4

Straminipila: minor fungal phyla

4.1 Introduction

The kingdom **Chromista** was erected by Cavalier-Smith (1981, 1986) to accommodate eukaryotic organisms which are distinguishable from the Protozoa by a combination of characters. Some of these are concerned with details of photosynthesis, such as the enclosure of chloroplasts in sheets of endoplasmic reticulum, and the absence of chlorophyll *b*, the latter feature being used for the naming of the kingdom. Other defining characters apply also to the non-photosynthetic members of the Chromista (Kirk *et al*., 2001). These are as follows:

1. The structural cell wall polymer is cellulose, in contrast to walls of Eumycota which contain chitin.

2. The inner mitochondrial membrane is folded into tubular cristae (Fig. 4.1a) which are also found in plants. In contrast, mitochondrial cristae are generally lamellate in the kingdoms Eumycota (Fig. 4.1b) and Animalia.

3. Golgi stacks (dictyosomes) are present; these are also found in the Protozoa (see p. 64). In contrast, in the Eumycota the Golgi apparatus is usually reduced to single cisternae (see Figs. 1.3, 1.10).

4. Flagella are usually present during particular stages of the life cycle; they always include one **straminipilous** flagellum (Lat. *stramen* = straw, *pilus* = hair). Dick (2001a) considered this feature to be of such high phylogenetic significance that he has renamed the kingdom Chromista as **Straminipila**. The straminipilous flagellum is discussed in detail in the following section.

5. The amino acid lysine is synthesized via the α,ε-diaminopimelic acid (DAP) pathway. Diaminopimelic acid originates from aspartic semialdehyde and pyruvic acid and is present in terrestrial plants, green algae, Chromista and prokaryotes. The alternative route, the α-aminoadipic acid (AAA) pathway, draws on α-ketoglutaric acid and acetyl-CoA and is found almost exclusively in members of the Eumycota. Yet other organisms, including animals and Protozoa, are auxotrophic for lysine (Griffin, 1994). Lysine biosynthesis has been used as a chemotaxonomic marker for some time (Vogel, 1964; LéJohn, 1972).

The kingdom Chromista/Straminipila currently includes the diatoms, golden and brown algae, chrysophytes and cryptomonads, as well as three phyla of straminipilous organisms traditionally studied by mycologists, i.e. the Oomycota, Hyphochytriomycota and Labyrinthulomycota. The first two groups are also called **straminipilous fungi** because of the similarity of their mode of life to the fungal lifestyle (Dick, 2001a). The Oomycota are by far the more important of these, and are considered in detail in Chapter 5. The Hyphochytriomycota and Labyrinthulomycota are treated briefly in the present chapter. The Straminipila as circumscribed above are a diverse but natural

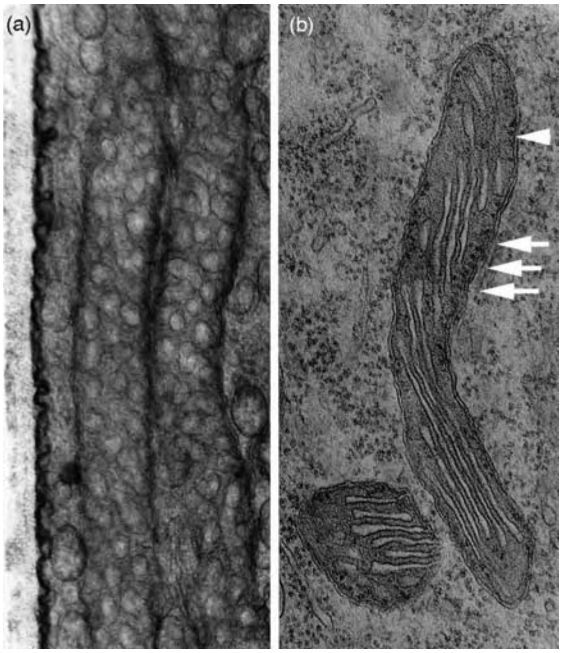

Fig 4.1 Mitochondrial ultrastructure observed by transmission electron microscopy. (a) Mitochondrion of *Phytophthora erythroseptica* (Oomycota). The inner mitochondrial membrane is folded into a complex tubular network. (b) Mitochondrion of *Sordaria fimicola* (Ascomycota) with the inner membrane appearing lamellate. Mitochondrial ribosomes (arrows) are also visible. Reprinted from Weber *et al*. (1998), with permission from Elsevier.

