6

Chytridiomycota

6.1 Introduction

The phylum Chytridiomycota comprises over 900 species in five orders (D. J. S. Barr, 2001; Kirk *et al*., 2001). Fungi included here are colloquially called 'chytrids'. Most chytrids grow aerobically in soil, mud or water and reproduce by zoospores with a single posterior flagellum of the whiplash type, although the zoospores of some members of the Neocallimastigales are multiflagellate. Some species inhabit estuaries and others the sea. Sparrow (1960) has given an extensive account of aquatic forms, Karling (1977) a compendium of illustrations, and Powell (1993) has provided examples of the importance of the group. Many members are saprotrophs, utilizing cellulose, chitin, keratin, etc., from decaying plant and animal debris in soil and mud, whilst species of *Caulochytrium* grow as mycoparasites on the mycelium and conidia of terrestrial fungi (Voos, 1969). Saprotrophs can be obtained in crude culture by floating baits such as cellophane, hair, shrimp exoskeleton, boiled grass leaves and pollen on the surface of water overlying samples of soil, mud or pieces of aquatic plant material (Sparrow, 1960; Stevens, 1974; Willoughby, 2001). From such crude material, pure cultures may be prepared by streaking or pipetting zoospores onto agar containing suitable nutrients and antibiotics to limit contamination from bacteria. The growth and appearance of chytrids in pure culture is variable and often differs significantly from their natural habit. This has led to problems in classification systems based on thallus morphology (Barr, 1990, 2001). The availability of cultures has, however, facilitated studies on chytrid nutrition and physiology (Gleason, 1976).

Some chytrids are biotrophic parasites of filamentous algae and diatoms and may severely deplete the population of freshwater phytoplankton (see p. 139). Two-membered axenic cultures of diatom host and parasite have been prepared, making possible detailed ultrastructural studies of comparative morphology, zoospores, infection processes and reproduction. Other chytrids such as species of *Synchytrium* and *Olpidium* are biotrophic parasites of vascular plants. *Synchytrium endobioticum* is the agent of the potentially serious black wart disease of potato. *Olpidium brassicae*, common in the roots of many plants, is relatively harmless, but its zoospores are vectors of viruses such as that causing big vein disease of lettuce. *Coelomomyces* spp. are pathogens of freshwater invertebrates including copepods and the larvae of mosquitoes. The possibility of using them in the biological control of mosquitoes has been explored. The most unusual group are the Neocallimastigales, which grow in the guts of herbivorous mammals, are obligately anaerobic and subsist on ingested herbage.

The cell walls of some chytrids have been examined microchemically by X-ray diffraction and other techniques. Chitin has been detected in many species (Bartnicki-Garcia, 1968, 1987), and in *Gonapodya* cellulose is also present (Fuller & Clay, 1993). The composition of the wall is of interest because chitin, a polymer of

N-acetylglucosamine, is also found in the walls of other Eumycota (i.e. Zygomycota, Ascomycota and Basidiomycota), whilst the cell walls of members of the Oomycota contain cellulose. Cellulose and chitin occur together in the walls of species of *Hyphochytrium* and *Rhizidiomyces*, members of the Hyphochytriomycota (Fuller, 2001; see Section 4.3).

The form of the thallus in the Chytridiomycota is varied. In biotrophic species such as *Olpidium* and *Synchytrium*, where the whole thallus is contained within the host cell, there is no differentiation into a vegetative and a reproductive part. At maturity the entire structure, except for the wall which surrounds it, is converted into reproductive units, i.e. zoospores, gametes or resting sporangia. Such thalli are termed **holocarpic** (Fig. 6.1). More usually, the thallus is differentiated into organs of reproduction (sporangia and resting sporangia) arising from a vegetative part which often consists of **rhizoids**. These serve in the exploitation of the substratum and the assimilation of nutrients. Thalli of this type are **eucarpic**. Eucarpic thalli may have one or several sporangia and are then termed **monocentric** or **polycentric**, respectively (Fig. 6.1). In some species there are both monocentric and polycentric thalli, so that these terms have descriptive rather than taxonomic significance. A further distinction has been made, especially in monocentric forms, between those in which only the rhizoids are inside the host cell whilst the sporangium is external (**epibiotic**), in contrast with the **endobiotic** condition in which the entire thallus is inside the host cell (Fig. 6.1). In monocentric thalli, the rhizoids usually radiate from a single position on the sporangium wall, but in polycentric forms a more extensive, branched rhizoidal system, the **rhizomycelium**, develops.

The zoosporangium is generally a spherical or pear-shaped sac bearing one or more discharge tubes or exit papillae. The method of zoospore release has been used in classification.

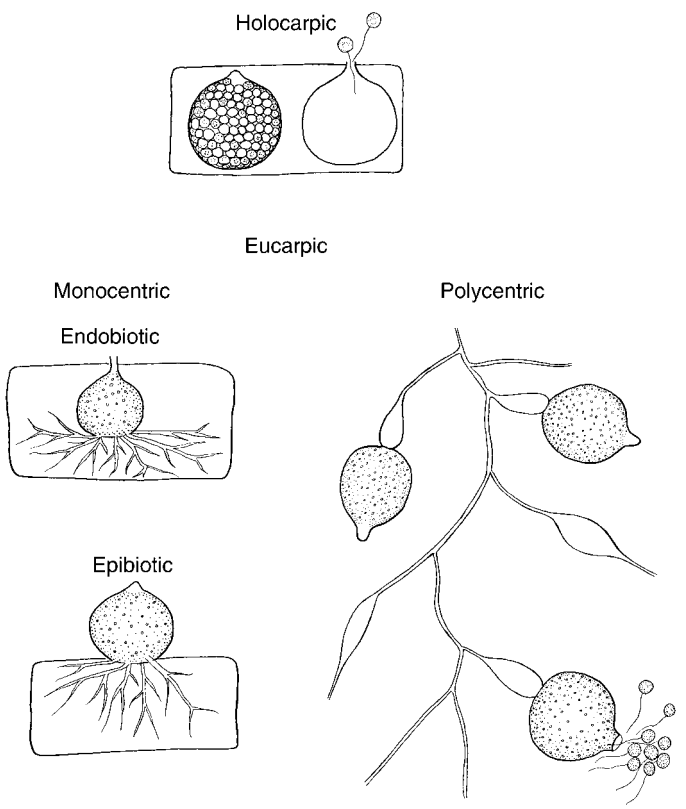

Fig 6.1 Types of thallus structure in the Chytridiales, diagrammatic and not to scale.

In the **inoperculate** chytrids such as *Olpidium*, *Diplophlyctis* and *Cladochytrium*, the sporangium forms a discharge tube which penetrates to the exterior of the host cell and its tip becomes gelatinous and dissolves away. In **operculate** chytrids such as *Chytridium* and *Nowakowskiella*, the tip of the discharge tube breaks open at a special line of weakness and is seen as a special cap or **operculum** after discharge (see Fig. 6.4b).

6.1.1 The zoospore

The number of zoospores formed inside zoosporangia of chytrids varies with the size of the spore and sporangium. Although the zoospore size is roughly constant for a given species, the size of the sporangium may be very variable. In *Rhizophlyctis rosea*, tiny sporangia containing only one or two zoospores have been reported from culture media deficient in carbohydrate, whereas on cellulose-rich media large sporangia containing many hundred spores are formed. The release of zoospores is brought about by internal pressure which causes the exit papillae to burst open. In studies of the fine structure of mature sporangia of *R. rosea* and *Nowakowskiella profusa* (Chambers & Willoughby, 1964; Chambers *et al*., 1967), it has been shown that the single flagellum is coiled round the zoospore like a watch spring. The zoospores are separated by a matrix of spongy material which may absorb water and swell rapidly at the final stages of sporangial maturation. When the internal pressure has been relieved by the ejection of some zoospores, those remaining inside the sporangium escape by swimming or wriggling through the exit tube. In some species the spores are discharged in a mass which later separates into single zoospores, but in others the zoospores make their escape individually.

The form of the zoospore is similar in all chytrids (with the exception of the multiflagellate members of the Neocallimastigales). There is a spherical or ellipsoidal body which in some forms is capable of plastic changes in shape, and a long trailing flagellum. When swimming, the zoospores show characteristic jerky or 'hopping' movements; additionally, abrupt changes in direction are sometimes made. The internal structure of the zoospore as revealed by light and electron microscopy is variable, but characteristic of particular genera (Lange & Olson, 1979). In view of the plasticity in morphology of the thallus under different growth conditions, zoospore ultrastructure is regarded as a more satisfactory basis of classification (D. J. S. Barr, 1990, 2001). Two features are of taxonomic importance, the flagellar apparatus and an assemblage of organelles termed the **microbody–lipid globule complex** (MLC) (D. J. S. Barr, 2001).

The flagellar apparatus

The whiplash flagellum resembles that of other eukaryotes, with a smooth membrane enclosing a cylindrical shaft, the axoneme, made up internally of nine doublet pairs of microtubules surrounding two central microtubules. As shown in Fig. 6.2, the base of the axoneme comprises three regions, the flagellum proper, the transitional zone and the kinetosome. The function of the kinetosome is to generate the flagellum. An interesting feature found in several species is a second kinetosome or the remainder of one, the **dormant kinetosome**. Its presence has led to the suggestion that the ancestors of the Chytridiomycota may have had biflagellate zoospores, the second flagellum having been lost in the course of evolution (Olson & Fuller, 1968).

In section, the kinetosome resembles a cartwheel (Fig. 6.2f), because to each of the nine outer microtubule doublets seen in the flagellum proper, a third microtubule is attached. This is called the C-tubule; in the doublets, that tubule with extended dynein arms is the A-tubule, and its partner is labelled B. These flagellar microtubules radiate as kinetosome props into the zoospore, perhaps providing structural support and anchorage of the flagellum (D. J. S. Barr, 2001). Microtubules may also be attached laterally to the kinetosome, contributing to the flagellar root system (Figs. 6.2c, 6.19). In the innermost (proximal) part of the transitional zone, the nine microtubule triplets of the kinetosome are converted into the doublets of the flagellum proper; concentric fibres, possibly arranged helically, surround the nine doublet pairs. Also within the transitional zone,

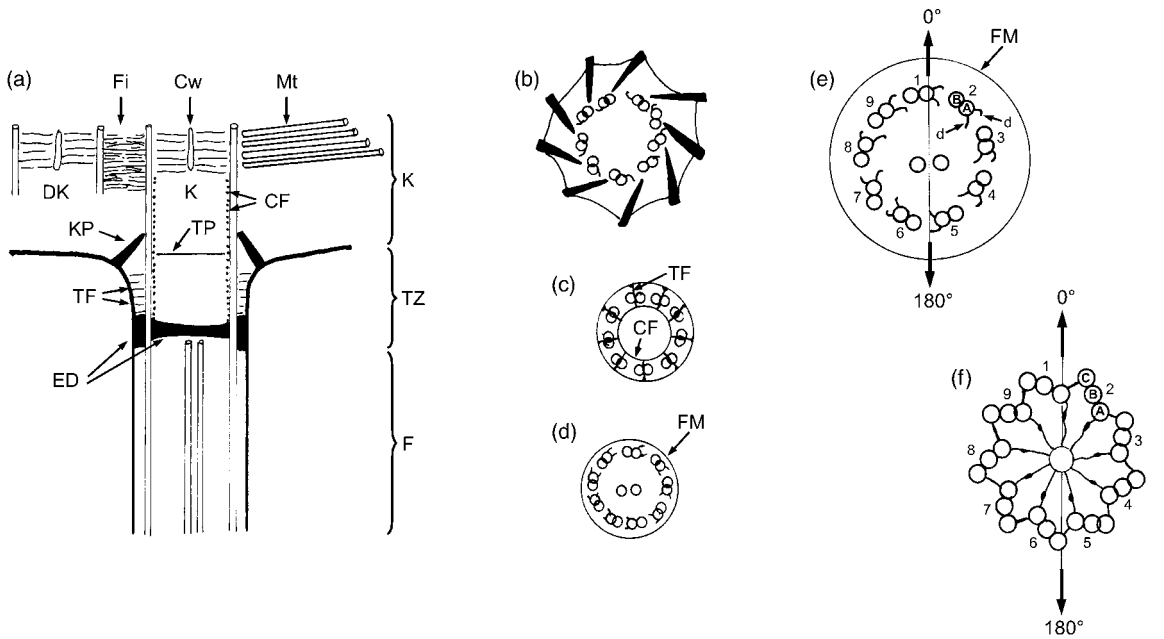

Fig 6.2 Flagellar apparatus typical of zoospores of Chytridiomycota. (a) Median longitudinal section of the junction of the flagellum with the body of the zoospore. The labels indicate the flagellum proper (F), transitional zone (TZ), kinetosome (K), electron-dense region (ED), concentric fibres (CF), transitional fibres (TF), kinetosome props (KP), terminal plate (TP), kinetosome (K) showing a cartwheel-like organization (Cw), dormant kinetosome (DK), fibrillar material (Fi) found in some taxa, and microtubular roots (Mt) extending from the side or end of the kinetosome into the body of the zoospore. (b) Transverse section near the terminal plate showing nine kinetosome props extending from doublet microtubules to the cell membrane. (c) Transverse section in the lower part of the transition zone showing concentric and transitional fibres. (d) Transverse section of the flagellum proper showing two central microtubules and nine peripheral doublet microtubules enclosed in the flagellar membrane (FM). (e) Schematic drawing of the flagellum proper in transverse section. The arrowed line 0°–180° shows an imaginary plane which coincides with the plane of undulation of the flagellum, passing through doublet pair 1 and between the central microtubules and doublet pairs 5 and 6. The convention used in labelling the outer doublet pairs of microtubules is shown: the microtubule with dynein arms (d) is the A microtubule and its partner is the B microtubule. (f) Kinetosome in transverse section showing the triplet arrangement of the peripheral microtubules by the addition of a third microtubule (C). Redrawn from Barr and Désaulniers (1988) by copyright permission of the National Research Council of Canada, Barr (1992). ©The Mycological Society of America, and D. J. S. Barr (2001) with kind permission of Springer Science and Business Media.

the two central microtubules arise near a terminal plate. The structure of the flagellum and kinetosome in transverse section is shown in Figs. 6.2e and f (Barr & Désaulniers, 1988).

The microbody–lipid complex

The MLC (Fig. 6.3) is made up of a microbody which is often closely appressed to a large lipid globule and to simple membrane cisternae or a tubular membrane system, the **rumposome**. This is defined as a cisterna in which there is an area with hexagonally arranged, honeycomb-like pores called fenestrae (Fuller, 1976; Powell & Roychoudhury, 1992). The rumposome may be involved in signal transduction from the plasma membrane to the flagellum because it is known that this organelle sequesters calcium. Regulation of external calcium concentrations has an effect on the symmetry of flagellar beat and hence on the direction of zoospore movement (Powell, 1983).

There are several distinct types of MLC (Powell & Roychoudhury, 1992) and Fig. 6.3 illustrates diagrammatically just one of them, that described for *Rhizophlyctis harderi*. In this species, the MLC includes several (3–5) lipid globules.

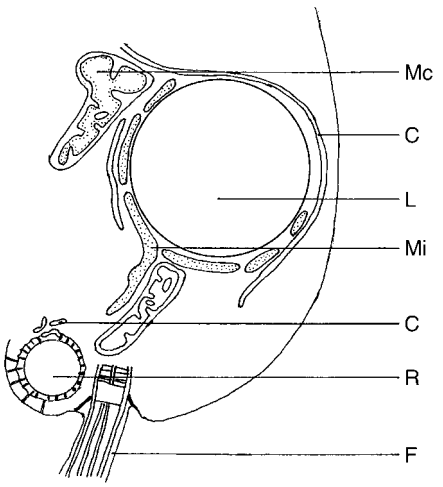

Fig 6.3 Schematic diagram of the microbody–lipid complex of the zoospore of *Rhizophlyctis harderi* as seen in a longitudinal section through the base of the zoospore and flagellum. The following organelles are drawn: mitochondrion (Mc), simple cisterna (C), lipid globule (L), microbody (Mi), flagellum (F) and rumposome (R). Redrawn from Powell and Roychoudhury (1992), by copyright permission of the National Research Council of Canada.

Those at the anterior of the cell are embedded in an aggregation of ribosomes. The surfaces of lipid globules close to the plasma membrane are partially covered by one to several simple cisternae, sometimes with irregularly scattered pores. Towards the centre of the cell the lipid bodies are clasped by cup-shaped microbodies. At the posterior of the zoospore near the kinetosome, 1–3 smaller lipid globules are partially covered by a rumposome, linked to the plasma membrane by short bridges and to the kinetosome by a microtubule root.

Other features

Patches of glycogen are located in the peripheral cytoplasm of the zoospore and it is likely that these and the lipid globules represent sources of energy used in respiration and propulsion. Mitochondria tend to be concentrated in the posterior of the zoospore close to the kinetosome; in *Allomyces* and *Blastocladiella* (Blastocladiales), the base of the flagellum passes through the perforation of a single large mitochondrion (see Fig. 6.19).

Most zoospores are uninucleate. The nucleus is surrounded in many cases (but not all) by a nuclear cap of uneven thickness. The nuclear cap is especially prominent in zoospores of members of Blastocladiales such as *Allomyces* and *Blastocladiella* (Fig. 6.19). It is rich in RNA and protein and also contains ribosomes.

