizes and (2) different relative proportions of reserve materials. The large oospores have four times the volume of the small oospores. The pie-chart diagrams between the two oospore sizes provide the percentages by volume of endospore:ooplast (the lipid reserve can only be estimated separately in eccentric oospores with a single lipid droplet). Note that the protoplasm:ooplast plus endospore ratios are reciprocal between diagram series* b *and* c. *Note that the endospore wall thickness (measured thickness) is the same for the large oospore in* a *and the small oospore in* b. *See Dick (1990c), Shahzad* et al. *(1992); diagram rearranged from Dick (1995).*

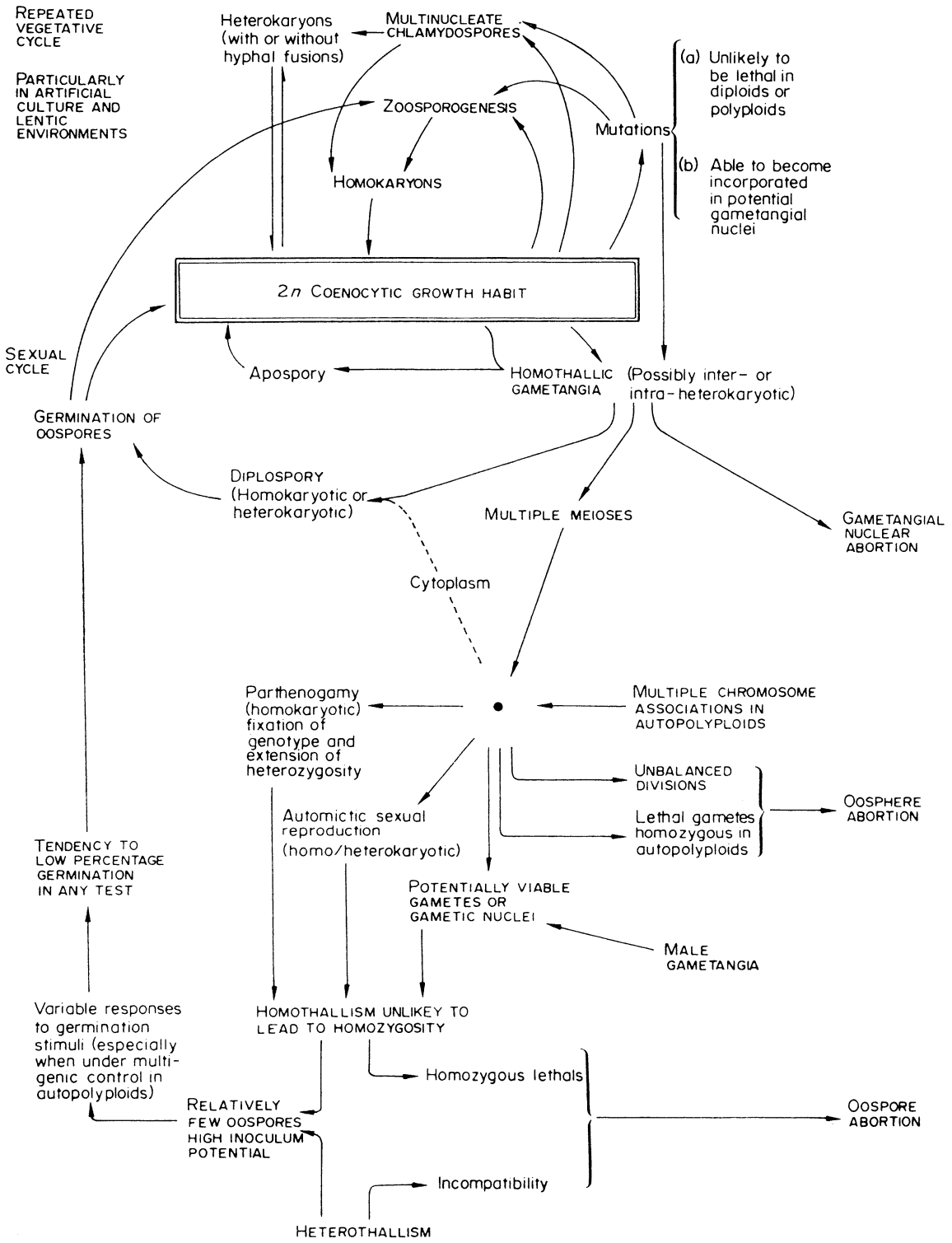


FIGURE I: 18. *Life-history of the* Peronosporomycetes *with emphasis on the nuclear developments, known events shown in upper case, unproven events in lower case. From Dick (1972: fig. 1)*

NUCLEAR CYTOLOGY: MITOSIS AND MEIOSIS

Mitosis

Early work on mitosis in the assimilative thallus was reviewed by Bakerspiegel (1960). Problems in resolving the irregularity of the spindle structure resulted in misinterpretations (binary fission) which were resolved by Heath (1974*a*, *b*, 1980*a*, *b*). Mitotic analysis is difficult and unlikely to be used as a diagnostic criterion.

Meiosis

Dick & Win-Tin (1973) reviewed the earlier accounts of nuclear behaviour in sexual reproduction of the *Peronosporomycetes* after Sansome (1961) had revived the earlier controversy regarding the site of meiosis. Despite some further controversy (Timmer *et al.*, 1970), it is now accepted that meiosis occurs in the gametangia of the *Peronosporomycetes* so that the ploidy cycle is haplomitotic B (Dick, 1987). The most recent and comprehensive overview of peronosporomycete nuclear cytology is that by Sansome (1987).

Much of the earlier work was based on paraffin wax sectioning, while more recent work has used squash techniques. The earlier work still has value because, although the interpretations of the division configurations may be obsolete, the spatial distribution and nuclear orientations will have been less disrupted than is the case after squash preparations. Table I: 3 provides a genus- and species-related summary of these earlier references from Dick & Win-Tin (1973) and Win-Tin & Dick (1975), with a few later citations.

Gametangia are meiogametangia in which meiosis occurs. Gametangia are coenocytic from the moment that the pro-gametangium is functionally predetermined; mitotic activity then ceases. All nuclei are diploid and apparently capable of undergoing meiosis. In paired gametangia the meioses are also either simultaneous, or nearly so, between the two protoplasts. The position of the meiotic nuclei in the gametangium is determined by the vacuolation of the protoplast in relation to centrifugal or centripetal oosporogenesis. Multiple concentric envelopes of ER may protect the functional nuclei (Dick, 1995). The numbers of nuclei entering the oogonial and antheridial initials are greater than the numbers of mature uninucleate haploid female gametes, which, in turn, are more numerous than the numbers of zygotes in the oogonium. A comparable reduction in the number of nuclei occurs in the antheridium (Dick, 1972). Figure I: 17, from Dick (1972) provides a chart of the probable patterns of nuclear abortion. Sterols have been implicated in relation to the patterns of nuclear abortion both prior to and after meiosis (Elliott & Sansome, 1977). Fertilization has been discussed on pp. 58-61; karyogamy may be precocious, as in *Achlya* (Win-Tin & Dick, 1965) or much delayed, as in *Phytophthora* (Jiang *et al.*, 1989).

All species of the *Peronosporomycetes* have a closed cruciform meiosis in which the nuclear membrane remains intact until the second telophase and the metaphase I and metaphase II spindle poles are in the same plane (Howard & Moore, 1970; Win-Tin & Dick, 1975). However, fenestration of the nuclear membrane, especially in the region of the poles, is a feature of *Olpidiopsis* (Bortnick *et al.*, 1985). Very few species have been examined by transmission electron microscopy for details of synaptonemal complexes (SCs). In *Achlya* the SCs are wider than in most other organisms, they tend to lack the central element, and fewer recombination nodules than would have been expected from the number of chromosomes present were found (Williamson & Yitzchak, 1991).

TABLE I: 4. *Cytology references from Dick & Win-Tin (1973) listed by species and augmented by later references*

Peronosporomycetidae

Peronosporales

Albugo bliti	Stevens (1899, 1901?)
Albugo candida	Davis (1900); Krüger (1910); Wager (1896*a*, *b*)
Albugo evolvuli	Thirumalachar, Whitehead & Boyle (1949)
Albugo ipomoeae-panduranae	Stevens (1904)
Albugo lepigoni	Ruhland (1902, 1904)
Albugo mysorensis	Safeeulla & Thirumalachar (1956)
Albugo platensis	Damle (1943)
Peronospora ficariae	Krüger (1910)
Peronospora parasitica	Wager (1889, 1900); Sansome & Sansome (1974)
Peronospora spp.	Tsang (1929)
Plasmopara alpina	Rosenburg (1903)
Plasmopara halstedii	Nishimura (1926)
Plasmopara viticola	Arens (1929); Bosc (1946); Berlese (1898)

Pythiales

Phytophthora cactorum	Blackwell (1943); Buddenhagen (1958); Erwin & McCormick (1971 - germination of oospores); Sansome (1965, 1966); Sansome & Harris (1962); Shaw & Elliott (1968)
Phytophthora cambivora	Allain (1934)
Phytophthora capsici	Timmer *et al.* (1970); Sansome (1976); Stephenson, Erwin & Leary (1974*a*, *b*)
Phytophthora cinnamomi	Brasier & Sansome (1975); Pratt *et al.* (1972)
Phytophthora drechsleri	Brasier & Sansome (1975); Sansome (1970)
Phytophthora erythroseptica	Murphy (1914, 1918); Sansome (1966)
Phytophthora fagi	Hartig (1880)
Phytophthora heveae	Bennett (1972)
Phytophthora himalayensis	Mundkur (1949)
Phytophthora infestans	Brasier & Sansome (1975); Gallegly (1968); Laviola (1969, 1971); Marks (1965); Sansome (1977); Sansome & Brasier (1973)
Phytophthora megasperma	Sansome & Brasier (1974)
Phytophthora megakarya	Brasier & Griffin (1979)
Phytophthora palmivora	Bennett (1972); Brasier & Medeiros (1978); Sansome (1977)
Phytophthora parasitica	Huguenin & Boccas (1970)
Phytophthora spp.	Elliott & McIntyre (1973); Galindo & Zentmyer (1967*a*, *b*); Gallegly (1970); Leal & Gomez-Miranda (1965); Sansome (1965, 1966); Sansome & Harris (1962); Shaw & Khaki (1971)
Pythium aphanidermatum	Seshadri & Payak (1970)
Pythium debaryanum	Miyake (1901); Sansome (1961, 1963*a*); Sansome & Harris (1962); Win-Tin & Dick (1975)
Pythium echinulatum	Win-Tin & Dick (1975)
Pythium multisporum	Win-Tin & Dick (1975)
Pythium torulosum	Patterson (1927*b*); Win-Tin & Dick (1975)
Pythium ultimum	Trow (1901); Win-Tin & Dick (1975)
Pythium spp.	Child & Haskins (1971); Marshall Ward (1883*b*); Saksena (1936)

(Table I: 4, continued)

Saprolegniomycetidae

Saprolegniales

Achlya ambisexualis	Raper (1940); Barksdale (1968); Ellzey (1974); Win-Tin & Dick (1975)
Achlya americana	Trow (1899)
Achlya apiculata	Win-Tin & Dick (1975)
Achlya benekei	Win-Tin & Dick (1975)
Achlya bisexualis	Raper (1936); Barksdale (1960, 1966); Mullins & Raper (1965)
Achlya caroliniana	Win-Tin & Dick (1975)
Achlya colorata	Patterson (1927*a*); Win-Tin & Dick (1975)
Achlya debaryana	Mäckel (1928)
Achlya dubia	Murdia (1938)
Achlya flagellata	Wolf (1938); Win-Tin & Dick (1975)
Achlya hypogyna	Clausz (1968); Cooper (1929*a*); Win-Tin & Dick (1975)
Achlya inflata	Win-Tin & Dick (1975)
Achlya klebsiana	Flanagan (1970)
Achlya polyandra	Mücke (1908)
Achlya prolifera	Pringsheim (1851); Mäckel (1928)
Achlya racemosa	Bakerspigel (1960); Carlson (1929); Win-Tin & Dick (1975)
Achlya radiosa	Win-Tin & Dick (1975)
Achlya recurva sensu Latham	Win-Tin & Dick (1975)
Achlya sparrowii	Win-Tin & Dick (1975)
Achlya treleasiana	Win-Tin & Dick (1975)
Achlya spp.	de Bary (1883); Sansome (1965, 1966); Sansome & Harris (1962)
Aplanopsis terrestris	Win-Tin & Dick (1975)
Brevilegnia diclina	Cooper (1929*b*)
Dictyuchus	Couch (1926)
Dictyuchus monosporus	Sherwood (1969, 1971)
Isoachlya anisospora var. *indica*	Bhargava (1946)
Pythiopsis cymosa	Win-Tin & Dick (1975)
Pythiopsis intermedia	Burton (1939)
Saprolegnia ferax	Mäckel (1928), Höhnk (1935), Bakerspigel (1960), Flanagan (1970), Win-Tin & Dick (1975)
Saprolegnia furcata	Beakes & Gay (1977); Win-Tin & Dick (1975)
Saprolegnia monoica	Reinke (1869)
Saprolegnia parasitica	Bakerspigel (1960)
Saprolegnia terrestris	Bryant & Howard (1969)
Saprolegnia spp.	Davis (1903, 1905); de Bary (1883); Hartog (1895, 1896, 1889, 1899); Marshall Ward (1883*a*); Moreau & Moreau (1935); Smith (1923); Slifkin (1967); Trow (1895, 1904, 1905); Ziegler (1953)
Thraustotheca clavata	Schrader (1937), Murdia (1938), Heath (1974*a*)
Apodachlya brachynema	Howard & Bryant (1971)
Aphanomyces laevis	Kasanowsky (1911)

Sclerosporales

Sclerospora graminicola	McDonough (1937)
Sclerospora sorghi	Safeeulla & Thirumalachar (1955 - as *Sclerospora andropogonis-sorghi*); Sansome (1963, 1966)
Sclerophthora macrospora	McDonough (1946*a*, *b*); Tasugi (1953)
Sclerophthora cryophila	Safeeulla, Thirumalachar & Shaw (1963)
Peronosclerospora sorghi	Safeeulla & Thirumalachar (1955)
Peronosclerospora sorghi	Sansome (1963*b*)

Order *incertae sedis*

Olpidiopsidales

Olpidiopsis achlyae	McLarty (1941)

The prevalence of self-fertilization and automictic sexual reproduction would appear to deny an outbreeding function for sexual reproduction in this group. The lack of genetic opportunity resulting from karyogamy of gametic nuclei derived from meioses of recent-sister diploid nuclei may be offset by the development of polyploidy or polysomy and B chromosome development, but further work on genome synteny (see below) is essential. The populations will nevertheless be heterogeneous because of the non-selection of presumptive meiotic nuclei from very large diploid-mitotic populations.