(monophyletic) grouping which has been confirmed by comparisons of the small-subunit (18S) ribosomal DNA sequences (e.g. Hausner *et al*., 2000; Fig. 4.2).

4.2 The straminipilous flagellum

The eukaryotic flagellum is a highly conserved structure. It is formed within the cytoplasm by a **kinetosome**, i.e. a microtubule-organizing centre resembling the centriole which co-ordinates the formation of the microtubular spindle during nuclear division. Like the centriole, the kinetosome contains an outer ring of nine triplets of microtubules surrounding two central microtubules (see Figs. 6.2 and 6.19). The flagellum extends outwards from the centriole as nine doublets of microtubules surrounding the two single central microtubules. This is the 9 + 2 arrangement. Where the eukaryotic flagellum protrudes beyond the cell surface, it is ensheathed by the plasma membrane. Within the flagellum, there are no obvious cytoplasmic features other than the microtubules which together are called the **axoneme**. Flagella which are entirely smooth or bear a coat of fine fibrillar surface material visible only by high-resolution electron microscopy (Fig. 4.3a; Andersen *et al*., 1991) are commonly called **whiplash** flagella. Dick (2001a) has pointed out that whiplash flagella in a strict sense are pointed at their tip due to the fact that the two inner microtubules are longer than the nine outer doublets (Fig. 4.3a).

A second type of flagellum is decorated with hair-like structures 1–2 μm long (Fig. 4.3b). This is the **tinsel** or **straminipilous** flagellum (Dick, 1997). The hairs are called **tripartite tubular hairs** (TTHs) because they are divided into three parts. They were formerly called mastigonemes, thereby naming the fungi which produced them Mastigomycotina, but both terms are no longer used. Each TTH is attached to the flagellum by a conical base pointed towards the axoneme. The main part of the TTH is a long tubular shaft thought to consist of two fibres of different thickness coiled around each other (Domnas *et al*., 1986). At the tip of the TTH, the two fibres separate from each other to form loose ends (Figs. 4.3b, 4.4). In the TTHs of some straminipilous organisms, only one loose end is visible (Fig. 4.7b). TTHs are assembled in anti-parallel arrays in Golgi-derived vesicles of the maturing zoospore, and are released by fusion of the vesicles with the plasma membrane (Fig. 4.5; Heath *et al*., 1970; Cooney *et al*., 1985). When a spore encysts, the flagellum may be withdrawn, shed or coiled around the spore. If it is withdrawn, the TTHs are sloughed off and left behind as a tuft on the surface of the cyst (Dick, 1990a).

TTHs are arranged in two rows along the axoneme. The cones of each row are adjacent to an outer microtubule doublet, and because there are nine such doublets, the two rows of TTHs are at an angle of about 160° rather than 180° to each other (Fig. 4.4a). In zoospores of