6.1.2 Zoospore encystment and germination

The period of zoospore movement varies. Some flagellate zoospores seem to be incapable of active swimming and amoeboid crawling may take place instead, or swimming may last for only a few minutes. In other spores, motility may be prolonged for several hours. Prior to germination, the zoospore comes to rest and encysts. The flagellum may contract, it may be completely withdrawn or it may be cast off, but the precise details are often difficult to follow. The subsequent behaviour also differs in different species. In holocarpic parasites the zoospore encysts on the host surface and the cytoplasmic contents of the zoospore are injected into the host cell. In many monocentric chytrids rhizoids develop from one point on the zoospore cyst and the cyst itself enlarges to form the zoosporangium, but there are variants of this type of development in which the cyst enlarges into a **prosporangium** from which the zoosporangium later develops. In the polycentric types, the zoospore on germination may form a limited rhizomycelium on which a swollen cell arises, giving off further branches of rhizomycelium. Germination may be from a single point on the wall of the zoospore cyst (**monopolar** germination) or from two points, enabling growth to take place in two directions (**bipolar** germination). The mode of germination is an important character in distinguishing, for example, the Chytridiales (monopolar) from the Blastocladiales (bipolar).

6.1.3 Life cycles of the Chytridiomycota

Most chytrids have haploid zoospores and thalli but some Blastocladiales show an alternation of haploid (gametothallic) and diploid (sporothallic) generations. Apart from differences in

the reproductive organs, the morphology of the two types of thallus is very similar, a phenomenon known as **isomorphic alternation of generations**.

Sexual reproduction, i.e. a life cycle which includes nuclear fusion and meiosis, may occur in several different ways (e.g. Figs. 6.6 and 6.22). In some chytrids it is by **gametogamy**, the fusion of gametes which are posteriorly uniflagellate. **Isogamous conjugation** occurs if there is no morphological distinction between the two fusing partners, but in some Blastocladiales (e.g. *Allomyces*) **anisogamy** takes place by fusion between a smaller, more actively motile male gamete with a larger, sluggish female gamete. **Oogamy**, fusion between an actively motile male gamete and a much larger, non-flagellate, immobile globose egg, is characteristic of Monoblepharidales. **Somatogamy**, the fusion of undifferentiated hyphae or rhizoids, has been well documented in cultures of the freshwater fungus *Chytriomyces hyalinus* by Moore and Miller (1973) and Miller and Dylewski (1981, 1987). As shown in Fig. 6.4, zoospores of *C. hyalinus* are released from the zoosporangium by the opening of a lid-like operculum. They germinate to form uninucleate rhizoidal thalli (contributory thalli) and the tips of the rhizoids from adjacent thalli, which are apparently not genetically distinct from each other, may fuse (Fig. 6.4c). At the point of fusion an incipient resting body develops (Fig. 6.4d) and swells while cytoplasm and a nucleus migrate into it from each contributory thallus. Nuclear fusion occurs in the resting body to form a diploid zygote nucleus. The resting body continues to enlarge and develops a thick wall. This type of sexual reproduction by somatogamous conjugation probably occurs in several genera of inoperculate and operculate chytrids (Moore & Miller, 1973).

Fusion of gametangia (**gametangio-gametangiogamy**) has been reported by Doggett and Porter (1996) for *Zygorhizidium planktonicum*, a parasite of the diatom *Synedra*. This species reproduces asexually by epibiotic zoosporangia. Germinating zoospores develop either new zoosporangial thalli or gametangial thalli of two sizes with globose uninucleate gametangia.

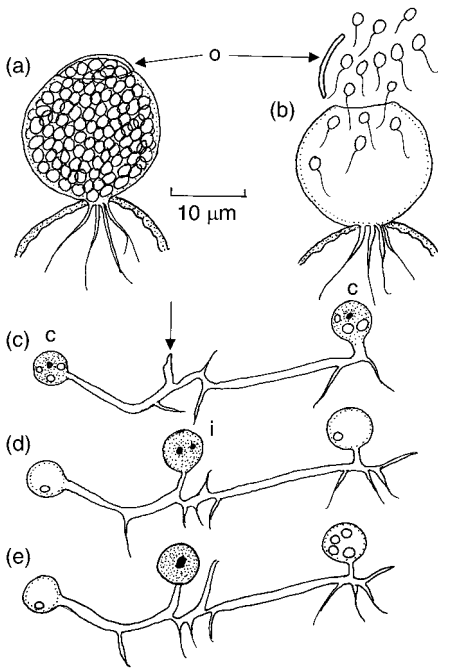

Fig 6.4 *Chytriomyces hyalinus* somatogamy. (a,b) Epibiotic fruiting thallus seated on a pollen grain into which rhizoids have penetrated. In (a) the zoosporangium, containing numerous zoospores, is seen shortly before discharge with a bulging operculum (o). In (b) the operculum has lifted off and the zoospores are escaping. (c–e) Stages in somatogamy. (c) Rhizoids from two uninucleate contributory thalli (c) have undergone anastomosis (arrow). (d) Cytoplasm and a nucleus from each contributory thallus have migrated towards the point of anastomosis, where the thallus swells to form a globose incipient resting body (i) which is binucleate and packed with cytoplasm, leaving the contributory thalli empty. (e) The two nuclei in the incipient resting body have fused. After C. E. Miller and Dylewski (1981).

Conjugation occurs when a conjugation tube grows from the smaller donor to the larger recipient gametangium (Fig. 6.5a). Following nuclear fusion, the larger gametangium develops a thick wall and functions as a diploid resting spore. After a period of maturation the resting spore acts as a prosporangium, giving rise to a thin-walled meiosporangium. Meiosis, as evidenced by the presence of synaptonemal complexes, occurs here, followed by mitosis and cytoplasmic cleavage to form zoospores (Fig. 6.5b). A variant of this form of sexual differentiation (**gametangio-gametogamy**) has

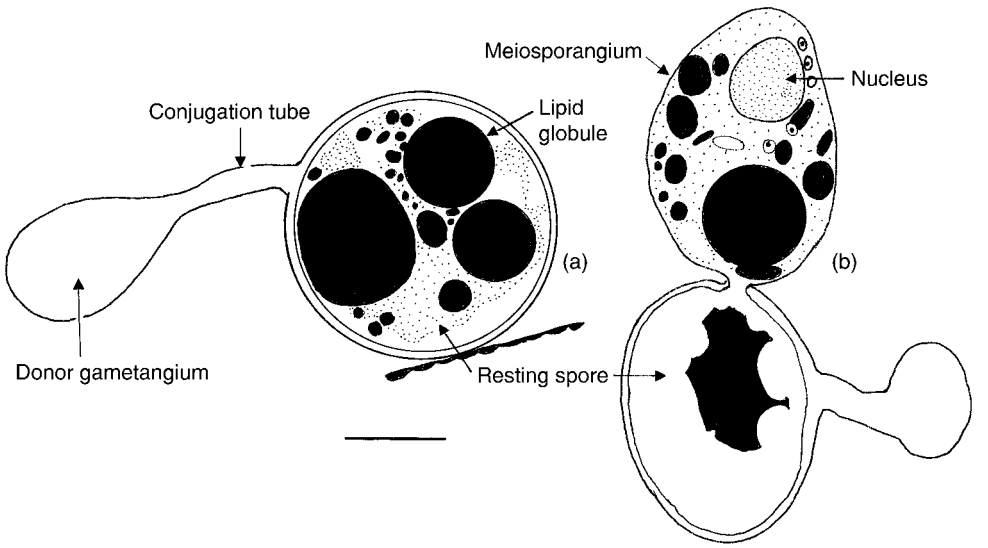

Fig 6.5 Sexual reproduction in *Zygorhizidium planktonicum*. (a) Empty donor gametangium to the left connected by a conjugation tube to a mature resting spore. (b) Near-median section of a fully formed meiosporangium which has developed from a germinating resting spore. The donor gametangium is on the right. Scale bar = 4 μm. After Doggett and Porter (1996).

been reported in species of *Rhizophydium* (Karling, 1977); this involves copulation between the gametangium of a rhizoid-forming thallus and a motile gamete that encysts directly on the gametangium.

Generally the product of sexual reproduction is a resting spore or resting sporangium with thick walls, but it is known that thick-walled sporangia may also develop asexually and in many chytrids sexual reproduction has not been described and possibly does not occur. Resting sporangia of some chytrids may remain viable for many years.

6.1.4 Classification and evolution

Fossil chytrids have been reported from the 400 million-year-old Rhynie chert, a site known for the discovery of fossil remains of the earliest known vascular land plants. Clusters of holocarpic, endobiotic thalli resembling the present day *Olpidium* have been found inside cells of a coenobial alga preserved within the hollow axes of a vascular plant, and epibiotic sporangia with endobiotic rhizoids have been seen attached to meiospores of a vascular plant, much like those of extant chytrids like *Rhizophydium* which grow on pollen grains (Taylor *et al*., 1992). Chytrid-like fossils have also been found in strata of the 340 million-year-old Pennsylvanian (Carboniferous) era (Millay & Taylor, 1978) and from the more recent Eocene strata (Bradley, 1967).

Formerly thought to have an affinity for the Oomycota, Hyphochytriomycota or protists, the Chytridiomycota are now accepted as members of the true fungi, the Eumycota. They are probably ancestral to other groups of true fungi, especially the Zygomycota (Cavalier-Smith, 1987, 2001; D. J. S. Barr, 2001). The inclusion of the chytrids in the Eumycota is supported by several DNA-based phylogenetic analyses (e.g. Bowman *et al*., 1992; James *et al*., 2000), but the delimitation of orders within the Chytridiomycota is still problematic. Particularly puzzling is the grouping of the Blastocladiales with the Zygomycota on the basis of 18S ribosomal DNA sequences (see Fig. 1.26).

D. J. S. Barr (2001) and Kirk *et al*. (2001) have classified the Chytridiomycota into five orders (Table 6.1) but the details of their distinguishing features need not concern us here. We shall study examples from each order.

Table 6.1. Orders of Chytridiomycota following D. J. S. Barr (2001) and Kirk *et al*. (2001).

Order	Number of described taxa	Examples
Chytridiales (see Section 6.2)	80 genera 600 spp.	*Cladochytrium*, *Nowakowskiella*, *Rhizophydium*, *Synchytrium*
Spizellomycetales (see Section 6.3)	13 genera 86 spp.	*Olpidium*, *Rhizophlyctis*
Neocallimastigales (see Section 6.4)	5 genera 16 spp.	*Anaeromyces*, *Caecomyces*, *Neocallimastix*, *Orpinomyces*, *Piromyces*
Blastocladiales (see Section 6.5)	14 genera 179 spp.	*Allomyces*, *Blastocladiella*, *Coelomomyces*, *Physoderma*
Monoblepharidales (see Section 6.6)	4 genera 19 spp.	*Gonapodya*, *Monoblepharella*, *Monoblepharis*

6.2 Chytridiales

This is by far the largest order, comprising more than 50% of the total number of chytrids described to date. It is difficult to characterize members of the Chytridiales because they lack any specific features by which species have been assigned to the other four orders. The classification of the Chytridiales has traditionally been based on thallus morphology (Sparrow, 1973) but, as pointed out by D. J. S. Barr (2001), this is unsatisfactory because of the great variability in thallus organization shown by the same fungus growing on its natural substratum and in culture. Future systems of classification will be based on zoospore ultrastructure and the comparison of several different types of DNA sequences, but too few examples have yet been studied to provide a definitive framework. Because of this we shall study genera which illustrate the range of morphology, life cycles and ecology of the Chytridiales without attempting to place them into families.

6.2.1 *Synchytrium*

In this genus the thallus is endobiotic and holocarpic, and at reproduction it may become converted directly into a group (sorus) of sporangia, or to a **prosorus** which later gives rise to a sorus of sporangia. Alternatively the thallus may turn into a resting spore which can function either directly as a sporangium and give rise to zoospores, or as a prosorus. The zoospores are of the characteristic chytrid type (Lange & Olson, 1978). Sexual reproduction is by copulation of isogametes, resulting in the formation of thalli which develop into thick-walled resting spores. *Synchytrium* includes about 120 species which are biotrophic parasites of flowering plants. Some species parasitize only a narrow range of hosts, e.g. *S. endobioticum* on Solanaceae, but others, e.g. *S. macrosporum*, have a wide host range (Karling, 1964). Most species are not very destructive to the host plant but stimulate the formation of galls on leaves, stems and fruits.

Synchytrium endobioticum

This is the cause of wart disease affecting cultivated potatoes and some wild species of *Solanum*. It is a biotrophic pathogen which has not yet been successfully cultured outside living host cells. Wart disease is now distributed throughout the main potato-growing regions of the world, especially in mountainous areas and those with a cool, moist climate. Lange (1987) has given practical details of techniques for studying the fungus but in most European countries handling of living material by

unlicensed workers is illegal. Diseased tubers bear dark brown cauliflower-like excrescences. Galls may also be formed on the aerial shoots, and they are then green with convoluted leaf-like masses of tissue (the leafy gall stage; Plates 3a,b). Heavily infected tubers may have a considerable proportion of their tissues converted to warts. The yield of saleable potatoes from a heavily infected crop may be less than the actual weight of the seed potatoes planted. The disease is thus potentially a serious one, but fortunately varieties of potatoes are available which are immune from the disease, so that control is practicable. The possible life cycle of *S. endobioticum* is summarized in Fig. 6.6.

The dark warts on the tubers are galls in which the host cells have been stimulated to divide by the presence of the fungus. Many of the host cells contain resting spores which are more or less spherical cells with thick dark brown walls folded into plate-like extensions (see Fig. 6.7a). The resting spores are released by the decay of the warts and may remain alive in the soil for over 40 years (Laidlaw, 1985). The outer wall (exospore) bursts open by an irregular aperture and the endospore balloons out to form a vesicle within which a single sporangium differentiates (Kole, 1965; Sharma & Cammack, 1976; Hampson *et al*., 1994). Thus the resting spore functions as a **prosporangium** on germination. Germination of the resting spore may occur spontaneously but can be stimulated by passage through snails. It is presumed that abrasion and digestion of the spore wall

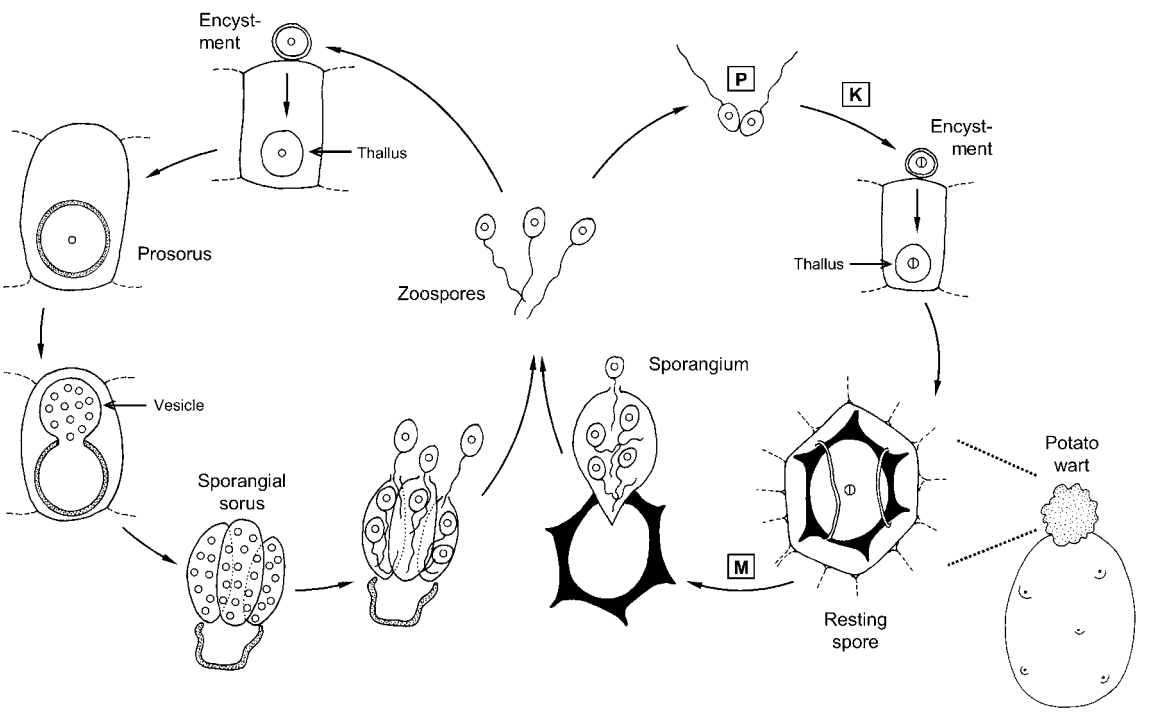

Fig 6.6 Schematic outline of the probable life cycle of *Synchytrium endobioticum*. Haploid and diploid nuclei are represented by small empty and larger split circles, respectively. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M). Resting spores within a warted potato contain a single nucleus which undergoes meiosis upon germination. Haploid zoospores are released from a single sporangium. If two zoospores pair up, a zygote is formed and penetration of a potato cell gives rise to a diploid thallus and, ultimately, a resting spore. Diploid infections cause host hyperplasia visible as the potato wart symptoms. If a zoospore infects in the haploid state, a haploid prosorus (summer spore) is formed, and hypertrophy of the infected and adjacent host cells ensues. A sorus of several sporangia is ultimately produced, with each sporangium releasing a fresh crop of haploid zoospores. *Synchytrium endobioticum* appears to be homothallic.