A comparative study of the cytotaxonomy of the *Saprolegniaceae* and *Pythium* (Win-Tin & Dick, 1975) indicated probable base chromosome numbers of $x=3$ for *Saprolegniaceae*, $x=4$ for *Leptomitaceae* and $x=5$ for *Pythiaceae*, with polyploid series in both *Saprolegniaceae* and *Pythiaceae* (Win-Tin & Dick, 1975; Sansome, 1965). Base numbers of $x=9$ or $x=10$ are also common. Much more detailed cytology of *Phytophthora* has been carried out by Sansome, Brasier and co-workers (see Sansome, 1987, for a review of recent literature).

Electrophoretic karyotypification of chromosome-length pieces of DNA by means of Contour-clamped Homogenous Electric Field electrophoresis (**CHEF**) has been published for *Phytophthora* (Howlett, 1989; Mao & Tyler, 1991; Tooley & Carras, 1992; Judelson *et al.*, 1993), *Bremia* (Francis & Michelmore, 1993) and *Pythium* (Martin, 1995*a*, *b*). In *Phytophthora*, Judelson *et al.* (1993) found 6-8 chromosome-sized bands ranging in length from 2·9 - 10·0 Mb, which is comparable with the 10-13 chromosomes estimated by Sansome & Brasier (1974). In *Bremia*, Francis & Michelmore (1993) found a minimum of 7 chromosome-sized bands ranging in length from 3·0 - >8·0 Mb, corresponding to chromosome numbers estimated by light microscopy (Michelmore & Sansome (1982), but in addition they found variable numbers of up to 5 smaller components, identified as possible B chromosomes or large plasmids. In the wider spectrum of 18 species of *Pythium*, Martin (1993*a*) found 7-20 chromosome-sized bands ranging in length from 18·8 - 41·5 Mb, again comparable but not identical with the estimates of Win-Tin & Dick (1975). Mao & Tyler (1991) concluded that some of the bands that they obtained could represent doublets or triplets, thus parity between light microscopical methods and CHEF electrophoresis might be difficult to establish. Intraspecific differences for both the number and the lengths of the chromosome-sized bands were reported for *Pythium* (Martin, 1995*a*). The ranges of chromosome lengths could provide taxonomic information if and when the criterion is shown to be species-stable for particular genera, but this is by no means certain at the present time.

A different approach to phylogeny, using comparative DNA-based data (Feulgen Image Analysis) is that of Voglmayr & Greilhuber (1998) who have produced data suggesting that *Peronospora* and *Plasmopara* are probably not closely related (cf. Dick, 1988). Electrophoretic Karyotype (EK) polymorphisms (determined by CHEF analysis) occur in *Pythium* species (Martin, 1995*a*), but only one DM, *Bremia lactucae*, has been assessed (Francis & Michelmore, 1993) so again, evolutionary predictions would be premature. Analyses of EK whch show intra-specific heterozygosity are already known for several species of *Pythium* (F. N. Martin, pers. comm.), therefore, a large database will be necessary to establish whether chromosome size and number also show consistent inter-specific differences.

Light-microscope nuclear cytology of the plasmodiophorids has been reviewed by Karling (1968*e*), who concluded that the data indicated a base number of $x=2$; Harris, Braselton & Miller (1980) provided a later summary. The nuclei of all taxa are very small and TEM analyses of synaptonemal complexes (Harris *et al.*, 1980; Braselton, 1982, 1983, 1984, 1989*a*, *b*, 1990; Braselton & Short, 1985; Braselton & Dylewski, 1986) suggest that estimates of chromosome number from light microscopy are likely to be underestimates (Harris *et al.*, 1980; Williamson & Yitzchak, 1991).

CYTOLOGY

The ultrastructure of nuclear division in the *Peronosporomycetes* was pioneered by Howard & Moore (1970) and has been reviewed by Beakes (1981*a*). Heath (1981) has provided a preliminary cluster analysis of nuclear cytological features of the *Peronosporomycetes* compared with other groups of protoctists. This analysis of mitosis was based on fourteen characters. Four of these diagnostic features are of relevance to this discussion:

1 the configuration of the centrioles
2 the persistence or reconstitution of the nuclear membrane
3 persistent telophase spindles (interzonal bridges)
4 the behaviour of the nucleolus

To these should be added two features of meiosis:

1 synaptonemal complexes
2 the arrangement of telophase II nuclei

Two patterns of centriole behaviour during mitosis have been described: in the *Peronosporomycetes* the spindle develops during polar separation of the centrioles, but in *Rhizidiomyces* and the chytrids polar migration is complete prior to spindle development. Perhaps related to this difference in synchrony, is the orientation of the two centrioles at the end of interphase. The centrioles are oriented end-to-end in the *Peronosporomycetes* (Berlin & Bowen, 1964: *Albugo*; Howard & Moore, 1970: *Saprolegnia*); the labyrinthulids (Perkins, 1970: *Labyrinthula*; Kazama, 1974*b*: *Thraustochytrium*), and the plasmodiophorids (Brazelton & Miller, 1973: *Sorosphaera*). Centrioles are approximately at right angles to each other in *Rhizidiomyces* (Fuller & Reichle, 1965), *Catenaria anguillulae* Sorokīn (Ichida & Fuller, 1968), *Blastulidium* (Manier, 1976) and some *Chytridiomycetes* (Whisler & Travland, 1973; McNitt, 1973).

In the *Peronosporomycetes* the nuclear membrane remains intact until the end of telophase in both meiosis and mitosis (Heath & Greenwood, 1968; Howard & Moore, 1970; Heath, 1974*a*; Ellzey, 1974). However, fenestration of the nuclear membrane, especially in the region of the poles, is a feature of *Olpidiopsis* (Martin & Miller, 1986*a*), *Rhizidiomyces* (Barstow, Freshour & Fuller, 1989), the labyrinthulids (Perkins & Amon, 1969), the plasmodiophorids (Braselton & Miller, 1973) and two orders of chytrids (*Spizellomycetales* (*Entophlyctis*): Powell,1975; *Chytridiales* (*Rhizophidium*): Powell, 1980) but not the *Blastocladiales* (*Catenaria anguillulae* - Ichida & Fuller, 1968). *Rhizidiomyces*, the plasmodiophorids and a few chytrids exhibit the type *iii* reconstitution behaviour (Heath, 1980*a*), whereby new nuclear envelopes are developed ***within*** the remnants of the original membrane (Garber & Aist, 1979*b*; Barstow *et al.*, 1989). In the thraustochytrid, *Ulkenia*, the nuclear membrane disappears completely at prophase (S. Raghu Kumar, 1982*b*).

Abstriction of the daughter nuclei may be achieved by nuclear membranes traversing the telophase spindle in two places rather than one, thus leaving a spindle remnant as an interzonal bridge (type *ii* reconstitution behaviour of Heath, 1980*a*). Such a pattern has been shown for *Olpidiopsis* (Martin & Miller, 1986*a*), *Thraustochytrium* (Kazama, 1975) and *Catenaria anguillulae* (Ichida & Fuller, 1968); it has also been reported for *Vaucheria* (Ott & Brown, 1972).

Pickett-Heaps (1970) has reviewed variations of nucleolar behaviour during mitosis. The nucleolus remains outside the spindle during mitosis in the oomycetes (Beakes, 1981*a*). In the plasmodiophorids the nucleolus also persists during mitosis, but has a more prominent role. The nuclei of the coenocyte undergo synchronous divisions prior to sporangial development (Karling, 1968*e*, Dylewski, 1990), and in these divisions there is an elongate nucleolus occupying the centre of the spindle. A comparable central structure is found in *Euglena* (Leedale, 1967) resulting from the fusion of numerous interphase nucleoli at prophase. In stained preparations the axile nucleolus of the plasmodiophorids gives the impression of a cruciform structure at metaphase, one axis being the nucleolus while the metaphase chromosomes form a plate perpendicular to the nucleolar axis (Miller, 1958*a*; Keskin, 1971; Braselton, Miller & Pechak, 1975; Dylewski, Braselton & Miller, 1978). The nucleolus is not persistent in meiosis and so cruciform configurations do not occur (Braselton & Miller, 1973). However, direct comparisons with *Euglena* are not possible because the arrangement of chromosomes in mitosis is different and meiosis is unknown.

Sparrow (1960, 1976) and Waterhouse (1973) suggested that, in the then present state of knowledge, the anisokont zoospore and the mitosis, with its persistent axile nucleolus at metaphase, should constitute the diagnosis for the *Plasmodiophoromycetes*. (Since the class and order are monotypic, this could be downgraded and interpreted as a family criterion.)

Synaptonemal complexes (SCs) have been repeatedly demonstrated in *Peronosporomycetes* (Howard & Moore (1970: *Saprolegnia*); Ellzey (1974) and Ellzey & Huizar (1977: *Achlya*); Hemmes & Ribiero (1977: *Phytophthora*); Beakes & Gay (1977: *Saprolegnia*); Gotelli (1979: *Sapromyces*); Traquair & McKeen (1980: *Aphanomyces*); Martin & Miller (1986*c*: *Olpidiopsis*); Williamson & Yitzchak (1991: *Achlya*). Williamson & Yitzchak (1991) regard the structure of the SC in oomycetes as "essentially unique" because they are wider and tend to lack a clearly defined central element, and although the SCs have recombination nodules, these were fewer than would have been expected from the chromosome number. The chromosome number determined by Williamson & Yitzchak (1991) was $n=15$, which is consistent with the base number proposed for the *Saprolegniaceae* by Win-Tin & Dick (1975). Martin & Miller (1986*c*) demonstrated the presence of SCs in the gametangia of *Olpidiopsis varians* but did not give an estimate of chromosome number.

Synaptonemal complexes have been more intensively studied in the plasmodiophorids (*Sorosphaera veronicae* (J. Schröt.) J. Schröt. - Harris *et al.*, 1980; *Plasmodiophora brassicae* - Braselton, 1982; *Polymyxa betae* - Braselton, 1983; *Polymyxa graminis* Ledingham - Braselton, 1984; *Plasmodiophora diplantherae* (Ferd. & Winge) Ivimey Cook - Braselton & Short, 1985; *Woronina pythii* Goldie-Sm. - Braselton & Dylewski, 1986; *Ligniera verrucosa* Maire & A. Tison - Braselton, 1989*a*; *Membranosorus heterantherae* Ostenf. & H. E. Petersen - Braselton, 1989*b*; *Tetramyxa parasitica* K. I. Goebel - Braselton, 1990; *Spongospora subterranea* - Braselton, 1992). Serial sections have revealed chromosome numbers of $n=9$ to $n=38$. Braselton (1989*a*, 1992) has noted that *Plasmodiophora brassicae* and *Woronina* have distinctly smaller nuclei and a shorter total length with less well-defined central zones to the SCs (cf. *Peronosporomycetes*, above) than the other plasmodiophorids.

For other straminipilous fungal groups, Moens & Perkins (1969) reported nine SCs in an unnamed species of *Labyrinthula*. The SCs of algal straminipiles have been much less intensively studied (see Berkaloff & Rousseau, 1979; Katsaros & Galatis, 1986; Markey & Wilce, 1976; Toth & Markey, 1973).

In the lagenidiaceous fungi, apart from *Olpidiopsis*, accounts of conventional nuclear cytology are limited to those on *Lagenisma* (Schnepf *et al.*, 1978*b*). The only comparative information relates to ultrastructural studies of *Salilagenidium callinectes* (Amerson & Bland, 1973) and *Lagenisma* (Schnepf *et al.*, 1978*b*). In the latter species the SCs appear normal, but in *S. callinectes* polycomplexes with a fine structure similar to synaptonemal complexes are described from nuclei of encysting [zoo]spores. These polycomplexes occurred in the presence of a nucleolus and the nuclear membrane appeared to be intact. Amerson & Bland (1973) did ***not*** claim that the polycomplexes established the site of meiosis. There must be some doubt as to whether these polycomplexes can be equated with synaptonemal complexes, although Beakes (1981*a*) and Williamson & Yizchak (1991) assume them to be synaptonemal complexes. Schnepf *et al.* (1978*b*) have also queried this interpretation. However, if the polycomplexes were to be considered equivalent to synaptonemal complexes, then meiosis would occur in the encysting [zoo]spore. The ploidy state of the life-history following germination, and the site of karyogamy, would need to be determined, but the life-history could hardly be considered peronosporomycetous. The lack of attachments between the polycomplexes and the nuclear membrane, and the absence of a regular series of true SCs, suggest that polycomplexes may be an unexplained chromosomal condensation which ***may not*** indicate the site of meiosis. Comparisons should be made with situations in which SCs are associated with mitotic ploidy reduction, as in endopolyploid oxymonads (Hollande & Carruette-Valentin, 1970) and radiolarians (Lécher, 1978), and in premeiotic mitoses (ascogenous hyphae - Zickler, 1973; pollen mother cells - MacQuade & Bassett, 1977).

The four telophase meiotic products can be arranged in three patterns depending on the orientations of the axes of the first and second metaphase spindles. If the spindles are all in the same orientation a linear array will be formed; if the spindles are in the same plane but with the second metaphase spindles at 90° to the first metaphase spindle a cruciform arrangement will result; if the two second metaphase spindle axes are at 90° to each other a tetrad will be formed. In the *Peronosporomycetes* the nuclear membrane persists until

the end of the second telophase and the spindle axes are all in the same plane (Howard & Moore, 1970), resulting in the cruciform arrangement. This appears to be the case in *Myzocytiopsis* (Dick, 1995). In the plasmodiophorids the haploid nuclei are not bound by a common nuclear membrane and there is an interval between the two divisions so that a precise pattern is not established.