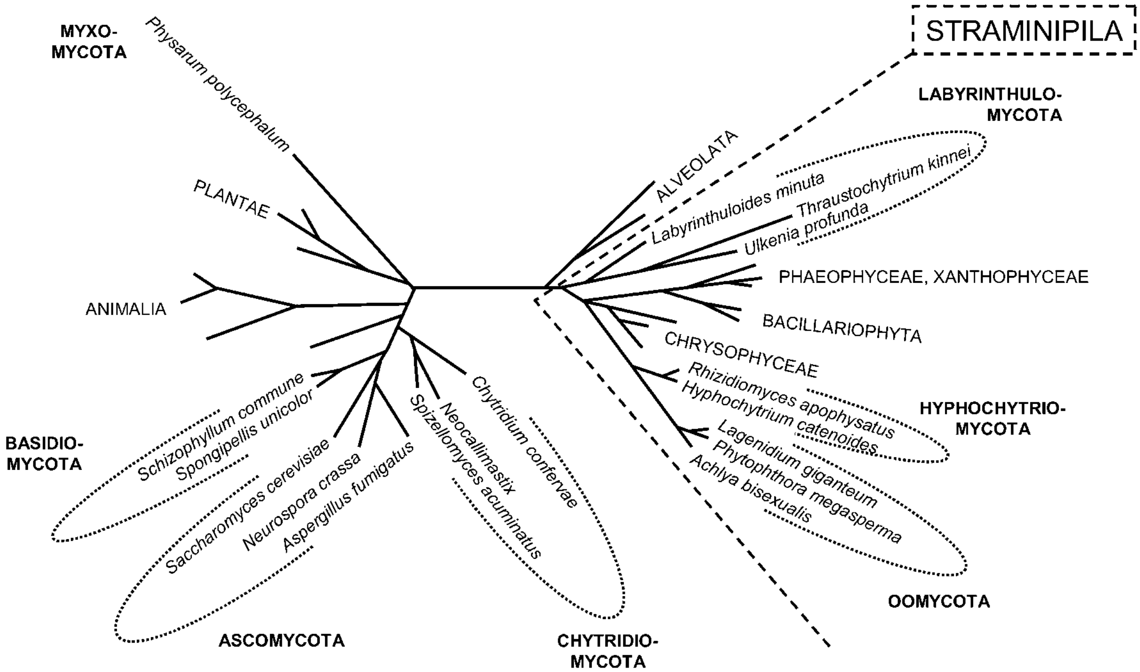

Fig 4.2 Unrooted phylogenetic tree of the Straminipila and members of other kingdoms, based on analyses of 18S rDNA sequences. Redrawn and modified from Hausner *et al*. (2000), by copyright permission of the National Research Council of Canada.

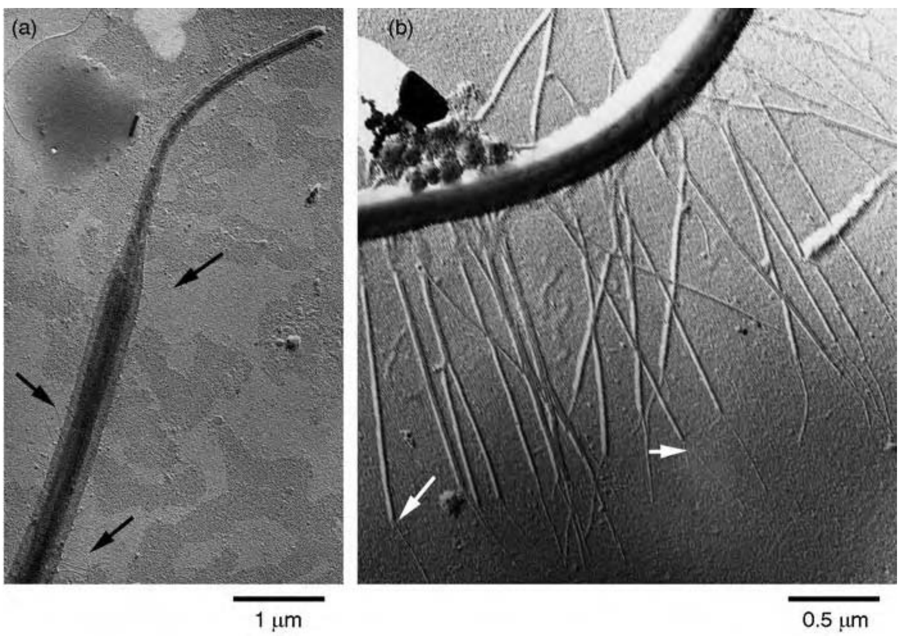

Fig 4.3 Ultrastructure of flagella in Straminipila. (a) Whiplash flagellum of *Pythium monospermum* (Oomycota). The tip is narrower than the main body of the flagellum because the two central microtubules are longer than the nine outer doublets. Arrows indicate the coating of the flagellum with very fine hairs. (b) Tinsel flagellum of *Achlya colorata* (Oomycota) with numerous TTHs. Each TTH ends in two fibres, one longer and thicker than the other (arrows). Original images kindly provided by M.W. Dick and I.C. Hallett.