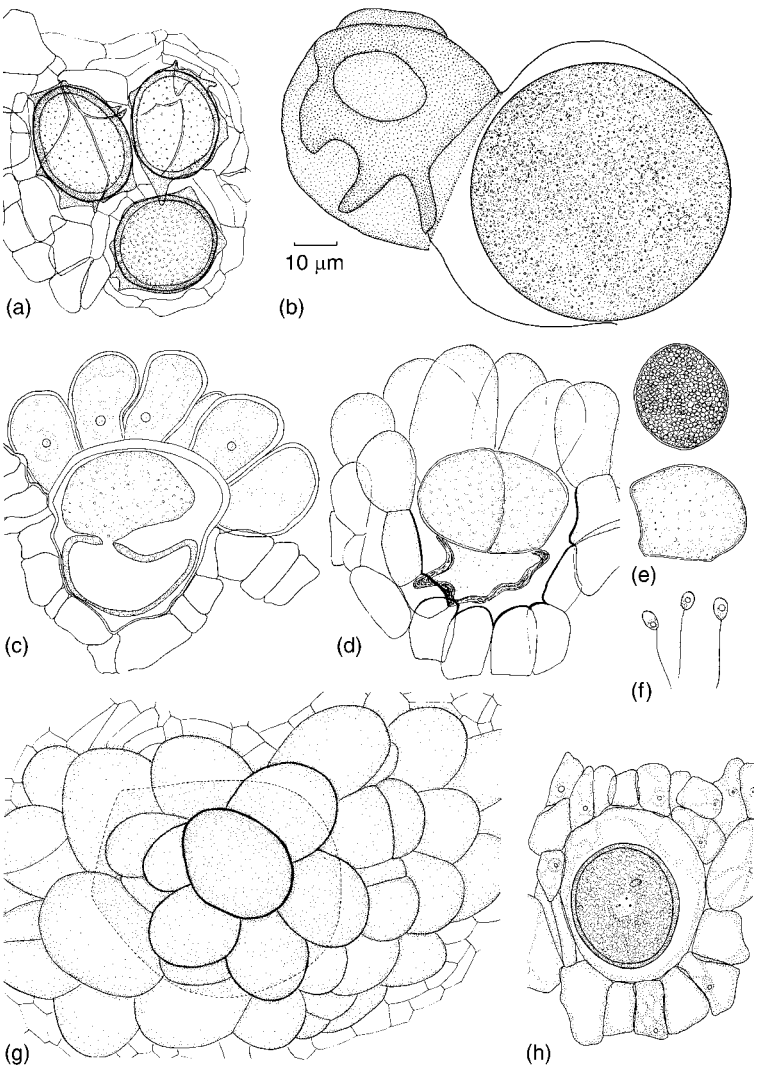

Fig 6.7 *Synchytrium endobioticum*. (a) Resting spores in section of wart. (b) Germinating resting spore showing the formation of a vesicle containing a single globose sporangium (after Kole, 1965). (c) Section of infected host cell containing a prosorus. The prosorus is extruding a vesicle. Note the hypertrophy of the infected cell and adjacent uninfected cells. (d) Cleavage of vesicle contents to form zoosporangia. (e) Two extruded zoosporangia. (f) Zoospores. (g) Rosette of hypertrophied potato cells as seen from the surface. The outline of the infected host cell is shown dotted. (h) Young resting sporangium resulting from infection by a zygote. Note that the infected cell lies beneath the epidermis due to division of the host cells.

in the snail gut causes breakdown of the thick wall which contains chitin and branched-chain wax esters, so overcoming dormancy related to the impermeability of the wall (Hampson *et al*., 1994).

The zoospores are capable of swimming for about two hours in the soil water. If they alight on the surface of a potato 'eye' or some other part of the potato shoot such as a stolon or a young tuber before its epidermis is suberized, they come to rest and withdraw their flagellum. During penetration, the contents of the zoospore cyst are transferred to the host cell whilst the cyst wall remains attached to the outside. When a dormant 'eye' is infected, dormancy may be broken and the tuber may begin to sprout. If the potato variety is susceptible to the disease, the small fungal thallus inside the host cell will enlarge. The infected host cell as well as surrounding cells also enlarge so that a rosette of hypertrophied cells surrounds a central infected cell (Fig. 6.7c). The walls of these cells adjacent to the infected cell are often thickened and assume a dark brown colour. The infected cell remains alive for some time but eventually it dies. The pathogen thallus passes to the bottom of the host cell, enlarges and becomes spherical. A double-layered chitinous wall which

is golden brown in colour is secreted around the thallus, now termed a prosorus or summer spore. Further development of the prosorus involves the protrusion of the inner wall through a pore in the outer wall, and its expansion as a vesicle which enlarges upwards and fills the upper half of the host cell (Fig. 6.7c). The cytoplasmic contents of the prosorus including the single nucleus are transferred to the vesicle. The process is quite rapid and can be completed in about 4 h. During its passage into the vesicle the nucleus may divide, and mitoses continue so that the vesicle contains about 32 nuclei. At this stage the cytoplasmic contents of the vesicle become cleaved into about 4–9 sporangia (Fig. 6.7d), forming a sorus. After the deposition of sporangial walls, further nuclear divisions occur in each sporangium, and finally each nucleus with its surrounding mass of cytoplasm becomes differentiated to form a zoospore. As the sporangia ripen, they absorb water and swell, causing the host cell containing them to burst open. Meanwhile, division of the host cells underlying the rosette has been taking place, and enlargement of these cells pushes the sporangia out onto the surface of the host tissue (Fig. 6.7e). The sporangia swell if water is available and burst open by means of a small slit through which the zoospores escape. There may be as many as 500–600 zoospores in a single large sporangium. The zoospores resemble those derived from resting sporangia and are capable of initiating further asexual cycles of reproduction throughout spring and early summer. Sometimes several zoospores succeed in penetrating a single cell so that it contains several fungal protoplasts.

Alternatively, zoospores may function as gametes, fusing in pairs (or occasionally in groups of three or four) to form zygotes which retain their flagella and swim actively for a time. Since zoospores acting as gametes do not differ in size and shape, copulation can be described as isogamous. However, the gametes may differ physiologically. Curtis (1921) has suggested that fusion may not occur between zoospores derived from a single sporangium, but only between zoospores from separate sporangia. Köhler (1956) has claimed that the zoospores are at first sexually neutral. Later they mature and become capable of copulation. Maturation may occur either outside the sporangia or within, so that in over-ripe sporangia the zoospores are capable of copulation on release. At first the zoospores are 'male', and swim actively. Later the swarmers become quiescent ('female') and probably secrete a substance which attracts 'male' gametes. After swimming by means of its two flagella, the zygote encysts on the surface of the host epidermis and penetration may then follow by a process essentially similar to zoospore penetration. Multiple infections by several zygotes penetrating a single host cell can also occur. Nuclear fusion occurs in the young zygote before penetration.

The results of zygote infections differ from infection by zoospores. The host cell reacts to zoospore infection by undergoing hypertrophy, i.e. increase in cell volume, and adjacent cells also enlarge to form the characteristic rosette which surrounds the resulting prosorus. In contrast, when a zygote infects, the host cell undergoes hyperplasia, i.e. repeated cell division. The pathogen lies towards the bottom of the host cell, adjacent to the host nucleus, and cell division occurs in such a way that the fungal protoplast is located in the innermost daughter cell. As a result of repeated divisions of the host cells, the typical gall-like potato warts are formed and fungal protoplasts may be buried several cell layers deep beneath the epidermis (see Fig. 6.7h). During these divisions of the host tissue the zygote enlarges and becomes surrounded by a two-layered wall, a thick outer layer which eventually becomes dark brown in colour and is thrown into folds or ridges which appear as spines in section, and a thin hyaline inner wall surrounding the granular cytoplasm (Lange & Olson, 1981). The host cell eventually dies and some of its contents are deposited on the outer wall of the resting sporangium, forming the characteristic brown ridges. During its development the resting spore remains uninucleate. Resting spores are released into the soil and are capable of germination within about 2 months. Before germination, the nucleus divides repeatedly to form the nuclei of the zoospores whose further development has

already been described. It has been claimed that the zygote and the young resting spore are diploid, and it has been assumed that meiosis occurs during germination of the resting sporangia prior to the formation of zoospores, so that these zoospores, the prosori and the soral zoospores are also believed to be haploid. These assumptions seem plausible in the light of knowledge of the life history and cytology of other species (e.g. Lingappa, 1958b), and an essentially similar life cycle has been described for *S. lagenariae* and *S. trichosanthidis*, parasitic on Cucurbitaceae, which differ from *S. endobioticum* in that their resting spores function as prosori instead of prosporangia (Raghavendra Rao & Pavgi, 1993).

Control of wart disease

Control is based largely on the breeding of resistant varieties of potato. It was discovered that certain varieties such as Snowdrop were immune from the disease and could be planted on land heavily infected with *Synchytrium* without developing warts. Following this discovery, plant breeders have developed a number of immune varieties such as Maris Piper. However, some potato varieties that are susceptible to the disease are still widely grown, including the popular King Edward. In most countries where wart disease occurs, legislation has been introduced requiring that only approved immune varieties be planted on land where wart disease has been known to occur, and prohibiting the movement and sale of diseased material. Within the British Isles, the growing of immune varieties on infested land has prevented the spread of the disease, and it is now confined to a small number of foci in the West Midlands, northwest England and mid and south Scotland. It has also persisted in Newfoundland. The majority of the outbreaks are found in allotments, gardens and smallholdings.

The reaction of immune varieties to infection varies (Noble & Glynne, 1970). In some cases when 'immune' varieties are exposed to a heavy inoculum load of *S. endobioticum* in the laboratory, they may become slightly infected, but infection is often confined to the superficial tissues which are soon sloughed off. In the field such slight infections would probably pass unnoticed. Occasionally infections of certain potato varieties may result in the formation of resting spores, but without the formation of noticeable galls. Penetration of the parasite seems to occur in all potato varieties, but when a cell of an immune variety is penetrated it may die within a few hours, and since the fungus is a biotrophic parasite, further development is checked. In other cases the parasite may persist in the host cell for up to 2–3 days, apparently showing normal development, but after this time the fungal thallus undergoes disorganization and disappears from the host cell.

Unfortunately, it has been discovered that new physiological races (or pathotypes) of the pathogen have arisen, capable of attacking potato varieties previously thought to be immune. About 20 pathotypes are now known, and the implications are obvious. Unless their spread can be prevented, much of the work of potato plant breeders over the past century will have to be started all over again.

Other methods of control are less satisfactory. Attempts to kill the resting spores of the fungus in the soil have been made, but this is a costly and difficult process, requiring large-scale fungicide applications to the soil. Copper sulphate or ammonium thiocyanate have been applied in the past at amounts of up to 1 ton $acre^{-1}$, and local treatment with mercuric chloride or with formaldehyde and steam has been used to eradicate foci of infection (Hampson, 1988). Control measures based on the use of resistant varieties seem more satisfactory. An interesting method of control developed in Newfoundland is the use of crabshell meal placed above seed tubers at the time of planting. This technique has resulted in significant and sometimes complete control (Hampson & Coombes, 1991) which may be due to selective enhancement of chitinolytic soil micro-organisms degrading the chitinous walls of the resting spores of *S. endobioticum*.

Other species of *Synchytrium*

Not all species of *Synchytrium* show the same kind of life cycle as *S. endobioticum*. *Synchytrium fulgens*, a parasite of *Oenothera*, resembles *S. endobioticum*

in that both summer spores and resting spores are formed (Lingappa, 1958a,b), but in this species the zoospores from resting sporangia can also function as gametes and give rise directly to zygote infections from which further resting spores arise (Lingappa, 1958b). It has been suggested that the same phenomenon may occasionally occur in *S. endobioticum*. In *S. taraxaci* parasitic on *Taraxacum* (Fig. 6.8; Plate 3c), as well as a number of other *Synchytrium* spp., the mature thallus does not function as a prosorus but cleaves directly to form a sorus of sporangia, and the resting spore also gives rise to zoospores directly. In some species, e.g. *S. aecidioides*, resting sporangia are unknown, whilst in others, e.g. *S. mercurialis*, a common parasite on leaves and stems of *Mercurialis perennis* (Fig. 6.9), only resting sporangia are known and summer sporangial sori do not occur. *Mercurialis* plants collected from March to June often show yellowish blisters on leaves and stems. The blisters are galls made up of one or two layers of hypertrophied cells mostly lacking chlorophyll, surrounding the *Synchytrium* thallus during its maturation to form a resting sporangium. In this species the resting sporangium functions as a prosorus during the following spring. The undivided contents are extruded into a spherical sac which becomes cleaved into a sorus containing as many as 120 sporangia from which zoospores arise. The variations in the life histories of the various species of *Synchytrium* form a useful basis for classifying the genus (Karling, 1964).

6.2.2 *Rhizophydium*

Rhizophydium is a large, cosmopolitan genus of about 100 species (Sparrow, 1960) which grow in soil, freshwater and the sea. The thallus is eucarpic, with a globose epibiotic zoosporangium which develops from the zoospore cyst, and endobiotic rhizoids which penetrate the host. Whilst some species are saprotrophic, others are biotrophic pathogens of algae and can cause severe epidemics of freshwater phytoplankton. Saprotrophic forms such as *R. pollinispini* and *R. sphaerocarpon* colonize pollen grains and are easily isolated by sprinkling pollen onto the surface of water overlying soil (Fig. 6.10). Within 3 days, sporangia with exit papillae are

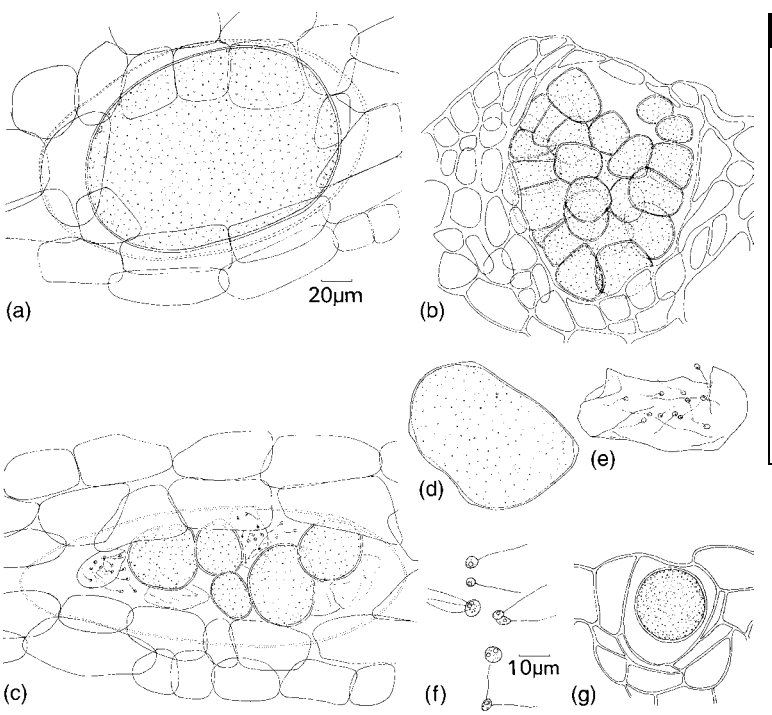

Fig 6.8 *Synchytrium taraxaci*.
(a) Undivided thallus in epidermal cell of scape of *Taraxacum*. Outline of host cell shown dotted. (b) Section of *Taraxacum* scape showing thallus divided into a sorus of sporangia. (c) A sorus of sporangia seen from above. Two sporangia are releasing zoospores. (d) A ripe sporangium. (e) Sporangium releasing zoospores. (f) Zoospores and zygotes. The triflagellate zoospore probably arose by incomplete separation of zoospore initials. (g) Section of host leaf showing a resting sporangium. (a–e) and (g) to same scale.

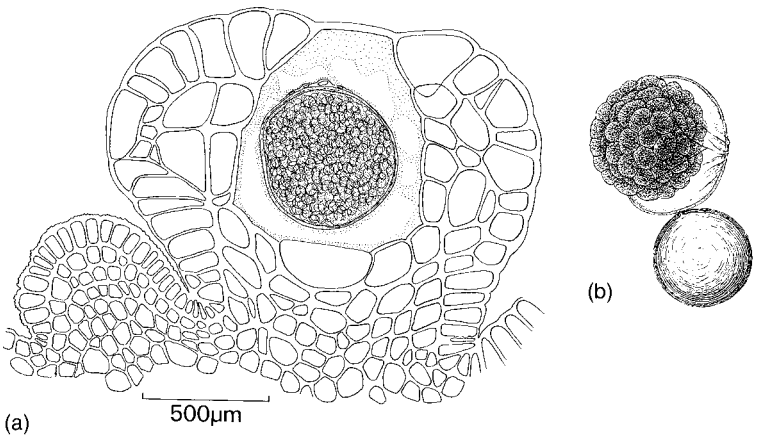

Fig 6.9 *Synchytrium mercurialis*. (a) Section of stem of *Mercurialis perennis* showing hypertrophied cells surrounding a resting sporangium. (b) Germination of a resting sporangium to release a sorus of zoosporangia. Thus in *S. mercurialis* the resting sporangium functions as a prosorus (after Fischer, 1892).