The diversity of nuclear cytology and the independence of some of the characters undoubtedly provides scope for the formulation of diagnostic criteria within the flagellate fungi. At present the number of organisms examined is insufficient to trace any evolutionary patterns.

Genome synteny

Genome synteny is the term which has been used to describe the total gene content of nuclei in relation to the chromosome number and the distribution of genes on the chromosomes. It is now viewed somewhat differently following the demonstration that while most of the genes in the genome are similar, they may be distributed differently between the chromosomes, so that, as in the grasses (Moore *et al.*, 1995), considerable differences in chromosome size and number conceal an underlying genome similarity. Genome synteny has been well established in animals and plants has not been documented or considered for eumycote fungi or straminipiles. The chromosomal distribution of genes may affect mode of action of the genes, with the possibility that a gene occurring in different positions may affect the biodiversity/speciation without much change in nucleotide sequence data. Polyploidy, as in *Achlya* (Win-Tin & Dick, 1975), *Pythium* (Win-Tin & Dick, 1975) and *Phytophthora* (Sansome, 1965) and polysomy, as in *Phytophthora* (Shaw, 1991, Shaw & Shattock, 1991) could affect gene function. When ploidy levels are different the breeding systems of the straminipilous fungi need to be taken into account, particularly when selfing and automictic sexual reproduction may be involved (Dick, 1972, 1987, 1995; Win-Tin & Dick, 1975). Brasier (1992) and Brasier & Hansen (1992) have reviewed the evolution of *Phytophthora* from a genetic standpoint.

It is also possible that differences in virulence could be attributed to Simple Sequence Repeats (SSRs ≡ microsatellite DNA). Infraspecific variation in *Pythium ultimum* is well-known, both at the varietal level (Drechsler, 1960) and within one of the varieties (Tojo *et al.*, 1998). Is the infraspecific variation in *Pythium ultimum*, with its varieties *P. ultimum* var. *ultimum* and *P. ultimum* var. *sporangiiferum* due to such changes in SSRs? Pathological distinctiveness (e.g., *formae speciales*) and interbreeding barriers, not be accompanied by morphological differences, may be the result of slight and recent genetic changes which will be of importance when determining species discontinuities. This possibility is relevant to molecular biological analyses which purport to determine conspecificity.

THE ASSIMILATIVE THALLUS

The assimilative thallus: morphology

The thallus is initiated from a uninucleate zoospore cyst or an aplanospore; a multinucleate asexual spore or propagule; or a uninucleate sexually produced oospore. The multinucleate asexual spores and oospores either germinate directly to give a thallus, hypha or conidiosporangiophore, or function as a zoosporangium. The thalli of members of the *Peronosporomycetes* may be filamentous, composed of hyphae forming a mycelium; or coralloid (eucarpic or holocarpic), allantoid or ellipsoid (holocarpic); or monocentric and eucarpic, having an assimilative system composed of branched rhizoids. Hyphae are ***analogous*** to hyphae of the *Eumycota* and show tip growth, first demonstrated in *Pythium* (Grove, 1970; Grove & Bracker, 1978; Grove, Bracker & Morre, 1970). Hill & Mullins (1979) used *Achlya* for a study of tip growth, and over a long period of time Heath and coworkers (e.g., Heath & Rethoret (1980) Gupta & Heath (1997) Kaminskyj, Garrill & Heath (1992) Heath & Steinberg (1999) have used *Saprolegnia ferax* as an experimental organism for investigating the fundamental processes of tip growth.

The protoplasm is coenocytic, with or without a conspicuous central tonoplast vacuole. Bidirectional cytoplasmic streaming along cytoplasmic strands can readily be seen in wide hyphae, where the cytoplasmic strands follow a long spiral. The nuclei are small, up to about 3 μm in diameter, but often deformed in hyphae. During active assimilative growth the nuclear cycle is short (about 36-76 min in *Saprolegniaceae* and 75-155 min in *Pythiaceae*, see Dick, 1990*a*, 2000*c*).

The mycelial habit has probably developed on several separate occasions (Dick, 1995, 2000*a, c*). Different views are held on the evolution of hyphal form (Barr, 1981; Dick, 1997*a*). The stout hyphae of the *Saprolegniaceae* have been regarded as a primitive character retained from an algal ancestor, but I regard this morphology as a more recent ecological specialization. The wide-diameter hyphal systems of the *Saprolegniaceae* may represent a modification from a monocentric kind of organization, with isodiametric growth being replaced by tip growth accompanied by a shift of assimilatory function from a well-defined rhizoidal system to these broader hyphae. This 'vegetative' system may thus be considered as an assimilative, growth-unlimited sporangio-gametangiophore. The origin of hyphae in the *Peronosporomycetes* was probably also recent, either from a sporangio-gametangiophore with indeterminate tip growth (wider hyphae), or from a narrow germ tube developed from an infection peg (narrower hyphae).

The possibility of different evolutionary origins of the myceliar habit might be supported from data on hyphal anastomoses. Wilde (1961) and Stephenson, Erwin & Leary (1974) have provided evidence for hyphal anastomoses in *Phytophthora*, but anastomoses have never been reported for the *Saprolegniomycetidae*. Stephenson *et al.* (1974) reported anastomoses between A^1 and A^2 sexual strains for three species of *Phytophthora*, but whether anastomosis is confined to union between hyphae differing ***only*** in the sexual mating type, or whether more general heterokaryotic union occurs has not been established. In eumycote fungi heterokaryon formation does ***not*** occur between sexually compatible strains so the mechanism and control must be different.

The assimilative thallus is bounded by a wall membrane at maturity, but initially may be naked in some endobiotic parasites. In mycelial forms, hyphae vary in diameter from 1·8-2·5 μm (*Pythiogeton, Verrucalvus*) up to 150 μm (after intussusception, in older hyphae of *Achlya*). Septa are normally present to delimit reproductive structures and sometimes develop as **retraction septa** in old mycelia of *Pythium*; plugs of wall material may replace septa at reproductive junctions in the *Rhipidiaceae*, *Peronosporaceae* and *Phytophthora*. In the *Saprolegniaceae* there is frequently excessive synthesis of wall material at the central closure of the septum, resulting in the development of an irregular peg or callus on one or both sides of the septum. Generalized intussusception of wall material occurs in the monocentric thalli of *Rhipidiaceae* and in the hyphae of some *Saprolegniaceae* as the structures increase in girth, but the mechanism of this synthesis is unresearched. Regular differential wall synthesis may result in ***thickened*** walls at the thallus

constrictions in the *Rhipidiaceae* (Dick 1973*b*). The entire wall of the basal cell of the *Rhipidiaceae* can become very thick, with exfoliation of wall material as the basal cell expands.

Septation of the mycelial thallus occurs in *Pythium*, and if *Lagenidium* is defined on the basis of differentiated gametangia, then there is hardly any discontinuity with *Pythium*. Similarly there is little distinction between a lobed thallus in an algal cell and the toruloid thallus typical of some saprotrophic members of the *Leptolegniellaceae*. Other taxa of *Leptolegniellaceae* (e.g., *Brevilegniella*) have a filamentous thallus with swollen intercalary reproductive segments. A thallus form such as that of "*Lagenidium* sp. on *Gracilaria*" (Johnson, 1957) is intermediate between that of *Brevilegniella* and the mycelium of certain species of *Pythium*. Diagram of diversity in thallus form are given in Figures I: 10 (p. 48-49) and I: 19 (p. 80).

It has long been recognized that experienced workers are able to suggest probable generic designatons for non-reproductive mycelia. This has been especially true for *Saprolegniaceae* (rounded or acute shape of hyphal tip and mean hyphal diameter distal from the tip), *Pythium* (sometimes 'fastigiate') and *Phytophthora* ('twiggy' and forming a 'chrysanthemum pattern'). Ho (1978) used nine parameters of hyphal branching systems and suggested that these characters might be useful in the delimitation of taxa. In addition, the modal angle of branching on agar is often useful. The water relations of *Achlya* on agar are such that extensive cracks develop in the agar well before the mycelium has reached the Petri dish circumference. With new programs for image analysis it should be possible to develop branching pattern as a diagnostic criterion, but it will be necessary to establish the reliability of these patterns under different cultural conditions.

Obligate parasites may be entirely confined ***within*** a single host protoplast (endobiotic), **intracellular** (some hyphae invading the protoplasts of a host thallus), or **intercellular** with specialized side branches (**haustoria**) that penetrate the cell walls, but not the protoplasts, of the host cells. In most of the endoparasitic fungi which have been studied by transmission electron microscopy, an extremely fine penetration tube of *ca* 0·1-0·2 μm diameter enters the host and subsequent tip expansion enables the formation of the first unit of the thallus within the protoplast of the host.

Thallus form has formerly been the principal determinant for placement in *Myzocytium* or *Lagenidium*. *Myzocytium* has a thallus which becomes articulated into swollen segments, often with the cross walls becoming thickened and hyaline, resembling *Catenaria* of the *Chytridiomycetes*. In some species these segments become disarticulated at maturity. In *Blastulidium* the irregularly loment thallus becomes septate and the segments become more or less spherical before disarticulating (Manier, 1976). On the other hand, *Lagenidium* has an allantoid, tubular branched or lobed thallus which may or may not be septate. If septate, the segments are not normally inflated. Obviously these forms intergrade, particularly in depauperate specimens or in small hosts, and reliance should not be placed on this criterion.

There is a fine distinction between the definition of a fungus as an osmotroph with a cell wall during the assimilative phase, and the naked intra-protoplasmic plasmodium of an endogenous parasite. This line of distinction appears to be crossed to different extents during the thallus development of species placed in the *Olpidiopsidales*, *Rozellopsidales* and *Plasmodiophorales*. The initial thallus development is plasmodial in *Olpidiopsis*, but a walled thallus, which continues expanding, soon becomes apparent. However, in *Rozellopsis* and *Rozella*, fungi which may or may not be related to either *Olpidiopsis* or the *Plasmodiophoromycetes*, more extensive plasmodial phases involving subdivision of the assimilative plasmodium into several independent units can occur (compare Held, 1980). The transition from a plasmodial to a walled assimilative state may be precocious or retarded in endoparasitic fungi, and thus not of great significance at high taxonomic hierarchies. Terminal elongation, with septation of the thallus and intercalary segments functioning as sporangia or gametangia, may be derived from a monocentric state and give rise to the 'hyphal' morphology.

Reliance on thallus/sporangial form, particularly when intermediate between a lobulate or saccate holocarpic form and a more or less minimalist ovoid (*Olpidium*- or *Olpidiopsis*-like) shape, has resulted in heterogeneous groups of organisms in genera such as *Olpidiopsis* and *Petersenia* (see pp. 215 and 228).

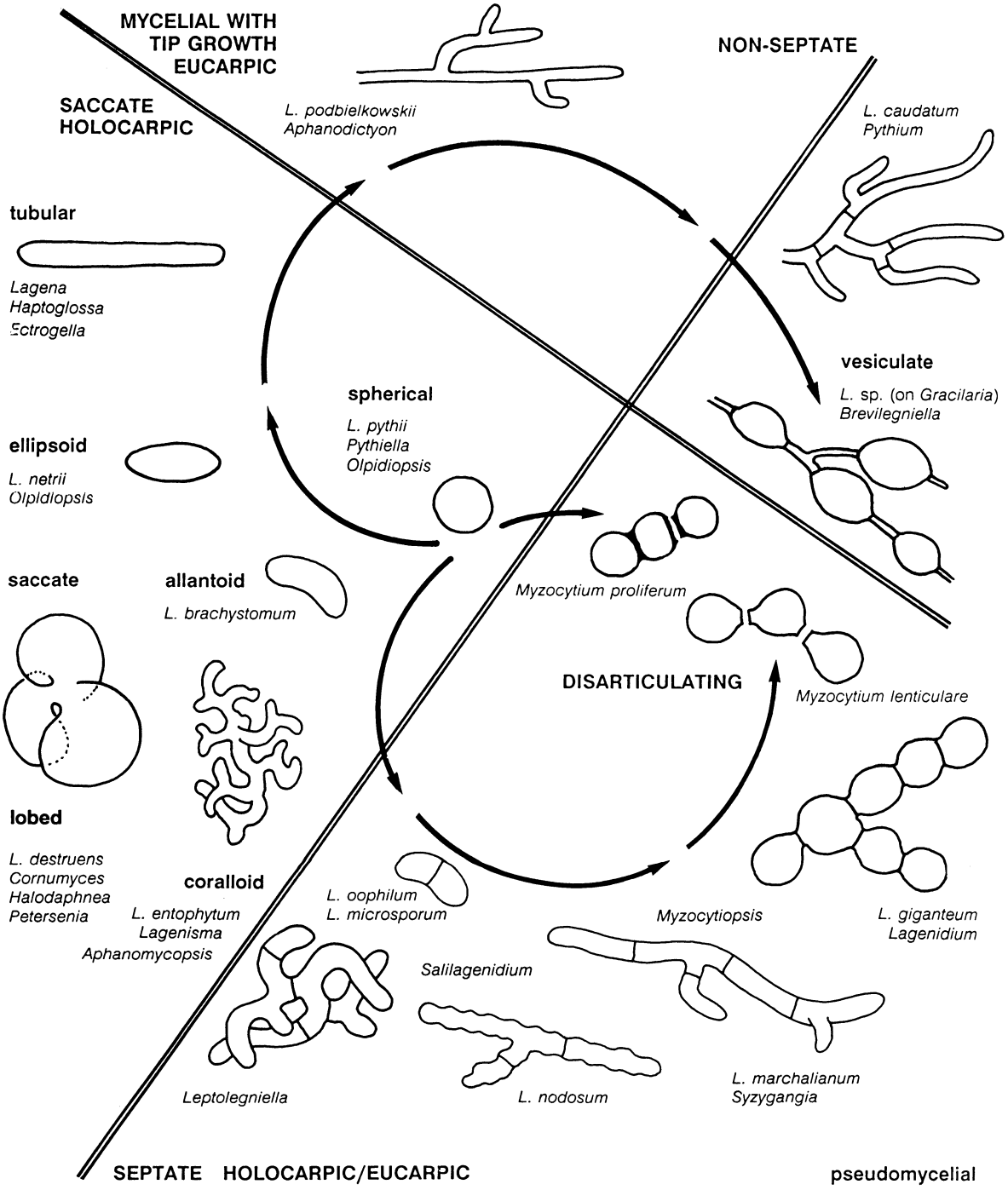


FIGURE I: 19. *Divergence and convergence in thallus form. Phylogenetic significance is not intended. The diagonal straight lines indicate major divisions with respect to holocarpy/eucarpy and mycelial tip growth/intercalary wall morphogenesis. Many states are represented by species of* Lagenidium sensu lato *(see PART IV), and by genera of the* Leptolegniellaceae.