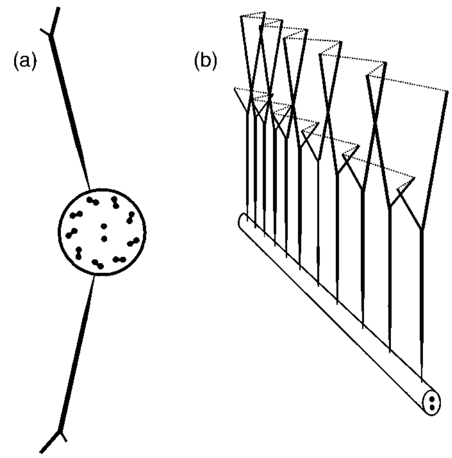

Fig 4.4 Organization of the straminipilous flagellum. (a) Postulated attachment of TTHs to the microtubule doublets 1 and 5 of the axoneme as seen in transverse section (after Dick, 2001a). (b) Longitudinal arrangement of TTHs along the axoneme of a straminipilous flagellum. Only one row of TTHs is drawn. The TTHs are thought to be arranged in an alternating fashion as regards the orientation of long and short fibres in adjacent TTHs. b redrawn from Dick (1990a). © 1990 Jones and Bartlett Publishers, Sudbury, MA. www.jbpub.com.

straminipilous fungi, the straminipilous flagellum always seems to point towards the direction of movement, and Dick (1990a, 2001a) has advanced a theory to explain how movement can be generated from a sinusoidal wave starting at the flagellar base, likening the straminipilous flagellum to 'a rowing eight with fixed oars and a flexible keel' (Fig. 4.4b; Dick, 2001a). An anterior straminipilous flagellum therefore pulls the spore through the water, whereas a backwardly directed whiplash flagellum pushes the spore.

The construction of the straminipilous flagellum is so elaborate that it is most unlikely to have arisen more than once during evolution (Dick, 2001a). The presence of a straminipilous flagellum, whether or not accompanied by another, smooth flagellum, therefore indicates membership in the Straminipila.

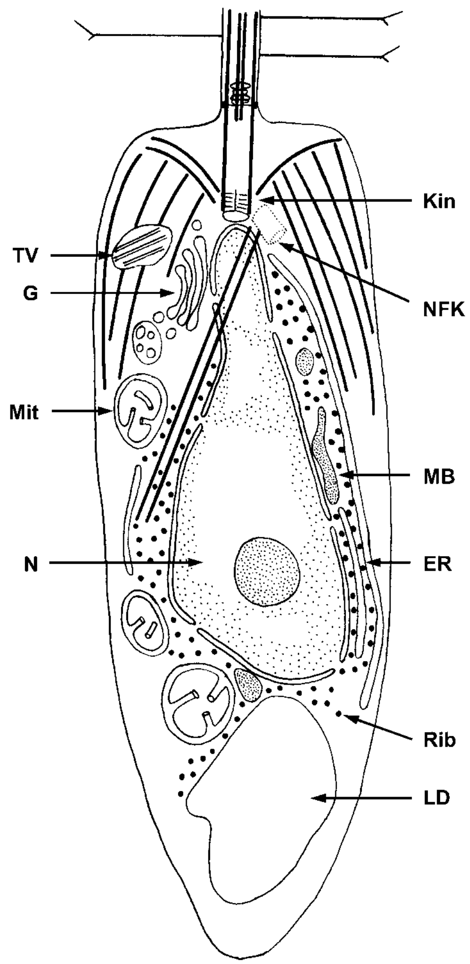

Fig 4.5 Schematic drawing of a L.S. of a zoospore of the hyphochytrid *Hyphochytrium catenoides*. The elongated shape of the zoospore and of the nucleus (N) is maintained by a system of 'rootlets' consisting of parallel bundles of microtobules (thick lines). The straminipilous flagellum arises from a kinetosome (Kin). A second, non-functional kinetosome (NFK) is interpreted as the base of a whiplash flagellum lost in the course of evolution from a heterokont ancestor. Mitochondria (Mit), TTH-containing vesicles (TV), a Golgi stack (G), ER, ribosomes (Rib), a large basal lipid droplet (LD) and microbodies (MB) are also visible. Some organelles of unknown function, e.g. electron-opaque bodies and osmiophilic bodies, have been omitted from the original for improved clarity. Redrawn and modified from Cooney *et al*. (1985).