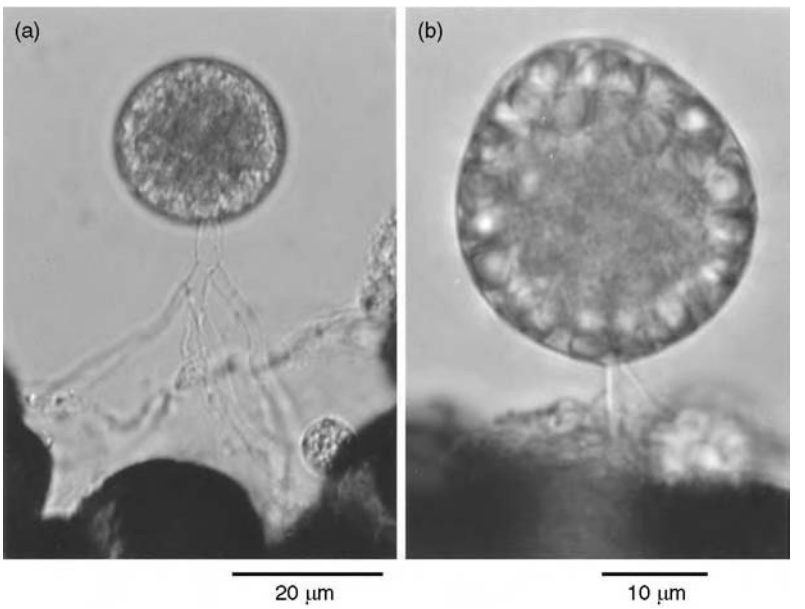

Fig 6.10 Pine pollen grains colonized by *Rhizophydium* sp. (a) The rhizoid system attaching the epibiotic sporangium to the colonized pollen grain. (b) Mature sporangium; the cytoplasm has become cleaved into numerous zoospores.

found in crude cultures on pine pollen. The zoospores are at first released into a hyaline vesicle which soon dissolves, allowing them to swim away. Gauriloff and Fuller (1987) have outlined techniques for growing *R. sphaerocarpon* in pure culture. This species can also grow parasitically on filaments of the green alga S*pirogyra*.

Douglas Lake (Michigan, USA) is surrounded by conifers shedding pollen which floats on the lake and becomes colonized by *Rhizophydium* spp. Using the MPN (most probable number) technique, Ulken and Sparrow (1968) have estimated that the number of chytrid propagules in the surface waters (epilimnion) can rise to over $900\,l^{-1}$ by late June. Some infected pollen grains sink through the hypolimnion to the mud at the floor of the lake. It is thought likely that these develop resting sporangia which survive the winter and provide inoculum to start off colonization of new pollen deposits in the following season.

Rhizophydium planktonicum

This species is the best-studied chytrid phytoplankton parasite. It is a biotrophic pathogen of

the diatom *Asterionella formosa*, an inhabitant of eutrophic lakes. This alga forms cartwheel-like colonies, the diatom frustules making up the spokes, cemented together by mucilage pads at the hub of the wheel. *Rhizophydium planktonicum* may form one to many thalli on each host cell (Fig. 6.11a). Dual cultures of the host and parasite have been established (Canter & Jaworski, 1978) and from such cultures a detailed picture of infection, development and zoospore structure has been built up (Beakes *et al*., 1993). Zoospores are attracted to the alga and encyst on it, forming monocentric rhizoidal thalli. The rhizoids penetrate between the girdle lamellae of the host (Fig. 6.11b). The rhizoids may extend throughout the whole length of the host cell and infection is often accompanied by loss of photosynthetic pigment, failure of cells to divide, and ultimately early death of the host cell. The zoospore is uninucleate and the nucleus is retained within the zoospore cyst, the rhizoids being devoid of nuclei. The zoospore cyst enlarges to form the sporangium. Synchronous nuclear divisions result in the formation of several nuclei lying within the cytoplasm, followed by the development of cleavage furrows which divide up the sporangial contents into zoospores. A septum develops at the base of the sporangium and, prior to cleavage, the upper part of the sporangium wall develops a thickened apical papilla which balloons out to form a vesicle into which the immobile zoospores are released. The complete cycle from infection to zoospore release depends on temperature and can be as short as 2–3 days. About 1–30 zoospores may be formed in a sporangium depending on the state of the host cells, in turn affected by external physical and chemical conditions. Breakdown of the vesicle allows the zoospores to swim away. No resting stage has been described for *R. planktonicum*.

A striking feature of the zoospore ultrastructure is the presence of several paracrystalline bodies near the nucleus in the peripheral part of the cytoplasm (Beakes *et al*., 1993). They consist of parallel arrays of regularly arranged crystals interconnected to each other with fibrous material. They appear late in sporangial development but disappear following encystment of zoospores. Similar structures have been reported from the zoospores of a few other Chytridiomycota, but their composition and function are unknown.

There have been several studies on the ecology of *Asterionella* subjected to parasitism by *R. planktonicum* (see Canter & Lund, 1948, 1953;

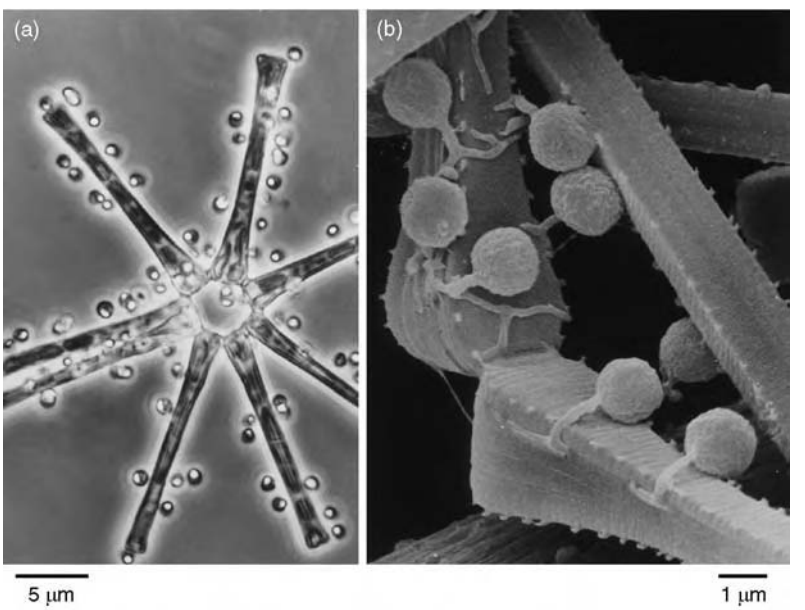

Fig 6.11 *Rhizophydium planktonicum* growing parasitically on the frustules of the colonial diatom *Asterionella formosa*. (a) Heavily infected colony from a dual-clone culture showing encysted zoospores. (b) Scanning electron micrograph of *Asterionella* cells showing heavy infection and zoospore cysts which have germinated and penetrated the host cells via the girdle lamellae. From Beakes *et al.* (1993), with permission from Elsevier; original images kindly provided by G.W. Beakes.

Canter & Jaworski, 1981; van Donk & Bruning, 1992). *Asterionella* is also parasitized by two other chytrids, *Zygorhizidium planktonicum* and *Z. affluens*, and some of the early studies in freshwater lakes may well have included a mixture of species.

Studies on the epidemiology of infection of *Asterionella* by *R. planktonicum* in lakes have shown that there are peak periods of *Asterionella* population density both in spring and in autumn, related to the availability of dissolved nutrients, water temperature, thermal stratification and its breakdown, daylength and light intensity. *Asterionella* cells infected with *Rhizophydium* can occur throughout the year, but epidemics in which a high proportion of cells are infected only occur at concentrations of around 10 host cells ml^{-1} (Holfeld, 1998). Interpretation of the conditions conducive to the occurrence of epidemics has been aided by experiments using dual cultures of pathogen and host in which effects such as light intensity, temperature and phosphorus concentration have been varied (van Donk & Bruning, 1992). The effects of light are complex. Although *Rhizophydium* zoospores are not phototropic, they are quiescent and incapable of infection in the dark or at low light intensity. Experiments by Canter and Jaworski (1981) have indicated that a light intensity below 200 lx is inadequate for zoospore settlement on host cells. In light-limited cultures of *Asterionella*, the sporangia of the pathogen and hence the number of zoospores produced are smaller than when light is not limiting (Bruning, 1991a). Similarly, zoospore production is also reduced when the concentration of phosphorus limits growth of the host (Bruning, 1991b). Temperature affects the rate of sporangium development and the size of sporangia, with maximum dimensions at 2°C at fairly high light intensities (Bruning, 1991a). It also affects the duration of swimming of zoospores and therefore their infective lifetime which can vary from about 10 days at 3°C to only 2 days at 20°C. Epidemic development may result from a combination of factors and there is a remarkable interaction between the effects of light intensity and temperature (Bruning, 1991c). At higher temperatures, optimal conditions for epidemic development occur at high light intensities, but at temperatures below 5–6°C epidemic development is encouraged by lower light intensities. This may explain why, in nature, epidemics can occur both in summer (high light intensity, high temperature) and winter (low light intensity, low temperature).

Rhizophydium planktonicum is a specialized parasite infecting only *Asterionella*. It is more compatible with certain clones of host cells than others, and cells from incompatible clones show hypersensitivity, undergoing rapid death following infection (Canter & Jaworski, 1979).

6.2.3 *Cladochytrium*

There are about a dozen species of *Cladochytrium* (Sparrow, 1960) which are widespread saprotrophs, mostly of aquatic plant debris. The thallus is eucarpic and polycentric and the vegetative system may bear intercalary swellings and septate **turbinate cells** (sometimes termed **spindle organs**). The sporangia are inoperculate. *Cladochytrium replicatum* is a common representative in decaying pieces of aquatic vegetation and can be distinguished from other chytrids by the bright orange lipid droplets found in the sporangia. It is frequently isolated if moribund aquatic vegetation is placed in a dish of water and baited with boiled grass leaves or cellulosic materials such as dialysis tubing. Lucarotti (1987) has given details of its isolation and growth in culture. The bright orange sporangia which are visible under a dissecting microscope appear on baits within about 5 days, arising from an extensively branched hyaline rhizomycelium bearing two-celled intercalary swellings. Sporangium development is encouraged by exposure to light. On release from the sporangium, the zoospores each contain a single orange lipid droplet and bear a single posterior flagellum. Lucarotti (1981) has described the fine structure of the zoospore. After swimming for a short time, the zoospore attaches itself to the surface of the substratum and puts out usually a single germ tube which can penetrate the tissues of the host plant. The germ tube expands to form an elliptical or cylindrical turbinate cell which is often later divided into two by a transverse septum (Fig. 6.12d). The

zoospore is uninucleate and during germination the single nucleus is transferred to the swollen turbinate cell which becomes a vegetative centre from which rhizoids are put out which in turn produce further turbinate cells (see Figs. 6.12b,d). Nuclear division is apparently confined to the turbinate cells, and although nuclei are transported through the rhizoidal system they are not resident there. The thallus so established branches profusely, and at certain points spherical zoosporangia form, either terminally or in intercalary positions. Sometimes one of the cells of a pair of turbinate cells swells and becomes transformed into a sporangium. In culture, both cells may be modified in this way. The spherical to pear-shaped zoosporangium undergoes progressive nuclear division, and the contents of the sporangium acquire a bright orange colour due to accumulation of lipid droplets containing the carotenoid lycopene. These lipid reserves are later found in the zoospores. Cleavage of the cytoplasm to form uninucleate zoospore initials follows. The zoospores escape through a narrow

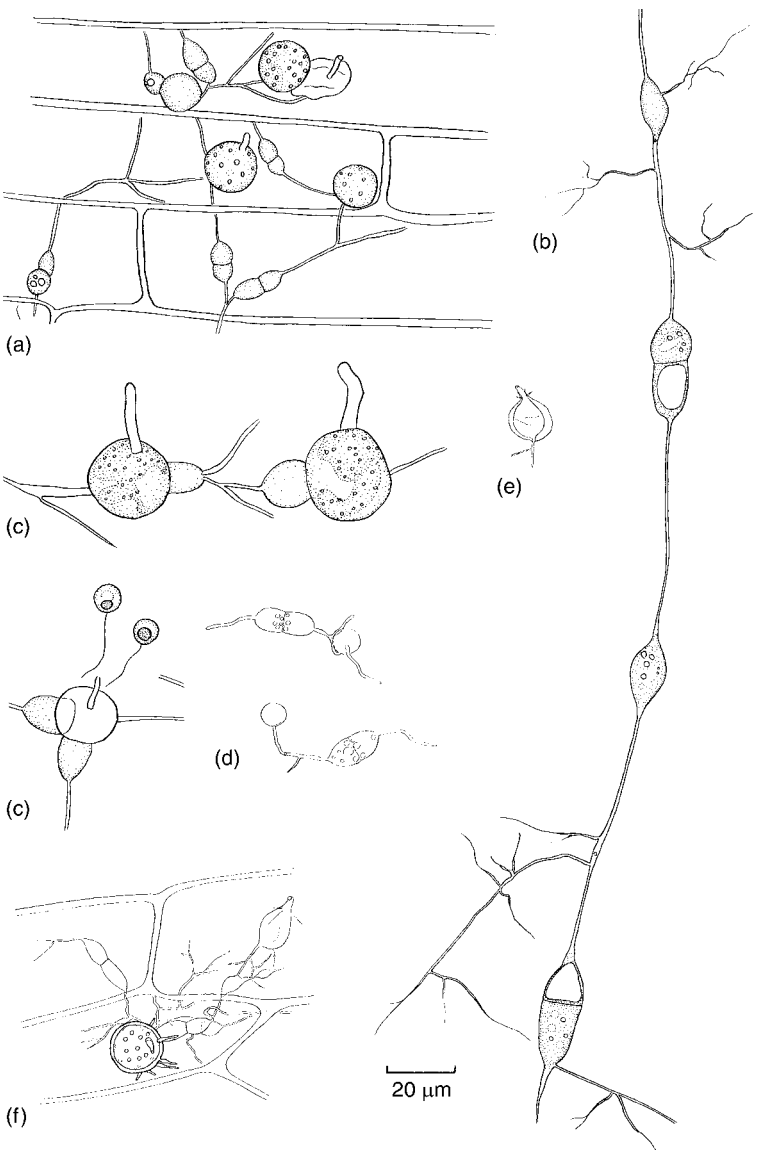

Fig 6.12 *Cladochytrium replicatum*. (a) Rhizomycelium within the epidermis of an aquatic plant bearing the two-celled hyaline turbinate cells and globose orange zoosporangia. (b) Rhizomycelium and turbinate cells from a culture. (c) Zoosporangia from a two-week-old culture. One zoosporangium has released zoospores, each of which contains a bright orange-coloured globule. (d) Germinating zoospores on boiled wheat leaves. The empty zoospore cysts are spherical. The germ tubes have expanded to form turbinate cells. (e) A zoosporangium which has proliferated internally to form a second sporangium. (f) Rhizomycelium within a boiled wheat leaf bearing a thick-walled, spiny resting sporangium.

exit tube which penetrates to the exterior of the substratum and becomes mucilaginous at the tip. There is no operculum. Sometimes zoosporangia may proliferate internally, a new zoosporangium being formed inside the wall of an empty one. Resting sporangia with thicker walls and a more hyaline cytoplasm are also formed either terminally or in an intercalary position on the rhizomycelium. In some cases the wall of the resting sporangium is reported to be smooth and in others spiny, and it has been suggested (Sparrow, 1960) that the two kinds of resting sporangia may belong to different species. However, studies by Willoughby (1962) of a number of single-spore isolates have shown that the presence or absence of spines is a variable character. The contents of the resting sporangia divide to form zoospores which also have a conspicuous orange droplet, and escape by means of an exit tube as in the thin-walled zoosporangia. Whether the resting sporangia are formed as a result of a sexual process is not known. Pure cultures of *C. replicatum* have been studied by Willoughby (1962), Goldstein (1960) and Lucarotti (1981). The fungus is heterotrophic for thiamine. Biotin, while not absolutely required, stimulates growth. Nitrate and sulphate are utilized, as are a number of different carbohydrates; a limited amount of growth takes place on cellulose.

6.2.4 *Nowakowskiella*

Species of *Nowakowskiella* are widespread saprotrophs in soil and on decaying aquatic plant debris, and can be obtained by baiting aquatic plant remains in water with boiled grass leaves, cellophane, dialysis tubing and the like. *Nowakowskiella elegans* is often encountered in such material, and pure cultures can be obtained and grown on cellulosic materials overlying agar, or directly in liquid culture media (Emerson, 1958; Johnson, 1977; Lucarotti, 1981; Lucarotti & Wilson, 1987). In culture, considerable variation in growth habit and morphology can result from changing the concentration of nutrients and the availability of water (Johnson, 1977). In boiled grass leaves the fungus forms an extensive rhizomycelium with turbinate cells (Fig. 6.13c). Zoosporangia are formed terminally or in an intercalary position (Fig. 6.13c) and are globose or pear-shaped with a subsporangial swelling (apophysis), and granular or refractile hyaline contents. At maturity some sporangia develop a prominent beak, but in others this is not present. When an operculum becomes detached, zoospores escape and initially remain clumped together at the mouth of the sporangium (Figs. 6.13b,c). The fine structure of the zoospore is very similar to that of *Rhizophydium* but paracrystalline bodies have not been observed (Lucarotti, 1981). It also has close resemblance to the zoospore ultrastructure of the inoperculate, polycentric *Cladochytrium replicatum*.

Yellowish resting sporangia (Fig. 6.13e) have been described (Emerson, 1958; Johnson, 1977; Lucarotti & Wilson, 1987). They develop as spherical to fusiform swellings in the rhizomycelium which become delimited by septa, develop thick walls and a large central vacuole surrounded by dense cytoplasm with small spherical lipid droplets. The resting sporangium is at first binucleate. After nuclear fusion the diploid nucleus divides meiotically. Further nuclear divisions are mitotic and the contents of the resting sporangium cleave into zoospores which may be released through a papilla in the sporangium wall. Alternatively, the resting sporangium may give rise to a thin-walled zoosporangium from which the zoospores are released, i.e. the resting sporangium may function as a prosporangium as in some other chytrids (Johnson, 1977).

In *N. profusa*, which is probably synonymous with *N. elegans* (Johnson, 1977), three kinds of sporangial dehiscence have been described: exo-operculate, in which the operculum breaks away to the outside of the sporangium; endo-operculate, in which the operculum remains within the sporangium; and inoperculate, where the exit papilla opens without any clearly defined operculum (Chambers *et al*., 1967; Johnson, 1973). Such variations within a single chytrid strain add emphasis to criticisms of the value of dehiscence as a primary criterion in classification.