While thallus form may be valuable at the species level, it cannot be accorded significance, on its own, at any higher taxonomic level until there is a series of much more precise, and rigorously applied, ontogenetic definitions.

Thallus form in the plasmodiophorids is initially plasmodial, and morphological distinctions between taxa only become evident when the plasmodium becomes sporogenous. After the mitotic phase a walled sporangium is formed which may take the form of an irregular chain of swollen segments, as in *Polymyxa betae*, or a convoluted branch system or reticulum, as in *Woronina pythii* (Dylewski, Miller & Braselton, 1978). The extent to which the protoplasm of these elements may be completely separated so that the elements function as individual sporangia is unclear. Ciafardini & Marotta (1988, 1989) have shown that the wall between contiguous parts of the sporangial complex of *Polymyxa betae* is more fragile than the other walls. In both of the above species there are resemblances to lagenidiaceous fungi (cf. Karling, 1968*e*: 95). Cystogenous plasmodia show cleavage to produce uninucleate segments of small volume *ca* 15-120 μm^3), each of which develops a smooth or spiny cyst wall. In *Octomyxa* these are commonly found in tetrads or octets, but larger groups occur in *Sorophaera* and *Sorodiscus*. Powell (1984) noted the probability of phagocytosis towards the end of the assimilative phase in *Rozella*, and there is a similar suggestion for the anisokont biflagellate parasite of protists, *Endemosarca* (Erdos & Olive, 1971, Erdos, 1973). Phagocytosis has not previously been regarded as an attribute of plasmodiophorids, but Clay & Walsh (1997) have provided evidence that it takes place in *Spongospora*.

The labyrinthulids are straminipilous heterotrophs, but they are not fungi because the assimilitive ectotrophic net, while osmotrophic, is never bounded by a cell wall. I therefore prefer to use the class name *Labyrinthista*, rather than the fungal hierarchical nomenclature *Labyrinthulomycota* (division) and *Labyrinthulomycetes* (class).

Intercellular hyphae of parasites

One of the striking features of the *Peronosporomycetes* (*Peronosporales*), the downy mildews (DMs), is the development of biotrophy from necrotrophy. Savile (1968, 1976) has suggested that the first step towards phytoparasitism would have been the development of ***systemic*** (whole plant) myceliar parasitism to protect the hyphae from desiccation (note the extremely narrow and vulnerable hyphae of the *Sclerosporaceae*), and that pathological lesions of limited mycelial extent would have evolved later. However, systemic infections are known to occur in the *Peronosporaceae* (Goosen & Sackston, 1968: *Plasmopara*; Heller, Rozynek & Spring, 1997, Ramsay, Smith & Wright, 1954, *Peronospora*; Marlatt, Lewis & McKittrick, 1962: *Bremia*) and *Albuginaceae* (Jacobson *et al.*, 1998). Infections caused by the DMs of panicoid grasses may also be systemic (Kenneth, 1981). Parasitism by the downy mildews must be contrasted with the parasitoidal associations of the *Myzocytiopsidaceae* with nematodes and algae (Dick, 1997*b*). These endobiotic parasites are always necrotrophic. Similarly, endobiotic *Saprolegniaceae* (*Aphanomyces parasiticus*), root-parasitic *Saprolegniaceae* (*Aphanomyces euteiches* and *A. cladogamus*) and *Pythiaceae* (many *Pythium* species) are necrotrophic.

Biotrophic obligate parasitism is not always fully developed, so that a limited range of host/parasite relationships may be covered by this phrase. Biotrophic obligate parasites, such as DMs, have advanced genetic and biochemical attributes often, but sometimes unjustifiably, equated to an evolutionary status. Significant cell damage is caused, for example, by *Peronospora tabacina* and *Plasmopara viticola* (Lafon & Bulit, 1981): the plasmamembranes of the host mesophyll cells become excessively leaky, resulting in a distinctive greasy or wet appearance to the infected part of the leaf. This is essentially a moderated manifestation of the symptoms associated with wet rots caused by certain species of *Phytophthora* (Keen & Yoshikawa, 1983) and probably resulting from a similar biochemical interaction. Biotrophic obligate parasitism certainly requires a degree of specialization and a constraint to variation: there must be elements of genome protection or conservation in both partners.

Haustoria

Another most important step would have been the development from mixed intra- and inter-cellular hyphae to mycelia solely of intercellular hyphae and haustoria (Fraymouth, 1956; Peyton & Bowen, 1963; Berlin & Bowen, 1964; Davison, 1968; Coffey, 1975). Parallel evolution of intercellular hyphae and haustoria (biotrophic parasitism) is manifest by the occurrence of these features in both the DMs and the phylogenetically unrelated *Uredinales* (*Puccinia*). Spencer-Phillips (Clark & Spencer-Phillips, 1993; Spencer-Phillips, 1997) has shown that the intercellular hyphae of the DMs retain the capacity for assimilation in the presence of haustoria. Differences could exist between the functions of haustoria in the nutrition of unrelated taxa. Thus, there is no reason to consider that this biotrophic development, even within the DMs, represents a monophyletic line. Indeed, the fact that the ***morphology*** of the haustoria is different in *Albugo* (small and spherical), *Peronospora* (frequently large and lobed), and *Sclerospora* (peg-like) could point to independent origins, each possibly with a characteristic physiology. Blackwell (1953) described haustoria from *Phytophthora infestans* and noted that about 15 other species formed haustoria although other species of the genus apparently lacked such a development.

Parasitism by the obligate, host-range specific, downy mildews

From the coevolutionary viewpoint, there are distinctions to be drawn between obligate parasitism, species-specific parasitism, and special-form relationships. A discussion on infra-specific differences could, in time, illuminate the processes of speciation compared with population diversity, but the data are too fragmentary at present. Whereas obligate parasitism merely requires the presence of a regular (but possibly periodic) and renewable (but possibly highly transient) nutrient availability from living protoplasm, species-specific parasitism implies a much more restricted range for potential complementary metabolisms. The concept of a 'tolerance range', probably much smaller *in planta* than *in vivo* and thus analogous to the ecological ranges of saprotrophs *in situ* in soils (Dick, 1992), might provide a better model than a search for a package of absolute metabolic requirements.

Potentially interacting organisms must be able to come into contact, and there must be sufficient compatibility for nutritional requirements to be satisfied. Frequently, this will be because new hosts are phylogenetically close to former hosts. Host populations at the frontiers of their realizable niches are more liable to become involved in new coevolutionary initiatives, but the development of a stable relationship will depend on the generation cycles of the parasite and its capacity for genetic change. The critical factors for the nutritional environment of the parasite, the pathways, or the specific metabolites produced, may occur in organisms of differing phylogeny; or, they may only become evident in certain populations because of environmental circumstances. Two facets interconnect: the coevolutionary reliance by the parasite on a host species, and the restrictive nature of this reliance to particular metabolitic pathways. The critical factors involved may require subtle definition. Obvious basic carbon and nitrogen sources are unlikely to be crucial, but sulphur and combined forms of carbon and nitrogen may be so for the DMs.

There must be physical or chemical similarities or analogues that enable an appropriate degree of association between previously separated populations. Too great a vulnerability will lead to an unstable and ephemeral (necrotrophic) relationship. The essence of coevolution is adaptive change in balanced relationships. It is possible that chance associations may lead to new relationships, as has been proposed by Baum & Savile (1985) for certain rusts. This may be more possible for parasites that produce a limited mycelium and for which physical rather than chemical environmental factors are more important. Chance associations leading to coevolution must be less likely for fungi that are essentially systemic, because there would be less likelihood that either host or parasite would survive long enough to reach reproductive maturity.

An obligate parasite that cannot be grown apart from its living host either requires particular metabolites that have not yet been identified, or the organism is intolerant of arbitrary levels of fluctuations in the concentrations and rates of supply of nutrients, or some other *in planta* factor is necessary. There are no suggestions that nutritional requirements are invariably linked to host range restriction in the DMs. The efficiency of waste removal may be a contributory factor. There is little evidence to support or refute any

of these contentions. Moreover, extrapolations made from studies of related fungi that can be grown axenically could be misleading.

If parasite dependence is not based on a demand for particular chemical units, the dependence must have a different origin. I have suggested (Dick, 1988) that this could be based upon an 'empathy' between certain crucial metabolic pathways of host and parasite, so that the catabolism and anabolism were in harmony. Different host pathways may be pre-eminent for different parasites, whether these are taxonomically related or not. Thus, individuals of a single host species may be infected by several parasites. The most notable example for DMs is the suggested synergism between *Peronospora* and *Albugo* in the *Brassicaceae* (Sansome & Sansome, 1974). However, my hypothesis of critical pathway differences would not only explain the occurrence of simultaneous parasitism of a host by different, but systematically related biotrophic obligate parasites: it would also allow for the possibility that these parasites may have different degrees of host specificity. Biphasic culture has been achieved for several genera and species (Ingram, 1980; Lucas *et al.*, 1991, Lucas, Hayter & Crute, 1995), but none is yet in axenic culture.

Whatever the biochemistry underlying attraction to a particular host, and stimulation to germination and colonization by the parasite, there are well-documented examples of parasite-mediated modification of host physiology after establishment. Green ear hyperplasia of pearl millet caused by *Sclerophthora* (Williams, 1984), hypoplasia of sunflower by *Plasmopara* (Sackston, 1981), and the well-known hypertrophy of crucifer stems by *Albugo* are three of the clearest examples relating to growth substance induction. The precise mechanisms of the biochemical modifications have not been researched.

Symptomless occurrence of *Peronosporales* and *Pythiales* in angiosperms suggests that the evolution of parasitism has achieved the ultimate balance in some associations. Haustoria are not essential. *Pachymetra* in *Imperata cylindrica* var. *major* in Queensland (R. C. Magarey, pers. comm.), *Phytophthora* in roots of raspberry and strawberry in Scotland (J. M. Duncan, pers. comm.), and *Pythium* in grass and herbaceous roots are all good examples of such symptomless associations. Symptomless association does not imply a 'no yield loss' situation.

The boundaries between obligate parasitism, species-specific parasitism, and special-form relationships are unclear: more research and discussion (cf. Skalický, 1964; Skidmore & Ingram, 1985) should elucidate the processes of speciation as opposed to different levels of infraspecific (population) diversity. Species-specific parasitism implies a much more restricted range for potential complementary metabolisms. This can be viewed as a ***tolerance range*** rather than a package of absolute metabolic requirements. The breadth of this tolerance range may well be extremely narrow *in planta*, in much the same way that saprotrophic *Pythium* species may co-exist in soil, but have very different patterns of relative frequency of occurrence *in situ* than might be predicted from growth studies *in vitro* (Dick, 1992). The endpoint of this progression is the race concept of the special form for which biochemical compatibility is presumed to be the only apparent distinguishing feature. This may be merely the result of extremely narrow tolerance ranges for a number of factors. But it may be, as with race induction in response to resistance cultivar production, a gene-for-gene evolution that may function through a variety of biochemical, physiological or morphological requirements. An hypothesis for absolute metabolite requirement in the absence of strong selective pressure might require an improbably large number of genetic lesions to explain race-specific parasitism (*formae-speciales*) between related parasites and related hosts. From the systematic viewpoint, the above environmental/host distinctions of the parasite rest uneasily with the infra-specific categories of variety and form, together with *formae speciales* which are not governed by the rules of the *International Code of Botanical Nomenclature* (*ICBN*).

Discussions of single-gene host resistance in different systems of host resistance and pathogen virulence (e.g., Keen & Yoshikawa, 1983) ignore the attraction and stimulation that enables both species to coexist. It is unlikely that studies concentrating on intraspecific differences will reveal underlying coevolutionary factors. There is a long-standing inverse relationship between the outlook and research momentum for plant pathology and the quest by mycologists for an understanding of species-specific coevolution.

It should be noted here that the straminipilous fungi have unique biochemical requirements and metabolic products, many of which are under-rated and some of which will be of significance to the establishment of parasitic relationships. Phylogeny inferred from assimilative morphology is not acceptable, although subtle differences in morphogenesis might be invoked.

Conidiosporangia, chlamydospores, hyphal bodies and gemmae

When the coenocytic asexual reproductive initial has a distinct shape and size, but shows no further differentiation or development after septation and is disseminated by disarticulation, it is functionally a conidium. However, it may germinate directly by a germ tube or retain the capability of germinating as a zoosporangium; this propagule would then most correctly be termed a multinucleate **conidiosporangium** (Snell & Dick, 1957). In some *Peronosporomycetes* asexual propagules lack a clearly defined shape or size and are released from the mycelium by decay or autolysis of the hyphal system; such structures are termed **hyphal bodies** (*Pythium*) or **gemmae** (*Saprolegniaceae*). If such structures also develop a thick wall they may be termed *chlamydospores*, but this development is infrequent in the Peronosporomycetes.