4.3 Hyphochytriomycota

This group, formerly called Hyphochytridiomycetes probably due to the perpetuation of a typographical error (see Dick, 1983), is a very small phylum currently comprising 23 species in 6 genera (Kirk *et al*., 2001). The Hyphochytriomycota (colloquially called hyphochytrids) are phylogenetically closely related to the Oomycota (van der Auwera *et al.*, 1995; Hausner *et al.*, 2000; see Fig. 4.2). Treatments of the group have been given by Karling (1977), Fuller (1990, 2001) and Dick (2001a). The diagnostic feature is the zoospore with its single anterior straminipilous flagellum (Fig. 4.5). This kind of zoospore is not found in any other known life form. The zoospore of hyphochytrids contains one prominent Golgi stack, one nucleus, and lipid droplets and microbodies (Barr & Allan, 1985; Cooney *et al.*, 1985). The latter are not arranged in a microbody–lipid complex like they are in chytrids (cf. Fig. 6.3). The TTHs are localized within Golgi-derived vesicles. The flagellum arises from a kinetosome, with microtubules rooting deeply within the spore and probably maintaining its shape. A second (dormant) kinetosome lies adjacent but at an angle, at the same position as that which gives rise to the backward-directed smooth flagellum in zoospores of Oomycota. This whiplash flagellum is missing in Hyphochytriomycota, and Barr and Allan (1985) have speculated that it could have been lost during evolution of the latter from the former. Like the Oomycota, hyphochytrids synthesize lysine by the α,ε-diaminopimelic acid (DAP) pathway (Vogel, 1964).

Hyphochytrids occur in the soil and in aquatic environments (both freshwater and marine) as saprotrophs or parasites of algae, oospores of Oomycota or azygospores of Glomales. *Hyphochytrium peniliae* was reported once as the cause of a devastating epidemic of marine crayfish (Artemchuk & Zelezinkaya, 1969), but no further cases have been observed since. Some species can be isolated into pure culture relatively easily (Fuller, 1990).

Zoospores encyst by withdrawing their flagellum and secreting a wall, leaving the TTHs dispersed on the surface of the cyst wall (Beakes, 1987). The cyst germinates by enlargement or by putting out rhizoids. Because of the similarity of their vegetative thalli with those of Chytridiomycota (see Chapter 6), hyphochytrids have been studied primarily by comparison with chytrids, and the same terminology has been used (see Fig. 6.1). Depending on the species, cysts germinate to develop in three different ways, which have been used to subdivide the Hyphochytriomycota into families: (1) **Holocarpic** thalli are produced by simple enlargement of the cyst. The entire content of the sac-like thallus ultimately becomes converted into zoospores (Anisolpidiae, e.g. *Anisolpidium* which parasitizes marine algae; Canter, 1950). (2) In **eucarpic monocentric** thalli, the cyst produces a bunch of rhizoids at one end, which anchor the enlarging thallus to the substratum and/or absorb nutrients (Rhizidiomycetidae, e.g. *Rhizidiomyces*; Wynn & Epton, 1979). (3) In **eucarpic polycentric** thalli, a broad hypha-like germ tube emerges, branches and produces several zoosporangia (Hyphochytriaceae, e.g. *Hyphochytrium*; Ayers & Lumsden, 1977). The asexual life cycle is completed when a fresh crop of zoospores is released. Sexual reproduction has not yet been reliably described for the hyphochytrids.

4.4 Labyrinthulomycota

Whereas the Hyphochytriomycota described in the previous section have a strong resemblance to true fungi (especially Chytridiomycota), the Labyrinthulomycota do not, and the only justification for mentioning them here is the fact that they have traditionally been studied by mycologists. They have been the subject of numerous taxonomic rearrangements, and are known under many different names such as Labyrinthomorpha, Labyrinthista and Labyrinthulea. Some 48 species are currently recognized (Kirk *et al.*, 2001). DNA sequence comparisons have placed them within the Straminipila (Fig. 4.2; Hausner *et al.*, 2000; Leander & Porter, 2001), and they are characterized by having **heterokont** flagellation, i.e. possessing a straminipilous and a whiplash flagellum with a pointed tip (Fig. 4.7). In addition, they have mitochondria with tubular cristae. Recent treatments of this group can be found in Moss (1986), Porter (1990) and Dick (2001a).