Goldstein (1961) has reported that *N. elegans* requires thiamine and can utilize nitrate,

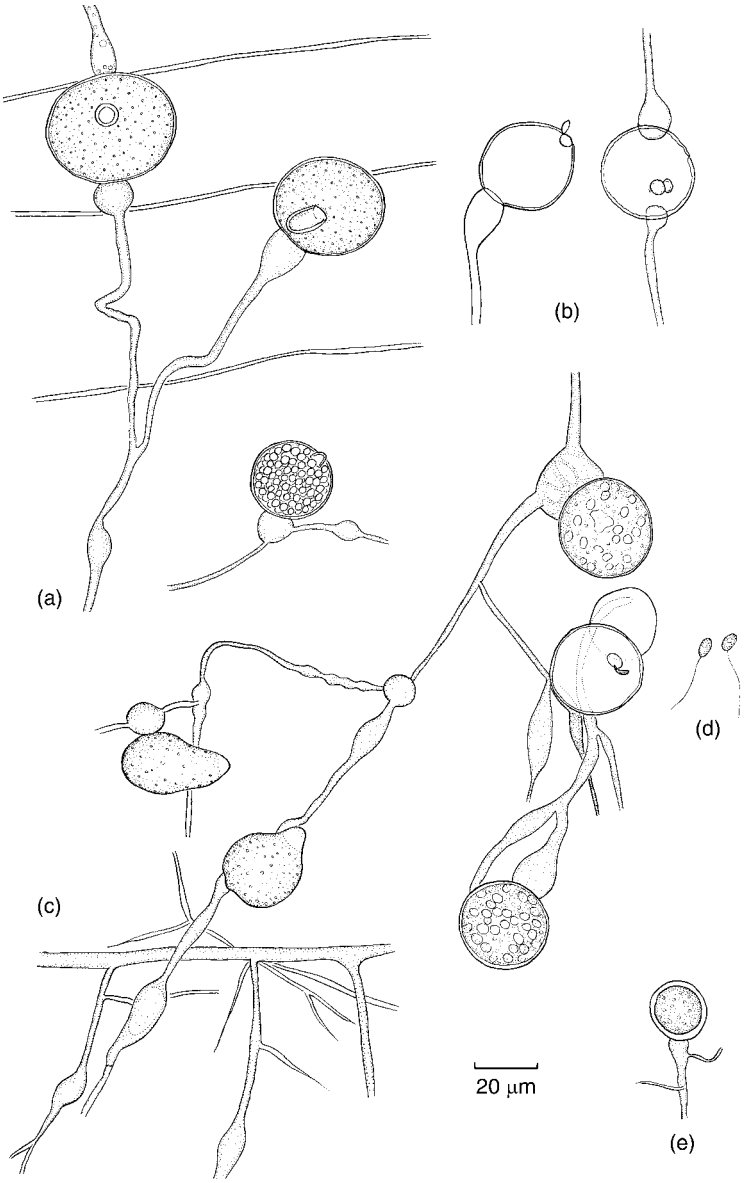

Fig 6.13 *Nowakowskiella elegans*. (a) Polycentric mycelium bearing zoosporangia. (b) Empty zoosporangia showing opercula. (c) Mycelium showing turbinate cells and zoosporangia. (d) Zoospores from culture. (e) Resting sporangium from culture.

sulphate and a number of carbohydrates including cellulose, but cannot utilize starch.

6.3 Spizellomycetales

Members of this order differ from the Chytridiales in possessing zoospores which contain more than one lipid droplet and are capable of limited amoeboid movement. Thalli are generally monocentric. The order takes its name from the genus *Spizellomyces* which in turn was named in honour of the chytrid pioneer F.K. Sparrow after *Spizella*, a genus of North American sparrows (Barr, 1980). Some 86 species of Spizellomycetales are currently recognized.

6.3.1 *Olpidium*

About 30 species of *Olpidium* are known, but the genus is in need of revision and possibly

some of the species should be classified elsewhere. Typical species are holocarpic. Some are parasitic on fungi and aquatic plants or algae, or saprotrophic on pollen (Sparrow, 1960). Others parasitize rotifers (Glockling, 1998), nematodes and their eggs (Tribe, 1977; Barron & Szijarto, 1986), moss protonemata or leaves and roots of higher plants (Macfarlane, 1968; Johnson, 1969). *Olpidium bornovanus* (= *O. radicale*) develops on various monocotyledonous and dicotyledonous plant roots following inoculation (Lange & Insunza, 1977). *Olpidium brassicae* is common on the roots of cabbages, especially when growing in wet soils, and is also found on a wide range of unrelated hosts, but some host specialization has been reported. Both *O. bornovanus* and *O. brassicae* are vectors of a number of plant viruses (Barr, 1988; Adams, 1991; Hiruki, 1994; Campbell, 1996) and this topic is discussed more fully below. Weber and Webster (2000a) have given practical details of how to grow *O. brassicae* for observation on *Brassica* seedlings. A film featuring *O. brassicae* is also available (Webster, 2006a).

Epidermal cells and root hairs of infected cabbage roots contain one or more spherical or cylindrical thalli, sometimes filling the whole cell (Fig. 6.14a). The cytoplasm of the thallus is granular and the entire contents divide into numerous posteriorly uniflagellate zoospores that escape through one or more discharge tubes which penetrate the outer wall of the host cell (Temmink & Campbell, 1968). Release of the zoospores takes place within a few minutes of washing the roots free from soil. The tip of the discharge tube breaks down and zoospores rush out and swim actively in the water. The zoospores are very small, tadpole-like, with

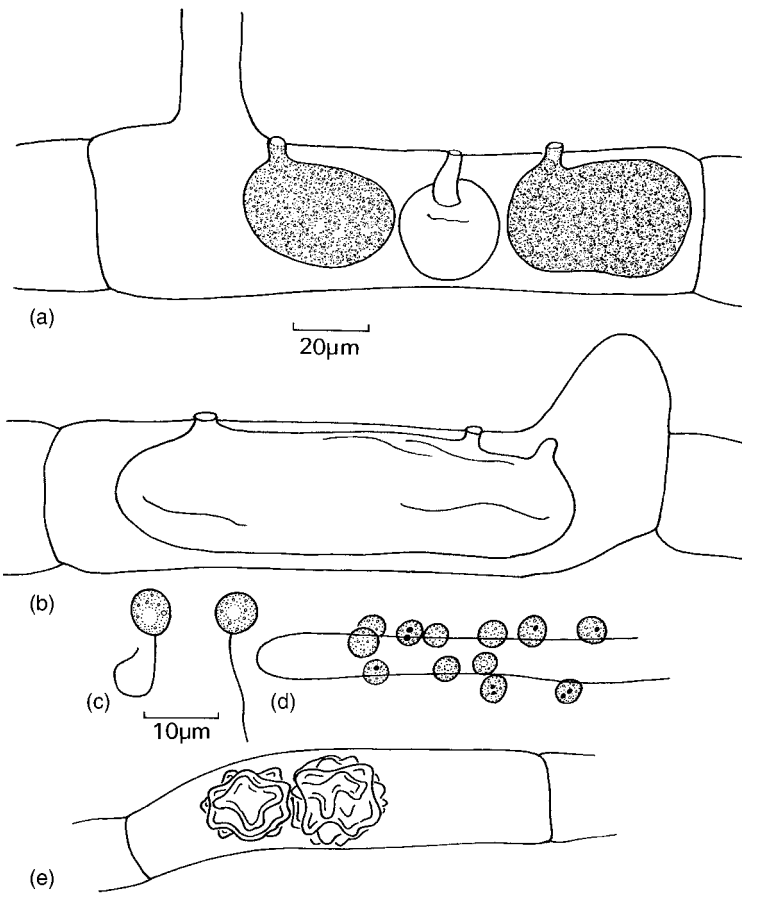

Fig 6.14 *Olpidium brassicae* in cabbage roots. (a) Two ripe sporangia and one empty sporangium in an epidermal cell. Each sporangium has a single exit tube. (b) Empty sporangium showing three exit tubes. (c) Zoospores. (d) Zoospore cysts on a root hair. Note that some cysts are uninucleate and some are binucleate. (e) Resting sporangia. (a,b,d,e) to same scale.

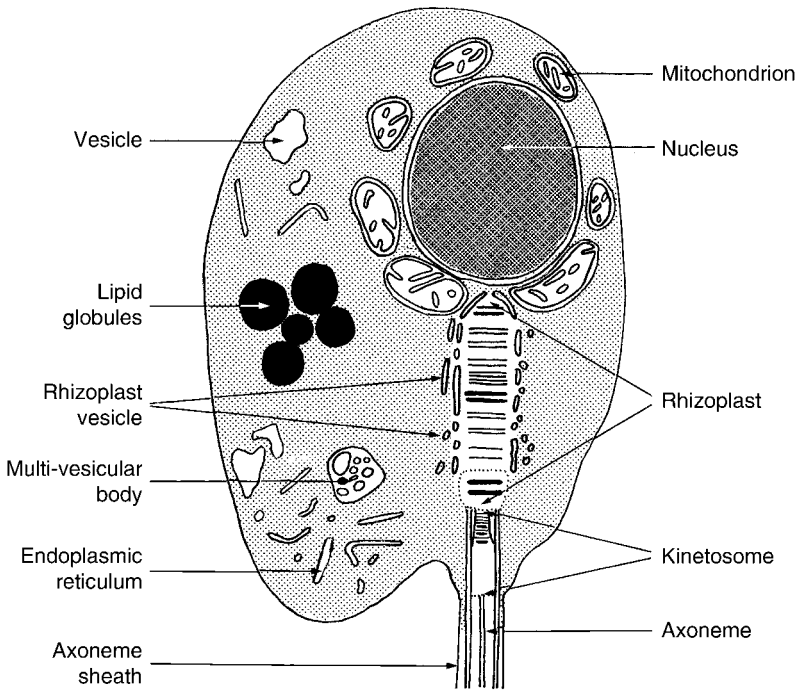

Fig 6.15 *Olpidium brassicae*. Diagrammatic representation of L.S. of zoospore (after Temmink & Campbell, 1969a).

a spherical head and a long trailing flagellum. The fine structure of the zoospore is summarized in Fig 6.15. A distinctive feature is the banded rhizoplast which connects the kinetosome to the nucleus (Temmink & Campbell, 1969a; Lange & Olson, 1976a,b; Barr & Hartmann, 1977). This structure has also been reported from the zoospore of the eucarpic chytrid *Rhizophlyctis rosea* (see p. 148; Barr & Hartmann, 1977).

The zoospores swim actively in water for about 20 min. If roots of cabbage seedlings are placed in a suspension of zoospores, these settle on the root hairs and epidermal cells, withdraw their flagella and encyst. The cysts are attached by a slime-like adhesive (Temmink & Campbell, 1969b). The cyst wall and the root cell wall at the point of attachment are dissolved and the root cell is penetrated. The cyst contents are transferred to the inside of the host cell, probably by the enlargement of a vacuole which develops inside the cyst, whilst the empty cyst remains attached to the outside. The process of penetration can take place in less than one hour (Aist & Israel, 1977). Within 2 days of infection, small spherical thalli can be seen in the root hairs and epidermal cells of the root, carried around the cell by cytoplasmic streaming. The thalli enlarge and become multinucleate. Within 4–5 days discharge tubes develop and the thalli are ready to release zoospores.

In some infected roots, stellate bodies with thick folded walls, lacking discharge tubes, are also found (Fig. 6.14e). These are resting sporangia. There is no evidence that they are formed as a result of sexual fusion either in *O. brassicae* or in *O. bornovanus* (Barr, 1988). Although biflagellate zoospores may occur in *O. brassicae*, these possibly result from incomplete cleavage (Temmink & Campbell, 1968) and zoospores with as many as 6 flagella have been observed (Garrett & Tomlinson, 1967). The resting sporangia are capable of germination 7–10 days after they mature, and germinate by the formation of one or two exit papillae through which the zoospores escape.

Virus transmission by *Olpidium*

Several plant viruses are transmitted by zoospores of *Olpidium*. By analogy with plant virus transmission by aphids, Adams (1991) arbitrarily

distinguished viruses with non-persistent and persistent transmission by fungi, although Campbell (1996) objected to the use of these terms, distinguishing instead between viruses which can be acquired in vitro (i.e. outside the plant) and those that can only be acquired in vivo (within the host cell).

Tobacco necrosis virus (TNV) and cucumber necrosis virus (CNV) are non-persistent viruses which can be acquired in vitro by zoospores of *O. brassicae* or *O. bornovanus* (respectively). Virus particles (virions) are adsorbed onto the plasmalemma of the zoospore and onto the flagellar axonemal sheath which is continuous with it (Temmink *et al*., 1970). Binding seems to occur between the virus coat and specific molecules at the zoospore surface, possibly oligosaccharide side chains of proteins (Kakani *et al*., 2003; Rochon *et al*., 2004). When the flagellum is withdrawn into the body of the zoospore at encystment, virus particles are introduced into the fungal cytoplasm and are then transmitted into the plant upon infection. Air-dried roots containing TNV virus and *O. brassicae* resting sporangia, or living virus-infected roots with resting sporangia treated with 5N HCl, were incapable of transmitting virus even though the resting sporangia survived these treatments, indicating that TNV is not carried inside the resting sporangia (Campbell & Fry, 1966).

Lettuce big vein virus, LBVV, in contrast, is an example of the persistent type (Grogan *et al*., 1958). In this case it has been shown that the virus can persist in air-dried resting sporangia for 18–20 years (Campbell, 1985). Here the virions are acquired in vivo and they are present inside the zoospores which emerge from sporangia and resting sporangia (Campbell, 1996).

Classification of *Olpidium*

Although previously classified within the family Olpidiaceae in the order Chytridiales, D. J. S. Barr (2001) has placed *Olpidium* in the order Spizellomycetales along with *Rhizophlyctis* on the basis of similarities in zoospore structure. Ribosomal DNA sequence comparisons are inconclusive in that they do not show any close similarity between *Olpidium* and either *Chytridium* or *Spizellomyces* (Ward & Adams, 1998).

6.3.2 *Rhizophlyctis*

There are about 10 known species of *Rhizophlyctis* with monocentric eucarpic thalli, growing as saprotrophs on a variety of substrata in soil, freshwater and the sea. *Rhizophlyctis rosea* grows on cellulose-rich substrata in soil, and it probably plays an active but currently underestimated role in cellulose decay (Powell, 1993). It can survive for prolonged periods in dry soil, even when this is heated to 90°C for two days (Gleason *et al*., 2004) and, in fact, the recovery of *R. rosea* is greatly enhanced if soil samples are air-dried prior to isolation experiments (Willoughby, 2001). Willoughby (1998b) has estimated that over 1000 thallus-forming units could be recovered per gram of air-dry soil or leaf humus fragments from Provence, France. These numbers may arise from one or a few sporangia, since a single sporangium about 100 μm in diameter may discharge up to 30 000 zoospores. Mitchell and Deacon (1986) have shown that zoospores of *R. rosea* accumulate preferentially on cellulosic materials.

The fungus is readily isolated and grown in culture, and details of techniques have been provided by Stanier (1942), Barr (1987), Willoughby (1998b) and Weber and Webster (2000a). The placing of a small crumb of soil onto moist tissue paper or cellophane overlying agar containing mineral salts, or the floating of squares of cellophane on water containing a soil sample, are followed within a few days by the development of thalli with bright pink sporangia. The sporangia are attached to coarse rhizoids which arise at several points on the sporangial wall and extend throughout the cellulosic substratum, tapering to fine points. Extensive corrosion of the substrate underneath the thallus and rhizoids points at the secretion of powerful cellulases (Fig. 6.17).

Although the fungus is usually monocentric, there are also records of some polycentric isolates. When ripe, the sporangia have pink granular contents which differentiate into numerous uninucleate posteriorly uniflagellate

zoospores (Fig. 6.16a). One to several discharge tubes are formed, and the tip of each tube contains a clear mucilaginous plug which, prior to discharge, is exuded in a mass from the tip of the tube (Fig. 6.16c). While the plug of mucilage dissolves, the zoospores within the sporangium show active movement and then escape by swimming through the tube. In some specimens of *R. rosea* it has been found that a membrane may form over the cytoplasm at the base of the discharge tubes. If the sporangia do not discharge their spores immediately, the membrane may thicken. When spore discharge occurs, these thickened membranes can be seen floating free within the sporangia, and the term endo-operculum has been applied to them. The genus *Karlingia* was erected for forms possessing such endo-opercula, including *R. rosea*, which is therefore sometimes referred to as *Karlingia rosea*, but the validity of this separation is questionable because the presence or absence of endo-opercula is a variable character (Blackwell & Powell, 1999).

Zoospores of *R. rosea* are capable of swimming for several hours. The head of the zoospore is often globose, but can become pear-shaped or show amoeboid changes in shape. It contains a prominent lipid body, several bright refringent globules, and bears a single trailing flagellum. Ultrastructural details resemble those of *Olpidium brassicae* in the presence of a striated rhizoplast connecting kinetosome and nucleus (Barr & Hartmann, 1977). On coming to rest on a suitable substratum, the flagellum is withdrawn and the body of the zoospore enlarges to form the rudiment of the sporangium, whilst rhizoids appear at various points on its surface. Within the sporangium, the flagella are tightly wrapped around the zoospores (Chambers & Willoughby, 1964).