Development from the thallus: conidiosporangiophores and sporangial regeneration

The patterns of growth and regeneration of the hyphae bearing sporangia are varied. When the axis is terminated by a sporangium the development is termed **determinate**, but when the sporangia are lateral or developed in intercalary positions development is termed **indeterminate**. Hyphal regrowth may take place through the sporangial septum (**internal renewal** in *Phytophthora*, *Saprolegnia*) or by a lateral branch (**cymose renewal** in *Achlya*). Sporangia may be produced in sequence on the same determinate axis (**basipetal development** in *Scoliolegnia*) or by limited internal renewal so that the successive sporangial septa are formed at approximately the same point on the axis (**percurrent development** in *Albugo*). The conidiosporangiophore may be differentiated from the assimilative hyphae to a greater (*Peronosporales*) or lesser (some *Phytophthora* species) extent; it may be swollen (*Basidiophora*, *Sclerospora*) with dichotomous (*Peronospora*), pseudodichotomous (*Sclerospora*) or more irregular branching (*Plasmopara*). In the *Peronosporales* the conidiosporangiophore is persistent and can be observed on herbarium material of the hosts, but in the *Sclerosporales* it is evanescent. The junction of the conidiosporangium on the conidiosporangiophore is pedicellate and plugged in the *Peronosporales* but delimited by a septum in the *Sclerosporales*. The evolution of sporangial form is also open to various interpretations which may depend on the evolution of thallus form. In *Pythium*, for example, there is a complete continuum from undifferentiated (and often intercalary) hyphal segments through coralloid branched sporangia, toruloid sporangia, sessile lateral inflated sporangia to spherical sporangia (Dick 1990*b*).

Mitochondrial morphology

Most mycology texts emphasise the difference between the tubular mitochondrial cristae of the biflagellate fungi and the plate-like cristae of the *Eumycota*. Cavalier-Smith (1981) distinguished beween plate-like cristae (true fungi); tubular cristae (chromistans); discoid cristae (euglenoids), and tubular - vesiculate cristae (protozoa) (Figure I: 20). However, this broad categorization masks minor but possibly significant differences between fungi, as noted by Dick & Abro (1990). Mitochondrial profiles can be observed in either the vegetative thallus or the planont. There has been no search for evidence of any difference in construction of the mitochondrion related to the phase of the life-history, even when the mitochondria occupy distinctive positions, as in chytrid zoospores (Lange & Olson, 1979) or in those cases (e.g., *Rozella*) where the mitochondria are uniquely positioned in relation to other organelles (Held, 1975).

In the *Peronosporomycetes* the profile of the mitochondrial crista is as a tube of more or less uniform diameter, and the cristae appear in TEMs in longitudinal sections with connection to the inner mitochondrial membrane and as transverse circular profiles (Figure I: 19).

Confusion has arisen because of the terminology used at different times. Mitochondrial cristae in the plasmodiophorids have been described as "with relatively few tubular cristae" (Williams & Yukawa, 1967) and "tubular" (Sleigh, 1989). They correspond to the vesicular category of Cavalier-Smith (1981) and a more accurate description would be: flattened finger-like lobes with ellipsoidal profiles in transverse section (e.g., *Polymyxa*, Barr & Allen, 1982: fig. 4, lowermost mitochondrion). Although by no means clear from the published TEMs, the mitochondria of *Gonimochaete* (Saikawa & Anazawa, 1985: figs 2, 7, 14) and *Haptoglossa* (Lee *et al.*, 1992) appear to have tubular/vesicular cristae; TEMs of *Haptoglossa* (Glockling, 1994) have similarly indefinite profiles, in contrast to those she obtained for *Myzocytiopsis*. Some micrographs of mitochondrial profiles of *Catenaria anguillulae* also show tubular/inflated finger-like cristae (Ichida & Fuller, 1968: figs 6, 8). Reference might also be made to the problematic protoctist *Nephromyces*, which is reported as having tubular mitochondrial cristae and chitin synthesis (Saffo, 1981; Saffo & Nelson, 1983; Saffo & Fultz, 1985).

Variations on the vesicular category of mitochondrial cristae can be seen in illustrations (e.g., *Rozella*, Powell, 1984), where the inflated lobes have a constricted attachment to the inner membrane, but such variations tend to be ignored in the two-category classification of tubular or plate-like cristal arrangement.

Mitochondrial profiles for many of the critical species of lagenidiaceous fungi are not available. However, Gotelli (1974*b*), Molina (1986) and Foissner (1987) have reported tubular cristae for mitochondria of *Salilagenidium callinectes*, *Petersenia* and *Ciliatomyces* respectively; Glockling (1994) obtained TEMs of mitochondria of *Myzocytiopsis* and *Chlamydomyzium* which have clearly defined tubular cristae.

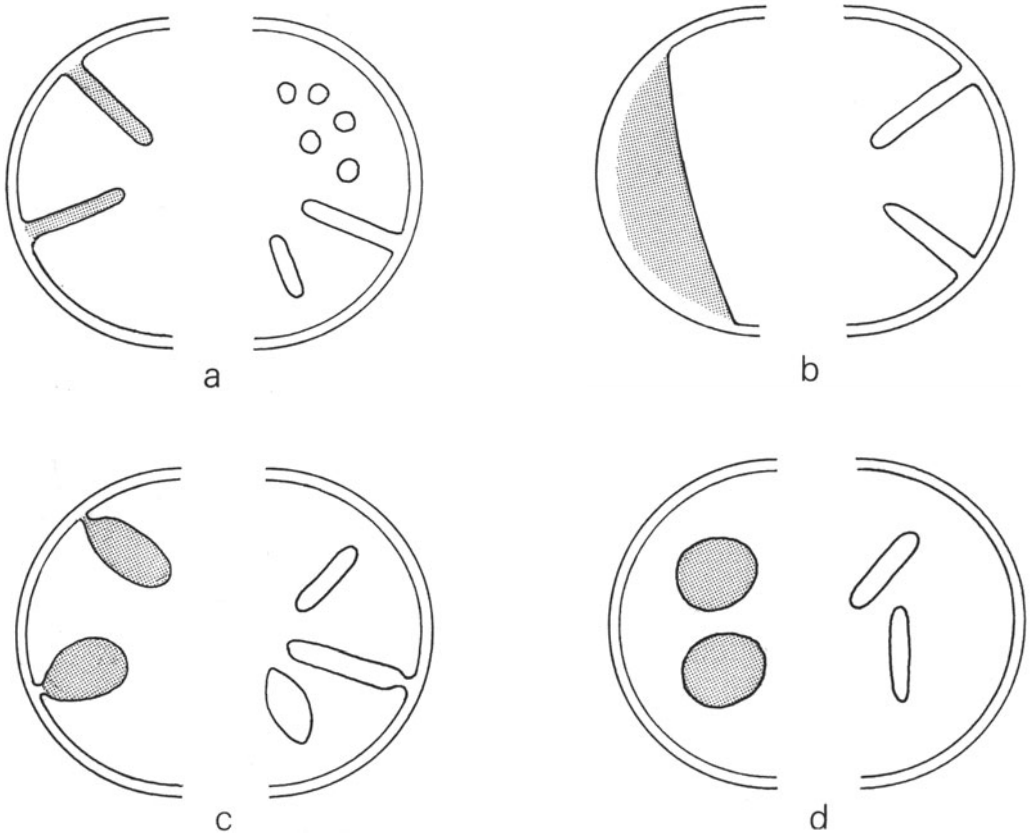


FIGURE I: 20. *Mitochondria: lateral diagrams (left) and observable ranges of transverse, longitudinal and oblique profiles (right) of cristae.*

a: tubular cristae; b: plate-like or shelf-like cristae; c: vesiculate or flattened, finger-like cristae; d: discoid cristae.

BIOCHEMISTRY

It is inevitable that the fungi used for biochemical investigations are those which are most readily culturable. Hence, the potentially powerful biochemical criteria are regrettably diminished to a role of providing confirmation of otherwise fairly obvious taxonomic affinities. Technical difficulties still need to be surmounted before these criteria can be employed where they are most needed, in the endobiotic obligate parasites.

The following sections relate only to those aspects of the biochemistry of the straminipilous fungi which have been accorded systematic significance. Different approaches have been used for these sections because of the different levels of phylogenetic significance and the extents of the research activity.

Cell walls

The physical and chemical composition of the walls of culturable peronosporomycete fungi has been extensively studied, with early work reviewed by Aronson (1965) and Bartnicki-Garcia (1966). The physical differences between peronosporomycete and chromophyte algal walls were highlighted by Parker, Preston & Fogg (1963) and these differences are probably only partly due to the different physiological requirements of heterotrophs and autotrophs. The amount of fibrillar material in the cell wall is much less in the fungi, and the cellulose (ß-1,4-glucan) tends to be masked by the much larger amounts of ß-1,3- and ß-1,6-glucans (reviewed by Cooper & Aronson, 1967, in relation to *Pythium*). Comparative studies (Novaes-Ledieu, Jiménez-Martínez & Villanueva, 1967; Sietsma, Eveleigh & Haskins, 1969; Dietrich, 1973, 1975; Vaziri-Tehrani & Dick, 1980*c*) indicate that there are differences in carbohydrate composition between species, at least in their characteristics after extraction.

The cellulosic nature of peronosporomycete cell walls has long been recognized and the use of chlor-zinc iodide histochemistry was in routine use for these fungi a century ago. This test is not always reliable; it sometimes gives different colour reactions which are difficult to interpret (see Couch, 1935*a*: *Pythium*: blue; *Olpidiopsis*: purplish; *Pythiella*: no reaction). Even for *Pythium ultimum*, a species which would be expected to have various glucans including cellulose, the stain reaction may be negative (Trow, 1901). The standard formulation of the cellulose stain (Zimmerman, 1901) was modified by Post & Laudermilk (1942) who also categorized some of the different colour reactions obtained when using this stain for cellulose from various plants. Until the techniques of gold-complexed lectins in association with glycosidic enzymes (Benhamou *et al.*, 1987) are shown to be applicable to foreign fungal walls within walled ***fungal*** protoplasts, the unsatisfactory chlor-zinc iodide test remains the only way of indicating the chemistry of the walls of endobiotic parasites.

Hegnauer & Hohl (1978) have distinguished two basic types of wall layer in the peronosporomycetes: the A layer, which is amorphous as perceived by TEM, and the FA layer, which is fibrillar with an amorphous component. The relative thicknesses and numbers of these alternating layers varies according to species and to wall origin, reaching an extreme development in the oogonial spines of *Pachymetra* (Dick *et al.*, 1989). The diversity of peronosporomycete zoospore cyst walls is well-known (Beakes, 1987; Dick, 1990*a*) but the chemistry of the ornamentations has not been established. Possibly similar alternating electron-opaque/electron lucent layers occur in the cyst spines of *Haliphthoros* (Overton *et al.*, 1983).

In the *Labyrinthista* the wall is composed of one or many layers of imbricated, dictyosome-derived, circular scales (Jones & Alderman, 1971; Perkins, 1973*a*, *b*; Darley, Porter & Fuller, 1973; Bahnweg & Jäckle,

1986; Moss, 1986). These walls contain 30-43% protein and variable amounts of carbohydrate, with the rare isomer L-galactose as the predominant component of the carbohydrate polymer, except for *Aplanochytrium*, in which fucose predominates (Bahnweg & Jäckle, 1986). The presence of sulphated polysaccharides, similar to those found in algae, has been demonstrated in *Thraustochytrium* (Chamberlain, 1980).

The unpolymerized precursor of chitin, *N*-acetyl-D-glucosamine occurs, and the presence of chitin has been inferred for saprolegniaceous fungi because of the effect of polyoxin D on hyphal diameters (Dietrich & Campos, 1978) and the occurrence of chitin has recently been confirmed by Bulone *et al.* (1992). Asiegbu, Lönneborg & Johansson (1996) have demonstrated the occurrence of relatively insignificant amounts of chitin in the middle layer of the hyphal wall of *Pythium*, masked by larger amounts of *N*-acetyl galactosamine, based on the use of fluorescine isothiocyanate and gold-labelled lectins. Chitin is known as a wall component for genera of the hyphochytrids (Nabel, 1939; Fuller, 1960; Fuller & Barshad, 1960; Clay *et al.*, 1991: *Hyphochytrium* and *Rhizidiomyces*) and *Leptomitaceae* (Lee & Aronson, 1975; Aronson & Lin, 1978; Huizar & Aronson, 1985, 1986; Bertke & Aronson, 1980: *Leptomitus, Apodachlya* and *Plerogone**). In the *Rhipidiaceae* glucosamine is absent (Bertke & Aronson, 1985: *Araiospora, Mindeniella*) or present only as a trace after digestion (Pau & Aronson, 1970: *Sapromyces*).

Clay *et al.* (1991) have demonstrated that *Hyphochytrium* and *Rhizidiomyces* contain both cellulose and chitin in their inner cell walls, and have shown that there are differences between these genera in the relative abundance (or ease with which they can be labelled) of these two wall components. The distribution of cellulose and chitin may differ in different walls (discharge tubes and septa) of the same organism. These authors stress that differences in wall composition may be of significance at low levels in the taxonomic hierarchy: such differences would thus have correspondingly less value at ordinal or class levels.

The walls of *Blastulidium* were merely noted as being "of polysaccharide" (Manier, 1976).

Proteins may be a large component of the dry weight of cell walls, being particularly high in the labyrinthulids (Darley *et al.*, 1973). Vaziri-Tehrani & Dick (1980*b*) reviewed the data then available for peronosporomycetes and other fungi, and produced a novel polygraphic display of relative amino acid ratios, which distinguished between the *Saprolegniomycetidae* (with *Sapromyces* of the *Rhipidiomycetidae*) and the *Peronosporomycetidae* (with *Atkinsiella dubia* (D. Atkins) Vishniac). One particular hydroxyproline-rich protein (HRP) is ubiquitous in plant walls and it has been suggested that HRP is involved in the control of growth (Lamport, 1970). Novaes-Ledieu *et al.* (1967) and Vaziri-Tehrani & Dick (1980*c*) have indicated that levels of HRP are greater in the *Pythiaceae* than in the *Saprolegniaceae*. Aronson & Fuller (1969) noted that the wall of *Atkinsiella dubia* was also rich in hydroxyproline. Takenaka & Kawasaki (1994) have used alanine-rich, hydroxyproline containing cell wall proteins for serological identification of *Pythium* species.