Resting sporangia are also found. They are brown, globose or angular and have a thickened

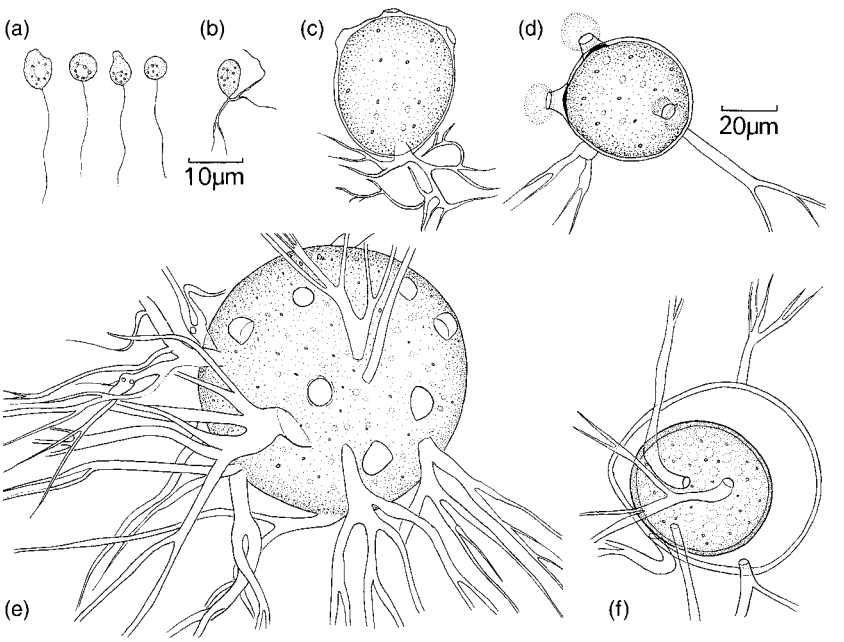

Fig 6.16 *Rhizophlyctis rosea*. (a) Zoospores. (b) Young thallus formed on germination of zoospore. The zoospore cyst has enlarged and will form the sporangium. (c) Older sporangium showing three discharge tubes. (d) Sporangium showing mucilage plugs at the tips of the discharge tubes and thickenings of the cell membrane at the bases of the tubes. Such thickenings are termed endo-opercula. (e) Globose sporangium and seven visible papillae. (f) Resting sporangium formed inside an empty zoosporangium. (a,b) to same scale; (c–f) to same scale.

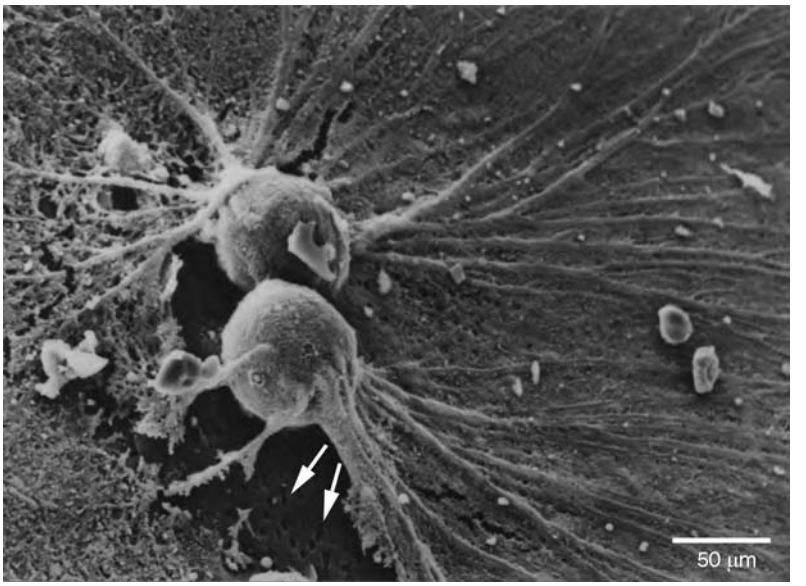

Fig 6.17 Scanning electron micrograph of two thalli of *Rhizophlyctis rosea* on a cellophane membrane. Pit corrosion is visible where a thallus has been lifted from the substratum (arrows).

wall (Fig. 6.16f). Whether they are formed sexually in *R. rosea* is not known. Couch (1939) has, however, put forward evidence that the fungus is heterothallic because single isolates grown in culture failed to produce resting sporangia whereas these structures did form when certain cultures were paired. Stanier (1942) has reported the occurrence of biflagellate zoospores, but whether these represented zygotes seemed doubtful. In the homothallic chitinophilic fungus *Rhizophlyctis oceanis*, Karling (1969) has described frequent fusions between zoospores. These fusions are possibly sexual, but unfortunately Karling was unable to cultivate the resulting thalli to the stage of resting spore development.

On germination, the resting sporangium of *R. rosea* functions as a prosporangium, although it is uncertain whether resting sporangia are important for survival in nature. Willoughby (2001) has shown that *R. rosea* could be recovered from cellophane baits in as little as 5–6 h after placing air-dried soil samples in water, and it was concluded that these zoospores were derived from sporangia instead of resting spores which need a longer time to produce zoospores.

The nutritional requirements of *R. rosea* are simple. It shows vigorous growth on cellulose as the sole carbon source but it can utilize a range of carbohydrates such as glucose, cellobiose and starch. The pink colour of the sporangia is due to the presence of carotenoid pigments such as γ-carotene, lycopene and a xanthophyll.

6.4 Neocallimastigales (rumen fungi)

A very interesting and unusual group of zoosporic fungi inhabits the rumens (foreguts) of ruminants (herbivorous mammals which regurgitate and masticate previously ingested food) like cows, sheep and deer. They have also been found in some non-ruminants such as horses and elephants and probably occur in the guts of many large herbivores. These fungi are obligate anaerobes which can flourish in the rumen because oxygen is depleted there by the intense respiratory activity of a dense population of protozoa and bacteria, some of which are facultative anaerobes capable of scavenging free oxygen. Their zoospores were at first thought to be protozoa and were not recognized as belonging to fungi because obligately anaerobic fungi

were not believed to exist. Further, microbiologists working on microbes from the ruminant gut studied only strained rumen fluid and therefore failed to see the thalli of fungi attached to herbage fragments. The view that the motile cells swimming in rumen fluid belonged to flagellates was challenged by Orpin (1974), who observed that there was an enormous increase in the concentration of 'flagellates' in the rumen of sheep within a short time of feeding. The ratio of minimum (pre-feeding) to maximum concentration of motile cells could vary between 1:15 and 1:296 (average 1:47), and if these were organisms reproducing by binary fission it would be necessary for them to undergo six successive cell divisions in 15 min. The explanation for the rapid increase in motile cells is that sedentary fungal thalli, anchored by rhizoids to partially digested food fragments floating in the rumen, are stimulated to release zoospores by soluble substances such as haems released from the newly ingested food material. The zoospores attach themselves in large numbers to the herbage fragments, and germinate to form rhizoidal or rhizomycelial thalli with sporangia capable of releasing further zoospores within about 30 h.

Some 5 genera and 15 species have now been distinguished (Theodorou *et al*., 1992, 1996; Trinci *et al*., 1994). They include *Caecomyces* which has mono- and polycentric thalli, *Anaeromyces* and *Orpinomyces* with polycentric thalli, and *Piromyces* and *Neocallimastix* which are monocentric. The zoospores of *Anaeromyces*, *Caecomyces* and *Piromyces* are uniflagellate whilst those of *Neocallimastix* and *Orpinomyces* are multiflagellate (see Fig. 6.18). They were classified within the order Spizellomycetales, family Callimasticaceae by Heath *et al*. (1983) and Barr *et al*. (1989) but are now placed in a separate order Neocallimastigales (Li *et al*., 1993; D. J. S. Barr, 2001). Special techniques and media are needed for isolating and handling anaerobic fungi, but the life cycle details of several have now been followed in pure culture. One of the best known is *N. hurleyensis*, isolated from sheep (Fig. 6.18). Minutes after the arrival of fresh food, globose ripe zoosporangia on previously colonized grass fragments release zoospores through an apical pore and these attach themselves to herbage fragments and germinate to produce rhizoids which penetrate and digest the ingested plant material. The walls of the thallus contain chitin. A single zoosporangium develops and is cut off from the rhizoidal system by a septum. The rhizoidal part is devoid of nuclei, but within the zoosporangium repeated nuclear divisions occur before the cytoplasm cleaves to form 64–128 zoospores. The life cycle of *N. hurleyensis* from zoospore germination to the release of a fresh crop of zoospores lasts about 29–31 h at 39°C (Lowe *et al*., 1987a). The zoospores bear 8–16 whiplash flagella inserted posteriorly in two rows.

The ultrastructure of zoospores has been described for several species of *Neocallimastix*, including *N. patriciarum* (Orpin & Munn, 1986), *N. frontalis* (Munn *et al*., 1981; Heath *et al*., 1983) and *N. hurleyensis* (Webb & Theodorou, 1988, 1991). There are differences in detail. For example, the zoospore of *N. frontalis* has a waist-like constriction, with the majority of the cytoplasmic organelles concentrated in the posterior portion near the insertion of the flagella. Characteristic organelles known from zoospores of aerobic chytridiomycetes such as mitochondria, Golgi bodies, lipid droplets or gamma particles (seen in zoospores of *Blastocladiella emersonii*; Fig. 6.19) are absent. In the posterior portion of the zoospore of *N. hurleyensis* near the point of insertion of the flagella, an irregularly shaped complex structure interpreted as a **hydrogenosome** has been reported in place of a mitochondrion. In zoospores of *N. patriciarum* there are many presumed hydrogenosomes concentrated around the region of flagellar insertion. Hydrogenosomes are organelles capable of the anaerobic metabolism of hexoses to acetic and formic acids. Protons (H^+) act as electron acceptors, so that gaseous H_2 is released by the activity of the enzyme hydrogenase (Müller, 1993; Boxma *et al*., 2004). The hydrogen, in turn, is used by anaerobic methanogenic bacteria to reduce CO_2 to CH_4 (methane) which escapes in profusion through the front and hind exits of the ruminant digestive tracts. Hydrogenosomes are found in several anaerobic lower eukaryotes and are believed to be derived

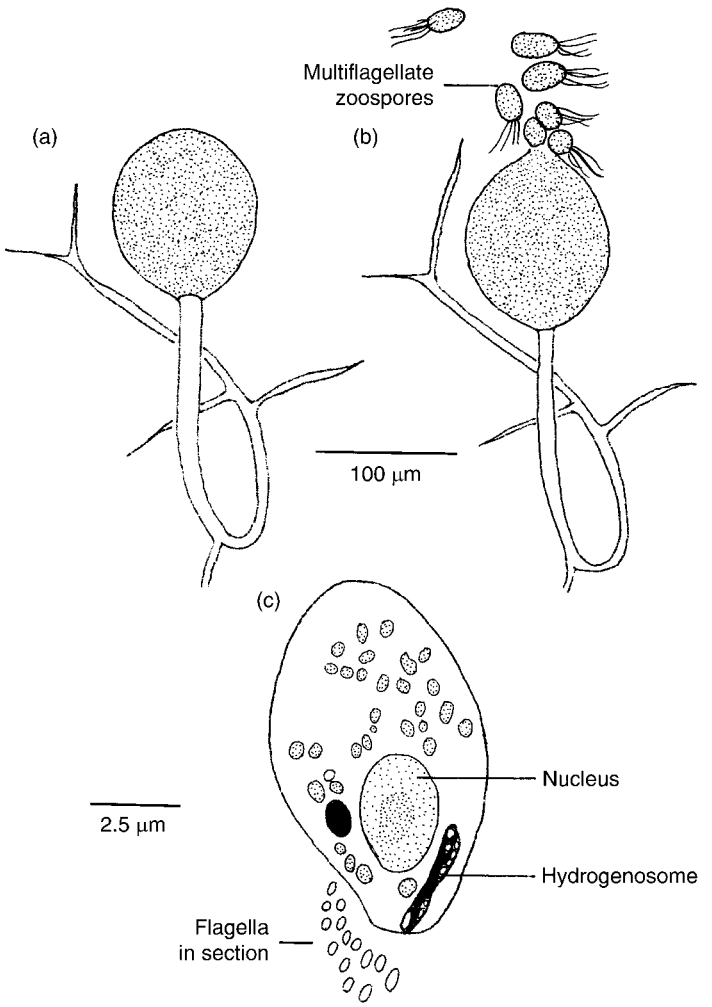

Fig 6.18 *Neocallimastix hurleyensis*. (a) Rhizoidal thallus with zoosporangium. (b) Release of zoospores. (c) Tracing of T.E.M. of zoospore with 14 flagella in longitudinal section. Diagrams based on Webb and Theodorou (1991).

from mitochondria (Embley *et al*., 2002). Whereas mitochondria of most fungi contain a limited amount of DNA, hydrogenosomes of rumen chytrids seem to have lost their genome altogether (Bullerwell & Lang, 2005).

Granular inclusion bodies which contain aggregates of ribosome-like particles and also free ribosome-like arrays are found anterior to the nucleus. Rosettes of glycogen represent the energy reserve of the zoospore. The shafts of the flagella contain the familiar eukaryotic $9 + 2$ arrangement of microtubules, but in *N. frontalis* the microtubules do not extend into the tips of the flagella which are narrower than the proximal part.

Ecologically, these anaerobic fungi play an important role in the early colonization of ingested herbage and have a wide range of enzymes which enable them to utilize monosaccharides, disaccharides and polysaccharides such as xylan, cellulose, starch and glycogen (Theodorou *et al*., 1992). They may play an active role in fibre breakdown. It is likely that colonization of straw particles by these fungi aids further attack by bacteria. The survival of anaerobic fungi outside the unusual and protective environment of the herbivore gut occurs in dried faeces in the form of cysts or as melanized thick-walled thalli whilst transmission to young animals takes place in saliva during licking

and grooming (Lowe *et al*., 1987b; Wubah *et al*., 1991; Theodorou *et al*., 1992).

6.5 Blastocladiales

6.5.1 Introduction

Species belonging to the Blastocladiales are mostly saprotrophs in soil, water, mud or aquatic plant and animal debris, and some are pathogens of plants, invertebrate animals or fungi. Most are obligate aerobes, but *Blastocladia* spp. are facultatively anaerobic, requiring a fermentable substrate and growing on submerged fleshy fruits, twigs or other plant materials rich in soluble carbohydrates (Emerson & Robertson, 1974). The life cycles of Blastocladiales show great variations and in some forms there is an alternation of distinct haploid **gametothallic** and diploid **sporothallic** generations. These terms are used in preference to the botanical terms gametophytic and sporophytic. Species of *Physoderma*, previously grouped with the Chytridiales (Lange & Olson, 1980), are biotrophic parasites of higher plants (Karling, 1950). They include *P. maydis*, the cause of brown spot of maize, and *P. alfalfae* (Lange *et al*., 1987). One genus, *Coelomomyces*, consists of obligate parasites of insects, usually mosquito larvae (Couch & Bland, 1985). This genus is unusual in that the vegetative thallus is a wall-less plasmodium-like structure lacking rhizoids. The life cycle is completed in unrelated alternate animal hosts, sporothalli occurring in mosquito larvae (Insecta) and gametothalli in a copepod (Crustacea) (Whisler *et al*., 1975; Federici, 1977). Attempts are being made to use *Coelomomyces* in the biological control of mosquitoes. *Catenaria anguillulae*, a facultative parasite of nematodes and their eggs, liver fluke eggs and some other invertebrates, can be grown in culture (Couch, 1945; Barron, 1977; Barstow, 1987), whilst *Catenaria allomycis* is a biotrophic parasite of *Allomyces* (Couch, 1945; Sykes & Porter, 1980).

With the exception of *Coelomomyces*, the thallus of members of the Blastocladiales is eucarpic. The morphologically simpler forms such as *Blastocladiella* (Fig. 6.22) are monocentric, with a spherical or sac-like zoosporangium or resting sporangium arising directly or on a short one-celled stalk from a tuft of radiating rhizoids. These simpler types show considerable similarity to monocentric Chytridiales of other orders such as *Rhizophlyctis rosea* (Figs. 6.16 and 6.17), and in the vegetative state they may be difficult to distinguish. The more complex organisms such as *Allomyces* are polycentric, and the thallus is differentiated into a trunk-like portion which has rhizoids below whilst branching above, often dichotomously, and bearing sporangia of various kinds at the tips of the branches. Chitin has been demonstrated in the walls of *Allomyces* and *Blastocladiella* (Porter & Jaworski, 1966; Youatt, 1977; Maia, 1994).

The zoospore of Blastocladiales

The zoospore of Blastocladiales has a single posterior flagellum of the whiplash type. Details of the fine structure of this kind of zoospore have been reviewed by Fuller (1976) and Lange and Olson (1979). The best known are *Blastocladiella emersonii* (Cantino *et al*., 1963; Reichle & Fuller, 1967) and *Allomyces macrogynus* (Fuller & Olson, 1971). The structure of the zoospore of *B. emersonii* is summarized diagrammatically in Fig. 6.19. The zoospore is tadpole-like with a pear-shaped head about $7 \times 9\,\mu m$ and a single, trailing flagellum about $20\,\mu m$ long. Under the light microscope, the most conspicuous internal structure is the dense crescent-shaped nuclear cap which surrounds the more transparent nucleus. The nuclear cap is rich in RNA and protein, and is filled with ribosomes. The zoospore of *B. emersonii* is unusual in that it contains only a single large mitochondrion, situated near the flagellar kinetosome.