On the basis of negative results from histochemical tests using chlor-zinc iodide (Wisselingh, 1898; Maire & Tison, 1911*a*; Pendergrass, 1950; Goldie-Smith, 1951, 1954, 1956), Waterhouse (1973) claimed that plasmodiophorid walls were chitinous. Work on plasmodiophorid walls (Moxham & Buczacki, 1983; Moxham, Fraser & Buczacki, 1983; Buckzacki & Moxham, 1983) has shown that the spore-cyst wall is composed of four layers, the inner two containing chitin, which accounted for 25% of the dry weight.

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*FOOTNOTE: As *Apodachlyella completa* (Humphrey) Indoh: the 1962 U.K. culture from M. W. Dick which was widely distributed in the U.S.A. by J. M. Aronson. This isolate was renamed by Dick (1986) on the rediscovery of Humphrey's fungus in the U.K. Cultures from American provenances of both *Apodachlyella* and *Plerogone* are now available (Longcore, Brooks & Homola, 1987).

Protein content of the entire wall was high, accounting for 33% of the dry weight. The outermost layer, and the next layer, which generated the spines, contained fibrillar protein. It is apparent that the range of variation within and between taxa is too high for generalizations to be established from the small number of species so far studied. See also Yano *et al.* (1994) for more recent data. Species comparisons using wall samples of different life-history origins need to be treated with caution.

Lysine synthesis

The two pathways for lysine synthesis are completely distinct, appear to be mutually exclusive and have major phylogenetic importance (Vogel, 1960, 1961, 1964). LéJohn (1972, 1974) has proposed that the DAP (α,ϵ-diaminopimelic acid) lysine synthesis pathway antedated the AAA (α-aminoadipic acid) lysine synthesis pathway, which correlates with chitinous cell walls, because the DAP pathway interferes with chitin biosynthesis. The control mechanisms of chitin biosynthesis and glutamate/glutamine biosynthesis appear to be connected, and the few organisms (notably the hyphochytrids) which possess both chitin and glucan polymers in their walls, lack a specific control of uridylates and UDP-amino sugar derivatives, which activate glutamic dehydrogenase. This would suggest potentially inefficient wall construction in the hyphochytrids. *Leptomitales* and *Saprolegniales* have yet to be tested for all these parameters. Rothschild & Heywood (1987) note another correlation, which might support LéJohn, namely that while AAA lysine synthesis is correlated with the presence of mitochondria with flat, plate-like cristae, DAP lysine synthesis may be associated with a range of mitochondrial types. The suggestion (Vaziri-Tehrani & Dick, 1980*a*) that high lysine:valine ratios, accompanied by generally lower but variable proline:valine ratios, may correlate with the DAP lysine synthesis pathway has been extrapolated by Buczacki (1983) to postulate that the plasmodiophorids have the AAA lysine synthesis pathway. However, the data of Vaziri-Tehrani & Dick relate to hyphal walls primarily of glucan polymers while the plasmodiophorid data were obtained from cyst walls, which are mostly protein (see above). There are no data to resolve these discrepancies, and there is no information for lagenidiaceous fungi with respect to combined lysine synthesis, wall composition and glutamine dehydrogenase characteristics. Paton & Jennings (1989) were unable to establish the presence of either pathway in *Thraustochytrium*. In the wider context it should be noted that euglenoids have the AAA pathway and mitochondria with discoid cristae, and the choanomastigotes have mitochondria with tubular cristae and produce investments of cellulose or chitin.

Lysine synthetic pathways cannot yet be used to resolve the classification of the lagenidiaceous fungi or the plasmodiophorids in the kingdoms *Straminipila* or *Protoctista*.

Sterol metabolism

Sterols fulfill two functions: as bulk sterols in membrane architecture where they act as stabilizing compounds, and as 'trigger' sterols for particular cellular activities (Leshem, 1992: 59). Bulk sterols will not be discussed here. In the *Peronosporomycetes* it was the 'trigger' function that was first given prominence by the study of the sterol requirements for sexual reproduction in *Pythium* (Haskins, Tulloch & Micetich, 1964). The same year Elliott *et al.* (1964), Harnish, Berg & Lilly (1964), Hendrix (1964) and Leal, Friend & Holliday (1964), and later, Elliott, Hendrie & Knights (1966) defined a steroid growth requirement for *Phytophthora*. Work on sterols was extended by Hendrix (1970, 1975), and Elliott & Sansome (1977) reviewed the role of sterol biosynthesis for meiosis. The interaction between sterol metabolism and progressive changes in phospholipases during the life-history of *Lagenidium giganteum* are under investigation (MacKichan, Tuininga & Kerwin, 1994).

Bulk sterols in membranes differ in the nature of the aliphatic side chain and in what groups are attached to the steroid skeleton, with sitosterol, campesterol and stigmasterol being commonly found in plants and cholesterol in animals, though cholesterol also occurs in plants. In a study which may be of relevance to sterol diversity and metabolism, Ellenbogen *et al.* (1969) pointed out that thraustochytrids, euglenoids and *Ochromonas* have retained a range of biochemical pathways for the biosynthesis of polyunsaturated fatty acids. These authors also pointed out the possible phylogenetic significance of particular polyunsaturated fatty acids, and since then the fatty acid 22:5w6 has been proposed as a signature compound for the thraustochytrids (Findlay *et al.*, 1986).

Detailed studies of sterol metabolism are confined to organisms which can be maintained in axenic culture. Nevertheless, information of taxonomic value is provided with respect to cycloartenol and lanosterol. Nes (1990, and references therein) has shown that lanosterol is formed from squalene oxide cyclization ***via*** cycloartenol in photosynthetic lineages, but ***directly*** in non-photosynthetic lineages. The *Peronosporomycetidae*, *Saprolegniomycetidae* and the hyphochytrids fall into the latter category, but differ in that fucosterol has not been detected in the hyphochytrids (Weete *et al.*, 1989). Nes *et al.* (1990) regard the different biosynthetic pathways as of a fundamental evolutionary significance equivalent to those for lysine synthesis. Some *Peronosporomycetes*, but not the *Pythiales*, can synthesize sterols from mevalonate. Domnas, Srebro & Hicks (1977) and Kerwin & Washino (1983, 1986*a*, *c*) have demonstrated the need for exogenous sterols for asexual and sexual reproduction by *Lagenidium giganteum*, but have also shown that this species differs, for example, from *Phytophthora* in being unable to use sterols with certain substituents. Dick (1988) suggested that there might be an interaction between the sterol requirements of obligate parasites such as the downy mildews and their host plant metabolisms. This suggestion was an extrapolation of the exogenous sterol requirements of *Phytophthora cactorum* (Lebert & Cohn) Schröt. (Nes, Saunders & Heftmann, 1982) in relation to the production of sitosterol by the host. The possible utilization of a principle based on reciprocity of sterol supply and demand for the understanding fungicide efficacy has been discussed in a perceptive review by Griffith, Davis & Grant (1992).

Warner, Sovocool & Domnas (1983*b*) have compared the sterol utilization by *Lagenidium giganteum* with that of *Salilagenidium callinectes*. Nes *et al.* (1986) and Berg & Patterson (1986) have taken this comparison further, showing that the similarity between *L. giganteum* and *Phytophthora cactorum* can be contrasted with the similarities between *S. callinectes* and *Achlya americana* Humphrey, *Dictyuchus monosporus* Leitg., *Saprolegnia ferax* (Gruith.) Thur. and *Plerogone helodes* M. W. Dick (see p. 88). The inference must be that while *L. giganteum* is closely related to the *Peronosporomycetidae*, *S. callinectes* is more likely to have affiliation with the *Saprolegniomycetidae*.

Some differences in sterol metabolism may prove to be of low evolutionary value, with loss of sterol anabolic pathways occurring at subgeneric levels, as may be inferred from sexual reproductive capacity within *Pythiaceae* (Kerwin & Duddles, 1989) and perhaps heterothallic and homothallic *Achlya* species. Vishniac (1955*b*, *c*, 1957) reported differences in steroid requirements between different isolates of *Labyrinthula*, with *L. minuta* possessing cholesterol. Bahnweg (1980) has provided data for *Haliphthoros* and *Halophytophthora epistomium* (Fell & Master) H. H. Ho & S. C. Jong on the stimulation of growth by exogenous sterols, but this provides little information of evolutionary or systematic significance.

MOLECULAR BIOLOGY

Molecular systematics, evolutionary origins and systematics

Molecular phylogeny is still in its infancy despite considerable research activity. The advantages and disadvantages of Linnaean classifications need to be evaluated, since an alternative system, based on molecular phylogenies, has been proposed which would challenge the nomenclatural hierarchy (Hibbett & Donoghue, 1998). Thus there exists and there will continue to be a tension between Linnaean/ICBN taxonomy and phylogenetic systematics (Brummitt, 1996; de Quiroz & Gauthier, 1994). Nevertheless, molecular phylogeny will provide information about relationships even if these relationships are not resolved into classifications. In addition to problems in translating molecular phylogeny into classifications (see PART II), diagnostic requirements raise several additional considerations summarized as follows.

To what extent should a clade node correspond to a 'classical' hierarchical level? Diversity within an ancient lineage may coexist with a more recently evolved, but fundamental attribute which so changes the evolutionary potential that the erection of a higher taxon is of practical value. Computer-generated similarity indices will reflect probable lineages, but these will not negate intra-subclass diversity in higher taxon concepts. Some higher taxa will encompass several nodes. Because of the progressively bifurcating nature of the cladogram, or lack of resolution for the origins of several lineages, phylogenetic approaches are not always best suited for establishing correlations (i.e., discontinuities) with currently recognized hierarchies in systematics. It is not always possible to distinguish between derived (apomorphous) and ancestral (plesiomorphous) character states. At ultimate branches of phylogenetic trees single cladistic characters may be insufficiently diagnostic, so that a 'suite' of characters is necessary for separation at species (and sometimes genus) level (see Donoghue, 1985). With finger-printing techniques separation proceeds through infraspecific taxa all the way to populations, clones and individuals (Lévesque *et al.*, 1994; Liew *et al.*, 1998; Panabières *et al.*, 1989).

The type concept is fundamental to systematics. Genera are defined by historically determined **type species**, irrespective of whether the type species is uncharacteristic of the taxa presently included in the genus. The type species is based upon a type, which again may deviate from the central tendency of the population from which it came. Although the type material may no longer be extant, or if extant no longer suitable for molecular analysis, it remains essential for the type species to be characterized before systematic changes can be justified. When the type material is not available, more recent isolates of the fungi (determined on morphological criteria) have to be used. These precepts are most pertinent to the systematics of the straminipilous fungi. The type species of *Plasmopara* (*Peronosporales*), *Phytophthora sensu lato* and *Pythium sensu lato* (both *Pythiales*) occupy extreme positions in the genera they characterize. Type species, characterized by their 18S rDNA data, which could be employed as outgroups now include: *Saprolegnia ferax*, *Leptolegnia caudata* and *Apodachlya brachynema* (*Saprolegniomycetidae*) and *Pythium monospermum* (*Peronosporomycetidae*) (Dick *et al.*, 1999).

There is no possibility of obtaining information from extinct taxa to qualify probabilities. In any systematic and phylogenetic (evolutionary) molecular reconstruction it is essential to recall that only relationships between ***extant*** species will be displayed. Taxa of extinct lines, which might have modified the cladograms, cannot be assessed.

The basis for phylogenetic placement and relationships within the straminipiles depends, very largely at present, on long sequences of nucleotides in the gene encoding for ribosomal RNA. It is possible that one part of one gene is sufficient to establish a robust cladistic framework, but justification and support is

normally required (see Doyle, 1992). In angiosperm phylogeny ***three*** independent genes are being used (Soltis, Soltis, Chase, *et al.*, 1998*a, b*; Soltis, Soltis & Chase, 1999; The Angiosperm Phylogeny Group (APG), 1998). For entirely understandable reasons, the independent, endosymbiont genes most studied in straminipiles are ***either*** in the photoendobiont ***or*** in the mitochondrial endosymbiont (heterotrophs), so that comparability is lacking across the whole kingdom. Other genes have not yet been studied in sufficiently large samples of straminipilous fungi or other straminipiles to enable construction of a robust phylogenetic hypothesis, similar to that for angiosperms.

Another question relates to the number of informative, variable sites within the sequences that are necessary to give adequate characterization and separation within a particular group of related taxa: the region for data analysis must contain sufficient differences in sequences to allow closely related species to be separated; these differences should be the result of a single base change and be free of length mutations. Berbee *et al.* (1998) have shown, with ascomycetes, that while shorter sequences are sometimes adequate, there are some taxa for which much longer sequences are essential. It will be necessary to characterize the straminipiles in this respect.

Evolution is on-going. Species concepts (both real and postulated) vary widely, even in a single genus. Incipient speciation (sibling species) will occur. Isolation and/or modification of the gene pool may not initially be correlated with or represented by morphological attributes. Molecular biologists concerned with identification markers have not yet addressed the problems posed by ***recent*** speciation caused either by ploidy change (e.g., from diploid to tetraploid) or by change in genes other than the rDNA gene, which so affect the physiology of the organism that a new, but as yet cryptic species (i.e., a species lacking any ***recognized*** morphological and diagnosable discontinuity) has evolved. In neither of these scenarios would differences in any part (18S- 28S- or ITS) of the rDNA necessarily show change. The molecular biology must not be given disparate weight with respect to morphology and physiology. Intra-specific population diversity and formally defined infra-specific taxa (subspecies, varieties and forms) require reassessment; genetically controlled host/parasite associations which are characterized ***only*** by their hosts, are known as *formae speciales*. Similarity, even identity, in nucleotide sequences with respect to one gene may be yoked to variation in another gene which codes for such host-specific functional differences.

There is no absolute time-scale for rates of molecular evolution, but eventually the molecular phylogeny should be integrated with geological time. The molecular clock will be influenced by rates of base substitutions, life-histories and sexual systems. Van der Peer *et al.* (1996) have provided a more accurate formula for calculating 'substitution rate calibration' to avoid anomalies caused by the presence of extremely long branch sequences with high evolutionary rates. If a molecular clock cannot be determined, the apparent evolutionary distance, as represented by nucleotide sequence changes, will not necessarily be the same as the absolute evolutionary time-scale for all organisms (the 'sloppy' clock hypothesis of Doyle, 1992). For the straminipiles generation times and population sizes will have a profound effect on the absolute time scale of change. The diatoms and other marine straminipilous unicells have enormous populations, short generation times and sexual reproduction is rare. *Hyphochytrium* (and all described members of the *Hyphochytriales*) and *Halophytophthora* are apparently constitutionally anamorphic; almost nothing is known of chromosomal or genetic stability in these two genera. In *Aphanomyces* there may be many asexual generations between sexual events (Dick, 1970). Furthermore, the rates of evolution of mitochondrial genes and nuclear genes may differ by a factor of 10 in other organisms. For a well founded phylogeny of straminipiles, the factorial differences between the genes selected should be clarified.

The stability of the cladistic arrangement has yet to be established. The placement of some ordinal branches within the *Peronosporomycetes*, such as the *Leptomitales* (Dick, *et al.*, 1999; Riethmüller, Weiss & Oberwinkler, 1999; Hudspeth, Nadler & Hudspeth, 2000; Cook, Hudspeth & Hudspeth, 2000), is still equivocal, even after analysis of long (>1800) nucleotide sequences from 18S rDNA. A comparable

situation holds for the photosynthetic straminipiles (Potter *et al.*, 1997). Association depends on the algorithm used. Additional, independent data are needed. Positioning of so few, deeply rooted taxa in cladograms can also be influenced by the size of the data base and the outgroups used. Divergent orders with very few known species, such as the *Leptomitales*, present problems when interpreting cladograms. It must also be recognized that the addition of new information may affect the branching of the cladogram. In all cases it is desirable to rationalize cladogram differences with structural features (the 'common sense' factor).

Diagnostic criteria based on molecular biology will become more firmly based when techniques have been developed and used for the extraction and sequencing of nucleic acids from unicellular endobiotic straminipilous parasites in straminipilous hosts. At present, three kinds of approach have been employed for readily cultured straminipilous fungi: the study of nuclear genomic DNA, initially as nucleotide ratios but now largely concerning the rDNA gene and its substructure; and mitochondrial DNA.

Nucleotide ratios

The first report of a DNA G:C ratio for a peronosporomycete was that for *Phytophthora infestans* (Clark *et al.*, 1968). Storck & Alexopoulos (1970) provided comparative data for other saprotrophic and facultatively parasitic species. Green & Dick (1972) gave a more detailed analysis for the DNA of other *Saprolegniaceae* and reported the presence of 'satellite' DNA, which was later shown to be mitochondrial DNA (Clark-Walker & Gleason, 1973; Neish & Green, 1976). Bahnweg & Jäckle (1986) have shown a wide range of G:C ratios for thraustochytrids. In CsCl-bisbenzimide gradients the rDNA repeating units produce a third band which facilitates separation (Belkhiri & Dick, 1988), and Martin (1990) has reported the presence, in some species, of a fourth band which he attributed to fragments derived from junctions between genomic DNA and rDNA. Belkhiri & Dick (1988) provided a basis for comparison between species of *Pythium* based on these gradients.

The rDNA gene

The rDNA gene has several regions which have been used for comparative sequencing: the small subunit (18S); the large subunit (28S, including the 5·8 segment); and the 5S and ITS1 and ITS2 regions. Most deep phylogeny still depends on the sequences of the small subunit (18S) of the rDNA gene. No other sequences can compare, in the numbers and diversity of organisms assessed, with 18S rDNA at this stage. Phylogenies based on 18S rDNA are well-established for straminipilous organisms and largely confirm prior taxonomic conclusions from kingdoms down to orders: relationships between families and genera are more open to debate.

Phylogenetic relationships based on the 18S rRNA analyses of a few members of the *Straminipila* have been made by Gunderson *et al.* (1987: *Achlya* and chrysophyte algae), Tan & Druehl (1993: *Fucophyceae*), Leipe *et al.* (1994: *Cafeteria* and *Labyrinthuloides*), Leipe *et al.* (1996: *Developayella*, *Labyrinthuloides* and *Proteromonas*) and Potter *et al.*, (1997: chromophytes). These data have been augmented by Förster *et al.* (1990: *Blastocladia, Phytophthora* and *Lagenidium*) and quoted in relation to other fungi by Illingworth *et al.* (1991) and Geber *et al.* (1992). Until recently the data for *Achlya*, *Phytophthora* and *Lagenidium* were the only 18S rDNA data available for deep phylogenetic analysis: note that the phylogenetic trees published by Illingworth *et al.* (1991) and Geber *et al.* (1992) showed different relative evolutionary distances for the same *Lagenidium* and *Phytophthora* data, perhaps because of the different algorithms used. A restriction map of the rDNA of *Lagenidium giganteum* shows that this species shares more sites with *Pythium* than it does with species of the *Saprolegniaceae* or *Leptomitaceae* (McNabb, 1989). Unpublished data (Lee &

Fuller, 1992) apparently provided information on the genetic relatedness of representatives of other major taxonomic groups of straminipiles, including *Salilagenidium*. The genetic distance between *Lagenidium* and *Phytophthora* is shorter than that between either and *Achlya*; the three *Peronosporomycetes* are more closely related to chrysophyte algae and diatoms than to other organisms. The phylogenetic value of genetic distance deduced by such analyses is not in dispute, but as Förster *et al.* (1990) have commented, the assessment of relatedness at particular genetic distances may depend on the appropriateness of the relative conservation (the numbers of informative sites) and not just the length of the subunit (number of nucleotides) that is selected. Förster *et al.* (1990) regard small sequences such as that for the 5S rRNA as inappropriate for extreme evolutionary distances, thus caution is necessary when interpreting data, for example, for the evolutionary position of the thraustochytrids (compare MacKay & Doolittle, 1982; Huysmans *et al.*, 1983; Hori, Lin & Osawa, 1985; Izzo, Lee & Porter, 1994).

Castlebury & Domier (1998) have presented 18S rDNA sequences for *Plasmodiophora brassicae*. More comparative data, based on about 800 bp-sequences for *Polymyxa* and *Plasmodiophora* has been provided by Ward & Adams (1998). They concluded that the wider relationships of the plasmodiophorids were unclear, but that the closest matches were with the straminipiles. A much larger range of outgroup organisms, a longer set of sequences for analysis and additional independent data will be necessary before a deep phylogeny is apparent.

The large subunit of the rDNA gene is now being used for phylogenetic analysis (Petersen & Rosendahl, 2000; Riethmüller *et al.*, 1999; Van der Auwera, Chapelle & De Wachter, 1994; Van der Auwera & De Wachter, 1997, 1998; Van der Auwera *et al.*, 1995). In general, the data support those from 18S rDNA, but the different ranges of organisms studied make comparisons difficult. The 28S rDNA data are particularly important in placing the hyphochytrids (Van der Auwera *et al.*, 1995). The relationships of the *Rhipidiales* and the *Leptomitales* with the *Saprolegniomycetidae* or *Peronosporomycetidae* are again inconclusive.

Restriction mapping has also revealed variability in the intergenic regions of the rDNA (Klassen, McNabb & Dick, 1987; Klassen & Buchko, 1990; Martin, 1990). Information (mainly restricted to shorter sequences) is known for straminipilous fungi, especially *Phytophthora* and *Pythium* (Briard *et al.*, 1995; Cooke *et al.*, 1996, 1999; Cooke *et al.*, 2000; Herrado & Klemsdal, 1998; Lévesque *et al.*, 1993, 1994, 1998; Moller, de Cock & Prell, 1993). The molecular data support, only in part, the hierarchical classification within the *Peronosporomycetes* (compare Grosjean, 1992 [pers. comm., J. M. Duncan, Scottish Crops Research Institute]; Panabières *et al.*, 1997; Ristaino *et al.*, 1998). The robustness of the cladogram branching order, as supported by Bootstrap and Jackknife procedures, is not always secure (values $<75\%$ should be viewed with caution; for the very much larger multigenic angiosperm database, this value could be set at $<50\%$, pers. comm., M. W. Chase, Royal Botanic Garden, Kew).

Genetic relatedness as assessed by ITS1 sequences may indicate centres of speciation but not necessarily the evolutionary phylogeny. The same may also apply to the position of the 5S rDNA relative to the NTS of the rDNA repeat. Shorter sequences such as pertain to the ITS region are frequently used for identification, but in *Pythium* there are length mutations in this region so that analysis becomes highly dependent on sequence editing. In spite of this complication, the ITS region can be effective in distinguishing between closely related species. Cock *et al.* (1996) have pointed out that heterogeneous base distribution can affect the efficacy of restriction enzymes and this should enable the optimal selection of restriction enzymes for determining similarity in restriction fragment patterns.

One of the problems in attempting to equate molecular identity with morphological criteria has been illustrated for the species pair *Pythium graminicola* and *P. aphanidermatum*. Chen (1990) originally concluded that the two species were conspecific, but later more detailed studies demonstrated their

distinctiveness (Chen, Hoy & Schneider, 1992). Chen (1993) preferred to use antheridial number to distinguish these two species whereas Dick (1990*c*) used oogonial morphometry.

Klassen *et al.* (1997) are now using amplification of the 5kb intergenic spacer (the D2 region) in the rDNA repeat unit to identify *Pythium* species. In a study of 5S rRNA gene family organization it was found that species of *Pythium* with globose sporangia (or sporangia unknown) do not have a 5S gene in the NTS of the rDNA repeat unit, but that species of *Pythium* with filamentous sporangia have an inverted copy of the 5S gene in the NTS (Belkhiri, Buchko & Klassen, 1992). The latter arrangement is also found in *Lagenidium giganteum*, *Pachymetra* and *Verrucalvus*. In *Phytophthora cryptogea* Pethybr. & Laff. and *Achlya klebsiana* Pieters the 5S gene is also present in the NTS, but in the non-inverted orientation. There is thus variation within both subclasses *Peronosporomycetidae* and *Saprolegniomycetidae* with respect to this character, although the relationship between *L. giganteum* and certain species of *Pythium* is probably close. Alignments in other unresolved regions may be similarly problematic elsewhere in the entire rDNA gene. Although the D2 region of the 28S rDNA gene appears to be less suitable for phylogeny (pers. comm., F. N. Martin, U.S.D.A., Salinas, Ca.), Briard *et al.* (1995) have used the variable D2 domain of the 5' end of the 28S rRNA to display relationships (as "phylogeny") between certain species of two genera of the *Pythiaceae*. More caution should be exercised when using limited (i.e., <200) sequences from relatively few species for phylogenetic conclusions.

Infra-generic molecular taxonomy

Förster *et al.* (1988), Förster, Oudemans & Coffey (1990) and Moller *et al.* (1993) have used the genomic and mitochondrial DNA to discuss relatedness within the genus *Phytophthora*. Förster & Coffey (1990) have also used similar data to provide sexual markers in heterothallic crosses of *Phytophthora* and Wu, Mathur & Rimmer (1994) have applied similar techniques for crosses between races of *Albugo*. Numerous studies have reported on the use of molecular biology for understanding the genetics of *Phytophthora infestans*, but this topic is outside the present considerations.

Intergenic repeats have been studied by Klassen & Buchko (1990), who found that *Pythium ultimum* isolates showed variation in a region downstream of the 3' end of the large subunit. Each isolate showed a unique pattern of heterogeneity in the subrepeat. They suggested that the simple sequence repeats (SSRs ≡ microsatellite DNA) may have implications with regard to pathogenicity and virulence. These data are therefore outside the criteria for taxon diagnosis.

The reverse blot technique, Random Amplified Polymorphic DNA (RAPD) technique and other comparable short sequence analyses are now being used to indentify individual species and to separate closely related species. Ultimately, techniques will be applicable to *in vitro* and in field studies (e.g., Wiglesworth *et al.*, 1994). At present, research has mostly been confined to *Phytophthora* (Belkhiri & Dick, 1988; Briard *et al.*, 1995; Cooke *et al.*, 1996; Förster & Coffey, 1991: *Ph. parasitica*; Förster, Learn & Coffey, 1995; Förster *et al.*, 1998; Förster, Oudemans & Coffey, 1990; Liew, Maclean & Irwin, 1998: *Ph. medicaginis*; Möller, Cock & Prell, 1993; Panabières *et al.*, 1989; Shumard-Hudspeth & Hudspeth, 1990) and *Pythium* (Belkhiri & Dick, 1988; Briard *et al.*, 1995; Herraro & Klemsdal, 1998: *P. aphanidermatum*; Klassen, Balcerzak & Cock, 1996; Klassen *et al.*, 1997; Lévesque *et al.*, 1993; Lévesque, Vrain & de Boer, 1994: *P. ultimum*; Martin, 1990, 1991, 2000; Martin & Kistler, 1990; Matsumoto *et al.*, 1999; Matthew, Hawke & Pankhurst, 1995; Rafin, Brygoo & Tirilly, 1995; Shumard-Hudspeth & Hudspeth, 1990). At present there is no consensus as to the most appropriate protocols to be followed. However, the screening for distinctiveness against rarer species has not always been thoroughly explored so that the reliability of the procedure is not yet assured. In any case, the distinctiveness of a species in its natural substratum or habitat will depend on features other that DNA characteristics.

The use of restriction fragment length polymorphism (RFLP) and other techniques for assessing infraspecific variation are being employed routinely for *Phytophthora* and *Pythium* but rarely for other genera of the *Peronosporomycetes*. References to work with *Phytophthora* include: *Ph. capsici*: Hwang *et al.* (1991); *Ph. infestans*: Carter *et al.* (1990); Judelson, Spielman & Shattock (1995); Newhouse *et al.* (1992); Tooley & Therrien (1987); *Ph. megasperma*: Förster *et al.* (1999); *Ph. megasperma* var. *glycinea*: Judelson *et al.* (1993); Mao & Tyler (1991); *Ph. sojae*: Mao & Tyler (1996); *Ph. parasitica*: Förster & Coffey (1990). References to work with other genera include: *Achlya*: Hudspeth *et al.* (1983), and *Bremia lactucae*: Hulbert *et al.* (1988).