The organization of the flagellum is essentially as described on p. 129. The nine triplet microtubules extend in a funnel-shaped manner from the proximal end of the kinetosome towards the nucleus and nuclear cap, maintaining its conical shape. Extending into the mitochondrion and linking up the kinetosome

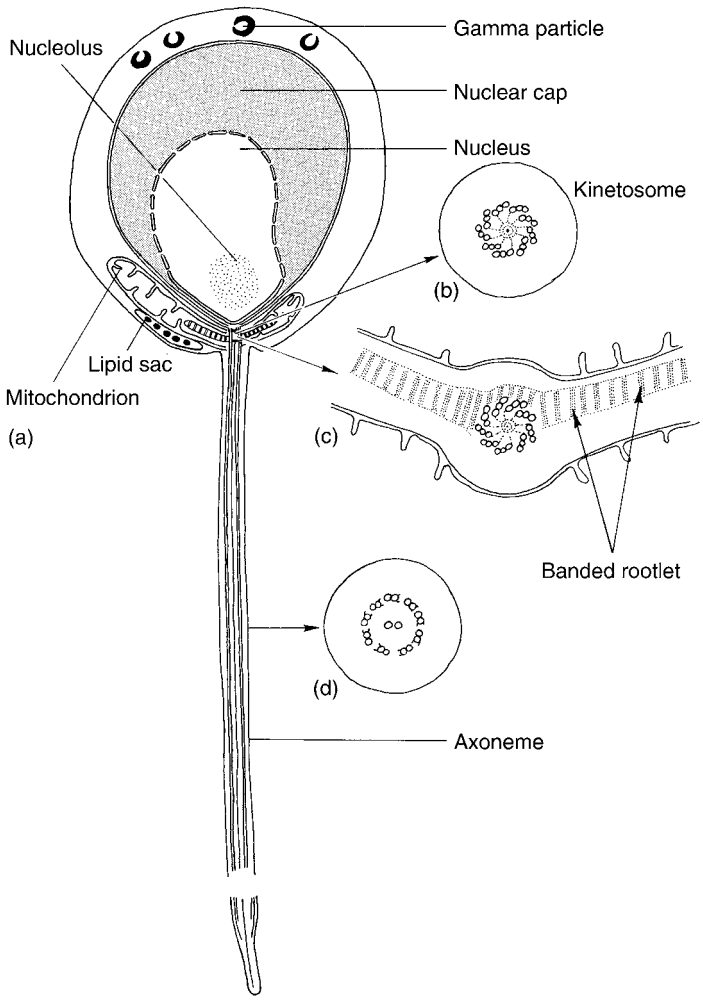

Fig 6.19 *Blastocladiella emersonii* zoospore, fine structure, diagrammatic and not to scale. (a) L.S. of zoospore along the axis of the flagellum. (b) T.S. of kinetosome showing nine triplets of microtubules. (c) T.S. of kinetosome at a slightly lower level showing the origin of two of the banded rootlets which extend into the mitochondrion. The cristae of the mitochondrion are close to the membrane which surrounds the banded rootlets. (d) T.S. of axoneme showing the nine paired peripheral microtubules and the two central microtubules.

with it are three striated bodies variously referred to as flagellar rootlets, striated rootlets or banded rootlets. They are contained within separate channels, and each is surrounded by a unit membrane. Since the energy for propulsion is generated within the mitochondrion, it is possible that the banded rootlets are, in some way, responsible for transmitting energy to the base of the axoneme. It is also possible that the banded rootlets serve to anchor the flagellum within the body of the zoospore.

There are two other obvious kinds of organelle within the body of the *Blastocladiella* zoospore. The **lipid sac** attached to the mitochondrion contains a group of lipid droplets which is surrounded by a unit membrane. It is not known whether lipid forms the energy reserve used in swimming, cytoplasmic glycogen deposits being a more plausible alternative (Cantino *et al*., 1968). In the anterior of the zoospore between the nuclear cap and the plasma membrane, there is a group of granules about 0.5 μm in diameter, called **gamma particles**. They consist of an inner core, shaped like an elongated cup and bearing two unequal openings at opposite sides of the cup. This cup-shaped structure is enveloped in a unit membrane (Myers & Cantino, 1974). Gamma particles are only present in developing and motile zoospores but disappear as the zoospore encysts. Formerly thought to represent the chytrid equivalent of the chitosome found in higher fungi (see p. 6), this notion has now been discarded (Hohn *et al*., 1984).

The zoospore of *Allomyces macrogynus* broadly resembles that of *B. emersonii* (Fuller & Olson, 1971). Gamma particles are present in the zoospore and, during encystment, these form vesicles which fuse with the plasma membrane. Fusion coincides with the appearance of wall material around the cyst (Barstow & Pommerville, 1980). The zoospore of *Allomyces* differs from that of *Blastocladiella* in some other ways. Although there is a large basal mitochondrion, many smaller mitochondria are also present, generally located along the membrane of the nuclear cap in the anterior part of the cell. A complex structure situated laterally at the base of the body of the zoospore, between the nucleus and the zoospore membrane, has been termed the **side body complex** by Fuller and Olson (1971). It consists of two closely appressed membranes separated by an electron-opaque material. These membranes subtend numerous electron-opaque, membrane-bound bodies, lipid bodies and a portion of the basal mitochondrion. In addition, there are membrane-bound non-lipid bodies termed **Stüben bodies** by Fuller and Olson (1971), whose function and composition are uncertain.

The zoospore is propelled forward by rhythmic lashing of the flagellum, and it can swim for a period even under anaerobic conditions. It is also capable of amoeboid changes of shape. On coming to rest, the flagellum is retracted into the body of the zoospore. There are different interpretations of the manner in which flagellar retraction is achieved. Cantino *et al*. (1968) have suggested that the flagellum is retracted by a revolving action of the nucleus, whereas in the 'lash-around' mechanism the flagellum coils around the body of the spore, the flagellar membrane fuses with the plasmalemma of the spore and the axoneme enters the spore cytoplasm (Olson, 1984). In *Allomyces*, the zoospore cyst produces, at one point, a narrow germ-tube which branches to form the rhizoidal system. At the opposite pole, the zoospore cyst forms a wider germ tube which gives rise to hyphae which branch and later bear sporangia. This **bipolar germination** pattern is a point of difference between the Blastocladiales and the Chytridiales, in which germination is typically unipolar. The rhizoids are strongly chemotropic and specialize in nutrient uptake and transport. An inwardly directed electrical current has been detected around the rhizoids, and an outwardly directed current around the hyphae and hyphal tips. The inward current at the rhizoids may be the consequence of localized proton-driven solute transport (de Silva *et al*., 1992).

Life cycles of Blastocladiales

A number of distinct life history patterns are found. In *Allomyces arbuscula*, for example, isomorphic alternation of haploid gametothallic and diploid sporothallic generations has been demonstrated. In *A. neo-moniliformis* (= *A. cystogenes*) there is no free-living sexual generation, but this stage is represented by a cyst (see below). In *A. anomalus*, only the asexual stage has been found in normal cultures, but experimental treatments may result in the development of sexual thalli. Similar variations in life cycles have been found in other genera such as *Blastocladiella*. A characteristic feature of the asexual thalli of the Blastocladiales is the presence of resting sporangia with chitinous, pitted walls impregnated with a dark brown, melanin-type pigment. The pits are inwardly directed conical pores in the wall. The inner ends of the pores abut against a smooth, colourless inner layer of wall material surrounding the cytoplasm (Skucas, 1967, 1968). The resting sporangia of *Allomyces* can remain viable for up to 30 years in dried soil. The ease with which certain members of the group can be grown in culture has facilitated extensive studies of their nutrition and physiology, and the results of some of these investigations are discussed below.

Four families have been recognized – Coelomomycetaceae, Catenariaceae, Physodermataceae and Blastocladiaceae – but of these we shall study only *Allomyces* and *Blastocladiella*, both representatives of the Blastocladiaceae.

6.5.2 *Allomyces*

Species of *Allomyces* are found in mud or soil of the tropics or subtropics, including desert soil,

and if dried samples of soil are placed in water and 'baited' with boiled hemp seeds, the baits may become colonized by zoospores. From such material, it is possible to obtain pure cultures by streaking or pipetting zoospores onto suitable agar media and to follow the complete life history of these fungi in the laboratory. Olson (1984) has given a full account of the taxonomy, life cycles, morphogenesis and genetics of different species of *Allomyces*, with practical details of how to grow and handle them. Good growth occurs on a medium containing yeast extract, peptone and soluble starch (YPsS), but chemically defined media have also been used. There is a requirement for thiamine and organic nitrogen in the form of amino acids.

Emerson (1941) isolated species of *Allomyces* from soil samples from all over the world. He distinguished three types of life history, represented by three subgenera.

Sub-genus *Eu-Allomyces*

The *Eu-Allomyces* type of life history is exemplified by *A. arbuscula* and *A. macrogynus* (Fig. 6.21; for a film, see Webster & Hard, 1998a). Resting sporangia are formed on asexual diploid thalli. They contain about 12 nuclei which undergo meiosis during the early stages of germination (Olson, 1974). The cytoplasm cleaves around the 48 haploid nuclei to form the zoospores. Since meiosis occurs in the resting sporangia, these have been termed **meiosporangia**, and the haploid zoospores **meiospores**. The meiospores are released when the outer wall of the brown pitted resting sporangium cracks open by a slit and the inner wall balloons outwards and eventually opens by one or more pores. The meiospores swim by movement of the trailing flagellum and, on coming to rest, encyst and germinate as described above to form a rhizoidal system and a trunk-like region which bears dichotomous branches.

The tips of the branches have been claimed to resemble the Spitzenkörper of higher fungi in being actin-rich, although secretory vesicles and/or microvesicles (chitosomes) have not been clearly shown (Srinivasan *et al*., 1996). Repeated nuclear division occurs to form a coenocytic structure, and finger-like ingrowths from the walls of the trunk-region and branches form incomplete septa, sometimes termed pseudosepta, with a pore in the centre through which cytoplasmic connections can be seen (Fig. 6.20d; Meyer & Fuller, 1985). The haploid thalli which develop from the meiospores are gametothallic, i.e. sexual. They are monoecious, and the tips of their branches swell to form paired sacs – the male and female gametangia. The male gametangia can be identified by the presence of a bright orange pigment, γ-carotene, whilst the female gametangia are colourless. In *A. arbuscula* the male gametangium is subterminal or **hypogynous**, i.e. beneath the terminal female gametangium, but in *A. macrogynus* the positions are reversed and the male gametangium is terminal or **epigynous** (Figs. 6.20e,i). The gametangia bear a number of colourless papillae on their walls, blocked by pulley-shaped plugs which eventually dissolve.

The contents of the gametangia differentiate into uninucleate gametes which differ in size and pigmentation. The female gametangium forms larger, colourless motile gametes (**swarmers**) whilst the male gametangium releases smaller, more active, orange-coloured swarmers. After escaping through the papillae in the walls of the gametangia, the gametes swim for a time and then pair off. A female gamete which fails to pair can function as a zoospore by germinating to form a new sexual thallus. A hormone, sirenin, is secreted by female gametangia during gametogenesis and by the released female gametes, and this stimulates a chemotactic response in male gametes at the extremely low concentration of 8×10^{-11} M (Machlis, 1972; Carlile, 1996a). The chemical structure of sirenin has been determined (Fig. 6.22), and both D- and L-forms have been synthesized. Only L-sirenin is active. It is a bicyclic sesquiterpene, probably derived from the parent hydrocarbon sesquicarene (Nutting *et al*., 1968; Plattner & Rapoport, 1971). A second hormone, parisin, which attracts female gametes, is secreted by male gametes. Its structure has not been determined, although it may well be related to sirenin (Pommerville & Olson, 1987).

The biflagellate zygote resulting from the fusion of two gametes may swim for a while

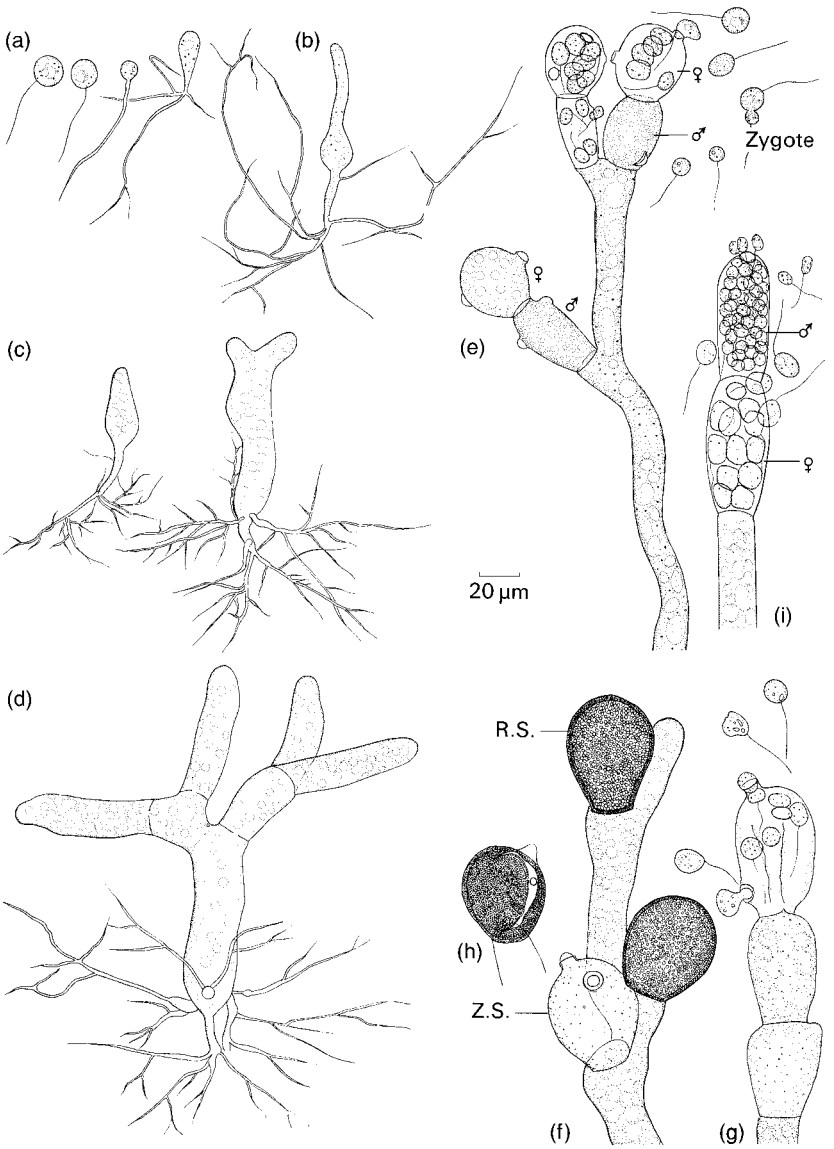

Fig 6.20 (a–h) *Allomyces arbuscula*. (a) Zoospores (haploid meiospores). (b) Young gametothalli, 24 h old. (c) Young sporothalli, 18 h old. (d) Sporothallus, 30 h old. Perforations are visible in some of the septa. (e) Gametangia at the tips of the branches of the gametothallus. Note the disparity in the size of the gametes (anisogamy). The smaller male gametes are orange in colour whilst the larger female gametes are colourless. Compare the hypogynous arrangement of the male gametangia with the epigynous arrangement in *A. macrogynus* shown at (i). (f) Meiosporangia (resting sporangia, R.S.) and mitosporangia (zoosporangia, Z.S.) on a sporothallus. (g) Release of mitospores from zoosporangia (= mitosporangia) on sporothallus. (h) Rupture of meiosporangium (= resting sporangium). (i) *Allomyces macrogynus*. Branch tip from gametothallus showing the arrangement of gametangia with terminal, epigynous male gametangia and anisogamous gametes.

before it encysts and casts off the flagella. Nuclear fusion then follows (Pommerville & Fuller, 1976). The zygote develops immediately into a diploid asexual thallus which differs from gametothalli in bearing two types of zoosporangia instead of gametangia. The first formed are thin-walled papillate zoosporangia formed singly or in rows at the tips of the

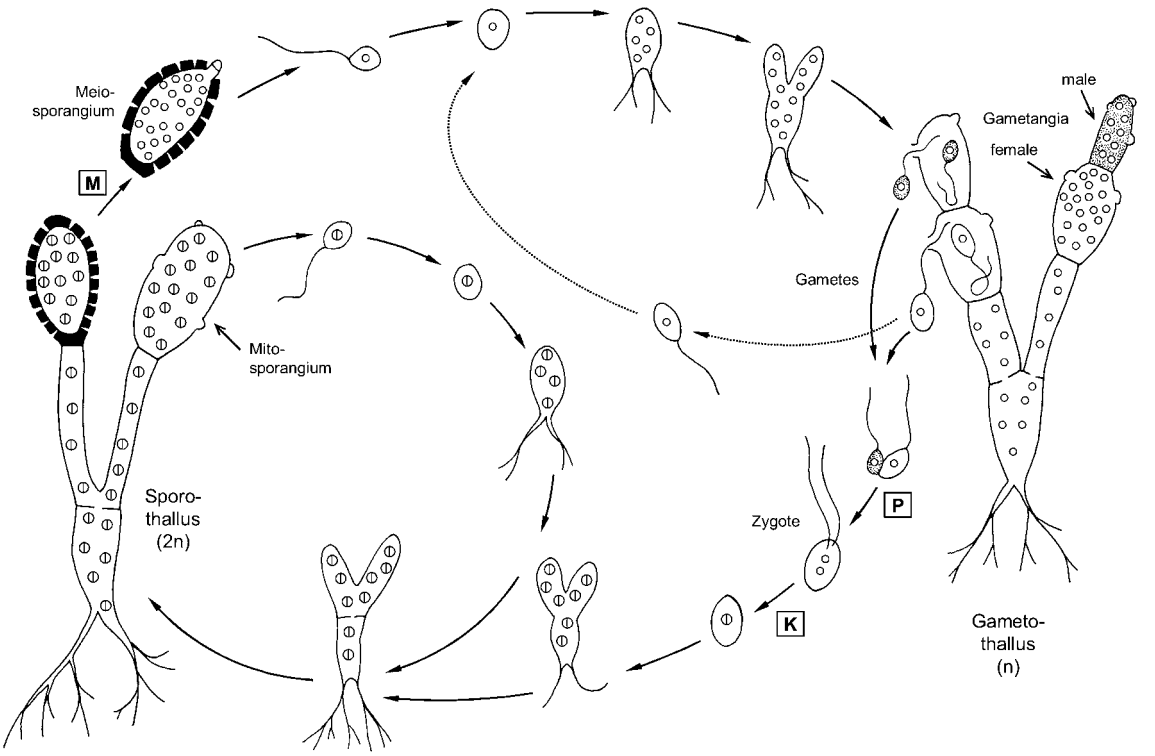

Fig 6.21 Life cycle diagram of *Eu-Allomyces* as exemplified by *A. macrogynus*. A diploid sporothallus may produce diploid mitospores from a colourless, thin-walled papillate mitosporangium, and haploid meiospores from a thick-walled pitted meiosporangium in which meiosis occurs. Meiospores germinate to form a haploid gamethothallus which produces two different gametangia and releases haploid gametes of two kinds, small carotenoid-rich (shaded) 'male' gametes and larger colourless 'female' ones. Upon copulation, a diploid zygote gives rise to a sporothallus. Alternatively, if failing to pair up, the female gametes may function as zoospores, in which case they give rise to a new gametothallus. Small open circles represent haploid nuclei whereas diploid nuclei are drawn larger and split. It should be noted that many field strains of *A. macrogynus* have a higher ploidy level, e.g. alternating between diploid (small circles) and tetraploid (large split circles) conditions. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M).