Genes other than rDNA

Other nuclear genes which might be suitable for providing data on the deeper phylogenies of the *Peronosporomycetidae* include the actin coding regions (Bhattacharya & Ehlting, 1995; Dudler, 1990; Hightower & Meagher, 1986; Uncles *et al.*, 1997) and ß tubulin (Cameron *et al.*, 1990; Sogin in Bhattacharya, Stickel & Sogin, 1991). Actin gene studies by Dudler (1990: *Phytophthora*) and Bhattacharya *et al.* (1991: *Achlya*) have confirmed the straminipile phylogeny, although the genetic distance between *Phytophthora* and *Achlya* appears to be greater than that between *Achlya* and the brown alga *Costaria* (*Laminariales*, *Fucophyceae*). The discrepancies noted by Bhattacharya *et al.* (1991) between the phylogenetic trees using actin or 18S/16S rRNA for protists/bacteria suggest that origins derived from these cladistics are still debatable. Complications arising from the use of actin gene sequences may arise because of gene duplication. In *Pythium irregulare* four 'copies' of the gene sequence occur. From sequence analyses, these 'copies' do not always fall in the same clade (one grouped with those for *Phytophthora* species, pers. comm., F. N. Martin, U.S.D.A., Salinas, Ca.). Another gene receiving attention, but which has not been used for straminipiles, is the DNA-dependent RNA polymerase (Klenk, Palm & Zillig, 1994; Klenk *et al.*, 1995; Palenik, 1992).

Mitochondrial DNA

The mitochondrial genome in straminipilous organisms is characteristically large. The length of the mitochondrial genome has been shown to vary between 36.4 and 73.0 kb (McNabb & Klassen, 1988). In some taxa Martin (1995*b*) found the mitochondrial genomes to be linear but he did not regard this feature as having phylogenetic importance. For straminipilous fungal genera such as *Pythium* and *Achlya* (i.e., in both subclasses), there is an inverted repeat in the mitochondrial genome (McNabb *et al.*, 1987). The length of this inverted repeat in *Pythium* (27-29 kb - kilobase pairs) is quite different from that in *Achlya* (10 kb) or mycote fungal mitochondria (4-5 kb), but it is very similar to that in chloroplasts (20-28 kb) (Whitfield & Bottomley, 1983). An inverted repeat of comparable size is present in the *Rhipidiales* (McNabb *et al.*, 1987; McNabb & Klassen, 1988) but *Phytophthora* (Shumard-Hudspeth & Hudspeth, 1990) has only a short (0·5-0·9 kb) inverted repeat. Shumard *et al.* (1986) concluded that the mere presence of this inverted repeat was not responsible for any evolutionary stability of this feature. The presence of an inverted repeat enables variation in the two unique regions which separate the repeat during replication.

Studies which have focused on the mitochondrial DNA include those of Boyd *et al.*, 1984; Cock *et al.*, 1992; Förster *et al.*, 1988; Hudspeth *et al.*, 1983; Hwang *et al.*, 1991; Klimczak & Prell, 1984; McNabb *et al.*, 1987; McNabb & Klassen, 1988; McNabb, Eros & Klassen, 1988; Möller, Cock & Prell, 1993; Shumard, Grossman & Hudspeth, 1986; Shumard-Hudspeth & Hudspeth, 1990). Alignments of the *Cox* II sequence from the mitochondrial genome have been prepared by Hudspeth *et al.* (2000) and Cook, Hudspeth & Hudspeth (2000) for a wide range of *Peronosporomycetes*, including *Pythiaceae*,

Saprolegniaceae, *Leptomitaceae*, *Rhipidiaceae* and *Lagenidium giganteum*. The most striking feature is the tripeptide indel sequence:

absent			from all *Saprolegniaceae* so far examined
present,	as	-Leu-Glu-Phe/Tyr-	in *Pythiaceae* and *Rhipidiaceae*
present,	as	-Tyr-Thr-Asp-	in *Leptomitaceae*
present,	as	-Leu-Glu-Tyr-	in *Lagenidium giganteum*

Further variations have been revealed by Cook *et al.* (2000) for some of the marine taxa:

present,	as	-Phe-Ser-Leu-	in *Atkinsiella dubia*
present,	as	-Thr-Asp-Leu-	in *Halodaphnea okinawensis*
present,	as	-Leu-Glu-Tyr-	in *Salilagenidium callinectes* (as for *L. giganteum*)

Lagenidium giganteum groups most closely with *Pythium ultimum* (D. S. S. Hudspeth, pers. comm., Northern Illinois University, DeKalb). According to D. S. S. Hudspeth (pers. comm.), the mitochondrially encoded cytochrome *c* oxidase subunit 2 locus (*cox2*) may be more informative of phylogeny where the 18S rRNA shows insufficient differences.

Summary

The deep phylogenetic divide between the *Peronosporomycetidae* and the *Saprolegniomycetidae* within the *Peronosporomycetes* (de Bary, 1866; Dick *et al.*, 1984) has now been confirmed with 18S rDNA data (Dick *et al.*, 1999) and 28S rDNA data (Riethmüller *et al.*, 1999; Petersen & Rosendahl, 2000). This divide is supported by the mitochondrially encoded cytochrome oxidase (*cox* II) data of Hudspeth *et al.* (2000), Martin (2000) and Cook *et al.* (2000). However, the placement of *Sapromyces* (*Rhipidiales*, *Rhipidiomycetidae*) may fall either in the *Saprolegniomycetidae* with the *Leptomitales* (Petersen & Rosendahl, 2000) or with the *Peronosporomycetidae* (Hudspeth *et al.*, 2000) depending on the molecular data used. Nevertheless, outgroups for phylogenetic analysis *within* the *Peronosporomycetidae* may be obtained from the *Saprolegniomycetidae* for the *Peronosporomycetidae* and *vice versa*. This will enable comparisons of longer nucleotide sequences, perhaps with additional variable and informative sites, than more distant outgroups taken from outside the *Peronosporomycetidae*.

Grosjean (1992), using ITS1, placed *Peronospora viciae* with *Phytophthora infestans*, and *Albugo candida* with *Pythium insidiosum* and *P. echinulatum*; both placements were at ultimate branches of their cladogram. Hudspeth (D. S. S. Hudspeth, pers. comm.), using mitochondrial *cox* II sequences, placed *Peronospora tabacina* and *P. nicotianae* with *Phytophthora megasperma*, also at ultimate branch points; in contrast, *Albugo candida* was basal to their phylogenetic tree, along with *Hyphochytrium* and *Sapromyces*. Cooke *et al.* (2000) again using ITS sequences, have placed a small sample of *Peronospora* species parasitic in the Rosids and Asterids (see PART III; Table III: 2 and Figure III: 1) with an intermediate branch of *Phytophthora* which includes *Ph. infestans* (the type species), *Ph. nicotianae* and *Ph. megakarya*. It is noteworthy that there is a measure of agreement with the molecular biological conclusions of Grosjean (1992) and Hudspeth (D. S. S. Hudspeth, pers. comm.) with respect to this particular group of *Peronospora* species with a similarly restricted group of *Phytophthora* species. There is no agreement concerning the placement of *Albugo*, although unpublished work generally places *Albugo* at some distance from *Phytophthora* and *Peronospora* (see PART III).

However, when *Peronospora rumicis* (the type species, Corda, 1837) has been studied and shown to belong to this group, then the genus *Phytophthora sensu stricto* (type species *Ph. infestans*) would become a

synonym of *Peronospora*, heralding a nomenclatural nightmare! One solution might be to set the monophyletic generic concepts at a very low heirarchical level so that neither *Phytophthora* nor *Peronospora* would need to be abandoned, but this would necessitate the simultaneous erection of numerous other genera from *Phytophthora sensu lato* and *Pythium sensu lato*. The alternative is to accept that the genus is paraphyletic. A meticulous taxonomic reassessment of historic generic names, and their type species, which have been placed in synonymy with *Phytophthora sensu lato* would be required for monophyly (see PART III: Table III: 3). Stamps *et al.* (1990) separated *Phytophthora* into six morphological groups but molecular studies have not entirely endorsed this division (compare Cooke *et al.*, 1996, 1999; Ristaino *et al.*, 1998). Dick (in Klassen, McNabb & Dick, 1987; Dick, 1990*c*: Venn diagram) suggested that there were perhaps five major centres of speciation within *Pythium*, based on morphological criteria, and exemplified by (1) *P. monospermum*, *P. torulosum* and *P. diclinum*, (2) *P. anandrum*, (3) *P. ultimum*, (4) *P. irregulare* and (5) *P. ostracodes* and *P. oedochilum*: all of these groups are supported by deep clades in the ITS data of Grosjean (1992), but again, these data should not be regarded as sufficiently robust at this stage. Data from Cook *et al.* (2000) suggest that *Phytophthora sensu lato* is similarly nested within *Pythium sensu lato* with *Lagenidium*.

The genera *Phytophthora sensu lato* and *Pythium sensu lato* may need to be retained for non-systematists, even though they may be paraphyletic, until, and even after, a concensus of relationships has been established.

The symplesiomorphic trait in the *Phytophthora* line that gave rise to the apomorphies of the genera of the Peronosporales is yet to be defined. To put nomenclatural order into the phylogenetic classification of the Peronosporales, it will eventually be necessary to make a large number of name changes at genus and family levels using comprehensive molecular, biochemical and morphological criteria. For example, this approach will be necessary for *Phytophthora undulata* (Dick, 1989; Mugnier & Grosjean, 1995) which is neither a *Phytophthora sensu stricto nor* a *Pythium sensu stricto*, while the *Pythium vexans* group almost certainly belongs to *Phytophthora sensu lato*, (cf. Dick, 1990*b* - morphology; Panabières *et al.*, 1997 - elicitor characteristics).

Genetic distance, as determined by a single gene, however important, ***on its own*** is ***insufficient*** to determine the limits of higher taxa. The fundamental criterion (or seminal event) that defines the node of the cladogram must also be identified. A diverse, species-rich and relatively ancient lineage will encompass extant species widely spaced by genetic distances. Some of these species will have lesser genetic distances from other species belonging to a much younger, derived, but significantly distinct major taxon. Thus short genetic distances, if they bridge the critical cladogram node, may appparently contradict robust higher taxon concepts based on several independent criteria.

The precise pattern of branching in the unrooted phylogenetic tree at large genetic distances may still be unreliable when data for whole groups of higher taxa are not available. The greatest problem lies in the fact that workers unfamiliar with a higher taxon (genus, family or order) may infer that data for one or two species placed in that higher taxon will adequately represent the higher taxon.

COEVOLUTION

Some attention must also be given to the possible evolution of obligate parasites, particularly the downy mildews and the thalloid endobiotic parasites. The evolution and coevolution of the downy mildews is considered in detail in PART III. It is concluded that a cophylogenetic coevolution is improbable. Coevolution is much more likely to depend on the availability of host secondary metabolites, particularly in relation to the more highly evolved angiosperms. The evolution of the downy mildews was probably instigated and driven by climate change in warm-temperate climates in the Cretaceous. Any coevolutionary pattern is therefore recent. It is possible that there was diversity in the geographic provenances of the different groups of downy mildews. The importance of physiological and biochemical attributes cannot be overstressed. It is remarkable that host/parasite relationships show a common pattern from the downy mildews, through the parasitic *Pythiaceae* and *Aphanomyces*, to the plasmodiophorids. Physiological traits and patterns of mycoparasitism (Pemberton *et al.*, 1990; Dick, 2000*c*) could also provide further insight to the interface between biochemistry and systematics.

In contrast to the advanced angiosperms, the evolution of the hosts for the thalloid endobiotic straminipilous fungi has a long geological history. Endobiotic parasites, such as the *Olpidiopsidales* and *Myzocytiopsidales*, appear to have a world-wide distribution. For many, the host range appears to be restricted and often within the *Charophyta* (*Charales*, *Zygnematales*), *Chlorophyta* (*Oedogoniales*), *Bacillariophyta*, *Peronosporomycetes*, *Aschelminthes* (*Rotifera* and *Nematoda*) and *Crustacea*. Most of these hosts are freshwater or wet-terrestrial, but a few are marine-littoral, estuarine or oligohaline. No evidence for a stenohaline requirement has been presented. Disjunct relict distributions could be invoked, but there is little evidence that could support this hypothesis. Similarly, morphological barriers do not seem to offer an explanation for host/parasite specialization. It is more probable that genetic adaptations have evolved which confine vulnerability to, and/or virulence of a pathogen by means of restricted ranges of chemical attractants. Thus, the endobiotic parasites would have evolved in association with their hosts. This would be particularly relevant if the association is parasitoidal (Newell *et al.*, 1977). Such a concept has been epitomized by creating a new binomial for each parasite in a novel host/parasite relationship, but this approach is not supported by cross-infection studies for *Myzocytiopsis* and *Haptoglossa* between nematode and rotifer hosts (Barron, 1977*b*, 1989*b*).

Proximity within a common habitat will facilitate the evolution of specific adaptations, as could occur between communities of *Spirogyra*, *Oedogonium*, desmids, *Lemnaceae* and other aquatic angiosperms, and animals and fungi associated with such a community. Close evolutionary connections between species which would involve both widely differing hosts ***and*** different ecosystems are rather less probable. Observations of host specificity in genera such as *Olpidiopsis* and *Petersenia*, where restriction is to particular species or groups of species in host genera (Shanor, 1940; Slifkin, 1961; Sparrow, 1960; Whittick & South, 1971,1972) is in accord with systems of biochemical recognition. Such data are difficult to reconcile with implications that species of a genus may be found in widely separated hosts and habitats, which in *Petersenia* range from marine red algae to grass leaves (*P. panicicola* Thirum. & Lacy), and in *Olpidiopsis* from freshwater *Saprolegniales* to marine red algae. In these parasitic associations I prefer to conclude that the existing taxonomy is suspect. While diversity in host organisms may occur for a particular genus, it is more probable that the more closely related taxa will share a common community, a related group of hosts, or both.