branches (Fig. 6.20g). Within these thin-walled sporangia the nuclei undergo mitosis. Initially the nuclei are arranged in the cortical region of the cytoplasm, but later they migrate and become uniformly spaced apart. Movement of the nuclei is controlled by forces generated by actin microfilaments whilst their spacing and positioning is controlled by microtubules (Lowry *et al*., 1998). Cleavage of the cytoplasm around the nuclei to form diploid colourless zoospores is initiated by the formation of membranes seen first at the plasmalemma, then extending into the cortex to form a complex membranous network (Fisher *et al*., 2000). The process of cytokinesis, i.e. the extension and fusion of membranes, seems to be mediated principally by the actin component of the cytoskeleton (Lowry *et al*., 2004). According to Barron and Hill (1974), the development of the cleavage

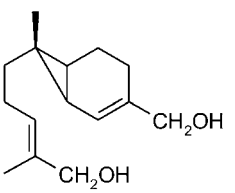

Fig 6.22 Chemical structure of the hormone L-sirenin which attracts male gametes of *Allomyces macrogynus*. The structure of parisin, attractive to female gametes, does not seem to have been elucidated as yet.

membranes is induced by the availability of free water. Zoospores are released from the sporangia after dissolution of the plugs blocking the exit papillae. Since nuclear division in the thin-walled sporangia is mitotic, these are termed **mitosporangia**, and the diploid swarmers they release are **mitospores**. The mitospores, after a swimming phase, encyst and are capable of immediate germination, developing into a further diploid asexual thallus.

The second type of zoosporangium is the dark brown, thick-walled, pitted resting sporangium (meiosporangium), formed at the tips of the branches. Meiotic divisions within these sporangia result in the formation of the haploid meiospores, which develop into sexual thalli. The life cycle of a member of the subgenus *Eu-Allomyces* is thus an isomorphic alternation of gametothallic and sporothallic generations (Fig. 6.21). Comparisons of the nutrition and physiology of the two generations show no essential distinction between them up to the point of production of gametangia or sporangia.

Emerson and Wilson (1954) have made cytological and genetic studies of a number of collections of *Allomyces*. Interspecific hybrids between *A. arbuscula* and *A. macrogynus* have been produced in the laboratory, and it has been shown that the fungus earlier described as *A. javanicus* is a naturally occurring hybrid between these two species. Cytological examination of the two parent species and of artificial and natural hybrids showed a great variation in chromosome number. In *A. arbuscula* the basic haploid chromosome number is 8, but strains with 16, 24 and 32 chromosomes have been found. In *A. macrogynus* the lowest haploid number encountered is 14, but strains with 28 and 56 chromosomes are also known. The demonstration that these two species each represent a polyploid series was the first to be made in fungi. The wild-type strain of *A. macrogynus* appears to be an autotetraploid which, after meiosis, produces diploid gametothalli (Olson & Reichle, 1978).

The behaviour of the hybrid strains is of considerable interest. As seen above, the parent species differ in the arrangement of the primary pairs of gametangia, *A. arbuscula* being hypogynous whilst *A. macrogynus* is epigynous. Following fusion of gametes derived from different parents, zygotes formed, germinated and gave rise to sporothalli. The meiospores from the hybrid sporothalli had a low viability (0.1−3.2%), as compared with a viability of about 63% for *A. arbuscula* meiospores, but some germinated to form gametothalli. The arrangement of the gametangia on these F_1 gametothalli showed a complete range from 100% epigyny to 100% hypogyny. Also, in certain gametothalli the ratio of male to female gametangia (normally about 1:1) was very high, with less than one female per 1000 male gametangia. It was concluded from these experiments that, since intermediate gametangial arrangements are found in hybrid haploids, this arrangement is not under the control of a single pair of non-duplicated allelic genes, but that a fairly large number of genes must be involved. Hybridization in some way upsets the mechanism which controls the arrangement of gametangia in the parental species. By treating meiospores of *A. macrogynus* with DNA extracted from gametothallic cultures of *A. arbuscula*, Ojha and Turian (1971) have demonstrated an inversion of the normal gametangial arrangement, i.e. a proportion of the DNA-treated meiospores developed colonies with hypogynous antheridia instead of the normal epigynous arrangement. Similar inversions were also obtained in converse experiments. In an isolate of the naturally occurring hybrid *A. javanicus*, Ji and Dayal (1971) have shown that although copulation between anisogamous gametes results in the formation of sporothalli bearing thin-walled and thick-walled sporangia, the swarmers from the thick-walled sporangia rarely develop into gametothalli, but into sporothalli. This is not surprising for a hybrid, and is possibly due to a failure of meiosis in the thick-walled sporangia.

Sub-genus *Cystogenes*

A life cycle different from *Eu-Allomyces* is found in *Allomyces moniliformis* and *A. neo-moniliformis*. There is no independent gametothallic generation, but this stage is probably represented by a cyst (C. M. Wilson, 1952). The asexual thalli resemble those of subgenus *Eu-Allomyces*, bearing

both thin-walled mitosporangia and brown, thick-walled, pitted meiosporangia. The mitospores encyst and germinate to form a further crop of asexual thalli. In the meiosporangium, meiosis takes place, but before cytoplasmic cleavage occurs, the haploid nuclei pair, the paired nuclei being united by a common nuclear cap. When cleavage does occur it therefore results in the formation of some 30 binucleate cells. When the meiosporangial wall cracks open, the binucleate cells are released as amoeboid bodies which may or may not bear flagella, and it is these cells which form the cysts. A mitotic division in each cyst results in four haploid nuclei, and cytoplasmic cleavage gives rise to four colourless uniflagellate isogametes. These copulate to form biflagellate zygotes, each of which can develop into an asexual sporothallus. In the *Cystogenes* life cycle there is thus a free-living diploid asexual sporothallic generation, whereas the haploid generation is reduced to the cysts and gametes.

Sub-genus *Brachy-Allomyces*

In certain isolates of *Allomyces* which have been placed in a 'form species' *A. anomalus*, there are neither sexual thalli nor cysts. Asexual thalli bear mitosporangia and brown resting sporangia. The spores from the resting sporangia develop directly to give asexual thalli again. The cytological explanation proposed by C. M. Wilson (1952) for this unusual behaviour is that, due to complete or partial failure of chromosome pairing in the resting sporangia, meiosis does not occur and nuclear divisions are mitotic. Consequently the zoospores produced from resting sporangia are diploid, like their parent thalli and, on germination, give rise to diploid asexual thalli again. Similar failures in chromosome pairing were also encountered in the hybrids between *A. arbuscula* and *A. macrogynus* leading to very low meiospore viability from certain crosses. In view of this it seemed possible that some of the forms of *A. anomalus* might have arisen through natural hybridization. In a later study, Wilson and Flanagan (1968) showed that there is a second way in which the life cycle of this fungus is maintained without a sexual phase. In certain isolates, meiosis does occur in the resting sporangia, followed by **apomixis**, i.e. the fusion of two meiosis-derived nuclei in the same thallus. Propagules from the resting sporangia are therefore diploid and the cysts develop into sporothalli. By germinating resting sporangia in dilute K_2HPO_4, a small percentage of zoospores were produced which developed into gametothalli, some of which were identified as *A. macrogynus* and some as *A. arbuscula*. No hybrids were found. Thus *A. anomalus* is not a single species, but represents sporothalli of these two species in which the normal alternation of generations has been upset by cytological deviations.

6.5.3 *Blastocladiella*

About a dozen species of *Blastocladiella* have been isolated from soil or water, and one is parasitic on the cyanobacterium *Anabaena* (Canter & Willoughby, 1964). The form of the thallus is comparatively simple, resembling that of some monocentric chytrids. There is an extensive branched rhizoidal system which is attached either to a sac-like sporangium or to a cylindrical trunk-like region bearing a single sporangium at the tip. In *B. emersonii* it has been shown that the rhizoids are chemotropic and function not only in attachment, but in absorption and selective translocation of nutrients (Kropf & Harold, 1982).

Different species of *Blastocladiella* have life cycles resembling those of the three subgenera of *Allomyces*, and Karling (1973) has proposed that *Blastocladiella* should similarly be divided into three subgenera, i.e. *Eucladiella* corresponding to *Eu-Allomyces*, *Cystocladiella* corresponding to *Cystogenes*, and *Blastocladiella* corresponding to *Brachy-Allomyces*. In some species there is an isomorphic alternation of generations, probably matching in essential features the *Eu-Allomyces* pattern, but cytological details are needed to confirm this. For example, in *Blastocladiella variabilis* two kinds of asexual thallus are found. One bears thin-walled zoosporangia which release posteriorly uniflagellate swarmers. These swarmers may develop to form thalli resembling their parents or may give rise to the second type of asexual thallus bearing

a thick-walled dark-brown sculptured resting sporangium within the terminal sac. The resting sporangium releases posteriorly uniflagellate swarmers which, after swimming, germinate to form sexual thalli of two kinds. About half of the sexual thalli are colourless ('female'), and about half are orange-coloured ('male'). However, in contrast to the anisogamy of *Eu-Allomyces*, in *Blastocladiella* there is no distinction in size between the gametes. The orange and colourless gametes pair to produce zygotes, which germinate directly to produce asexual thalli. In other species (e.g. *B. cystogena*) the life cycle is of the *Cystogenes* type, i.e. there are no gametothalli.

In yet other species there is no clear evidence of sexual fusion. In *B. emersonii* (Fig. 6.23), the resting sporangial thallus contains a single globose, dark reddish brown resting sporangium with a dimpled wall. Meiosis occurs during development of the resting sporangium (Olson & Reichle, 1978). After a resting period, the wall cracks open and one to four papillae protrude from which swarmers are released. The swarmers germinate to form two types of thallus bearing thin-walled zoosporangia. About 98% of the swarmers give rise to thalli bearing colourless sporangia (Fig. 6.23a), and about 2% to thalli with sporangia coloured orange due to the presence of γ-carotene. The colourless thalli develop rapidly and are ready to discharge zoospores within 24 h. These have about twice the DNA content as the swarmers released from resting sporangia (Horgen *et al*., 1985). Thus young colourless thalli are at first haploid, but release diploid zoospores. The manner in which the diploid state of the colourless thalli or of the resting sporangia is brought about is not known. The life cycle of *B. emersonii* thus corresponds to that of the sub-genus *Brachyallomyces*.

Blastocladiella emersonii has a number of other unusual features. If zoospore suspensions are pipetted onto yeast–peptone–glucose (YPG) agar, the majority of thalli which develop will be of the thin-walled colourless type. On the same medium containing 10 mM bicarbonate, resting sporangial thalli develop. The addition of 40–80 mM KCl, NaCl or NH_4Cl, or exposure of cultures to ultra-violet light, will similarly induce the formation of resting sporangia (Horgen & Griffin, 1969). Thus, by means of simple manipulation of the environment it is possible to switch the metabolic activities of the fungus into one of two morphogenetic

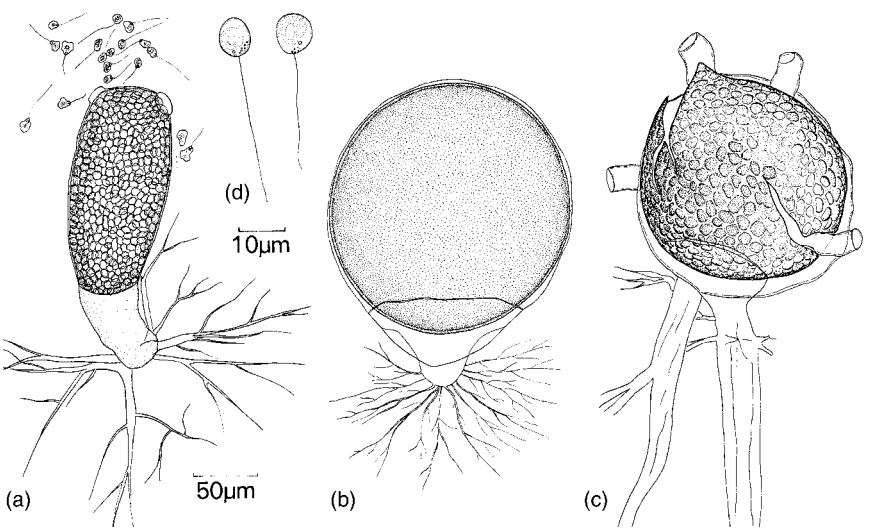

Fig 6.23 *Blastocladiella emersonii*. (a) Thin-walled thallus releasing zoospores. (b) Three-day-old thallus with immature resting sporangium. (c) Thallus with germinating resting sporangium showing the cracked wall and four exit tubes. (d) Zoospores from thin-walled thallus.

pathways. There are important differences in the activities of certain enzymes (Cantino *et al*., 1968; Lovett, 1975). In the absence of bicarbonate, there is evidence for the operation of a tricarboxylic acid cycle, whereas in the presence of bicarbonate, part of this cycle is reversed, leading to alternative pathways of primary carbon metabolism. In addition, a polyphenol oxidase, absent in the thin-walled thallus, replaces the normal cytochrome oxidase. There is also increased synthesis of melanin and of chitin in the presence of bicarbonate. The effect of bicarbonate can be brought about by increased levels of CO_2.

Another unusual feature is that *B. emersonii* fixes CO_2 more rapidly in the light than in the dark. In the presence of CO_2, light-grown thalli show a number of differences when compared with dark-grown controls. Illuminated thalli take about three hours longer to mature, and are larger than dark-grown thalli. They also have an increased rate of nuclear division and a higher nucleic acid content. The most effective wavelengths for this increased CO_2 fixation (or lumisynthesis) lie between 400 and 500 nm, i.e. at the blue end of the spectrum. This suggests that the photoreceptor should be a yellowish substance. Attempts to identify the photoreceptor have as yet been unsuccessful, but it is known not to be a carotenoid.

6.6 Monoblepharidales

This group includes about 20 species and is represented by 5 genera, namely *Monoblepharis*, *Monoblepharella*, *Gonapodya*, *Oedogoniomyces* and *Harpochytrium*. Fungi belonging to this order can be isolated from soil samples or from twigs or fruits submerged in freshwater, sometimes under anoxic conditions (Karling, 1977; Fuller & Clay, 1993). Whisler (1987) has given details of isolation techniques. Most species are saprotrophs and several are available in culture. In all genera the thallus is eucarpic either with rhizoids or a holdfast, and with branched or unbranched filaments. The walls contain microfibrils of chitin (Bartnicki-Garcia, 1968), but the walls of *G. prolifera* also contain cellulose (Fuller & Clay, 1993). A characteristic feature is the frothy or alveolate appearance of the cytoplasm caused by the presence of numerous vacuoles often arranged in a regular pattern. Asexual reproduction is by posteriorly uniflagellate zoospores which are borne in terminal, cylindrical or flask-shaped sporangia. Sexual reproduction, where known, is unique for fungi in being oogamous with a large egg and a smaller, posteriorly flagellate spermatozoid. The egg may be retained within the oogonium or may move to its mouth by amoeboid movement in some species of *Monoblepharis*, or propelled by the lashing of the flagellum of the spermatozoid in *Monoblepharella* and *Gonapodya*.

6.6.1 The zoospore

The fine structure of zoospores is similar in representatives of all five genera (Fig. 6.24; see Mollicone & Longcore, 1994, 1999). In all cases the body of the zoospore is oval, the narrow part facing forward and with a long whiplash flagellum trailing from the wider posterior. Amoeboid changes of shape may occur and swimming zoospores may develop pseudopodia anteriorly. The body of the zoospore is differentiated into three regions: an anterior region which is often devoid of organelles apart from lipid globules, a few vacuoles and tubular cisternae; a central region which contains the nucleus, surrounded by ribosomal aggregations (sometimes termed the nuclear cap), microbodies and spherical mitochondria with flattened cristae; and a posterior 'foamy' region at the base of which are the functional kinetosome, a non-functional kinetosome and a rumposomal complex. The functional kinetosome is surrounded by a striated disc, apparently anchored to annular cisternae. From an electron-dense region of the striated disc, about 31–34 microtubules extend outwards into the body of the zoospore. Water expulsion vacuoles have been identified in the anterior part of the zoospore of *G. prolifera*. Another distinctive feature in this fungus is the presence of a pair of paraxonemal structures, solid cylindrical fibres which are