Labyrinthulomycota occur in freshwater and marine environments where they are attached to solid substrata by means of networks of slime within which individual vegetative cells are contained. For this reason, they are sometimes referred to as 'slime nets' (Porter, 1990). The vegetative cells possess a wall which, uniquely, is produced from Golgi-derived scales of a polymer of L-galactose (Dick, 2001a). These scales are located between the plasma membrane and the inner membrane of the slime net. The slime net is delimited by an inner and an outer membrane and is produced by specialized organelles termed **sagenogens** or bothrosomes; the net membranes are continuous with the plasma membrane at the sagenogen (Perkins, 1972). Labyrinthulomycota feed by absorption (osmotrophy) of nutrients. The nets contain degradative enzymes which can lyse plant material or microbial cells. Two orders are distinguished.

4.4.1 Labyrinthulales

Members of this order, especially of the genus *Labyrinthula*, can be readily isolated from marine angiosperms such as *Zostera* and *Spartina* or from seaweed by placing a small piece of one of these substrata directly on low-nutrient sea water agar augmented with penicillin and streptomycin (Porter, 1990). Within a few days, a fine network of strands can be seen extending over the agar surface (Fig. 4.6). *Labyrinthula* spp. can be kept in monoxenic culture with yeasts or bacteria as food source. These are presumably lysed by the enzymes contained in the slime net.

A closer examination shows that the network consists of branched slime tubes within which spindle-shaped cells move backwards and forwards (Fig. 4.7a; see Webster, 2006a). Movement of a speed up to 100 μm min^{-1} has been reported and is due to a system of contractile actin-like proteins in the slime net (Nakatsuji & Bell, 1980). Cells occasionally aggregate to form sporangia containing numerous round cysts. Following meiosis, eight heterokont zoospores (Figs. 4.6a, 4.7b) are released by each cyst. These possess a pigmented eyespot not found in other types of heterokont zoospore (Porter, 1990). It is, however, unclear whether zoospores can establish new colonies (Porter, 1990). Asexual reproduction occurs by division of spindle cells within the slime net, and fragments of such a colony can establish new colonies (Porter, 1972). Further details of the life cycle appear to be unknown at present.

Labyrinthula spp. were implicated as pathogens in a wasting epidemic of eelgrass (*Zostera marina*) at the west coast of North America in the 1930s (Young, 1943; Muehlstein *et al.*, 1991), causing considerable disturbance to the littoral ecosystem and collateral damage to the local fisheries industry. However, although *Labyrinthula* spp. are still frequently associated with pieces of moribund *Zostera* shoots, no further epidemics seem to have occurred since. Instead, a new species, *L. terrestris*, has recently been identified as the cause of a rapid blight of

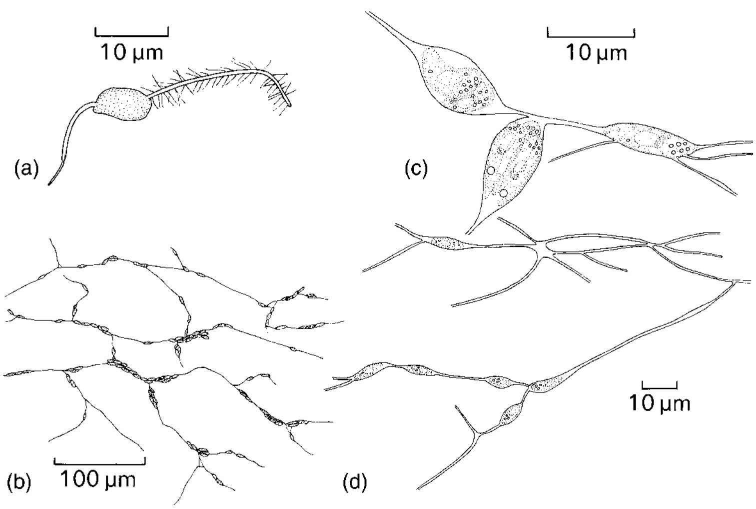

Fig 4.6 *Labyrinthula*. (a) Zoospore with long anterior straminipilous flagellum and a short posterior whiplash flagellum with a pointed tip (after Amon & Perkins, 1968). (b–d) Portions of colonies at different magnifications. In (c) spindle cells are seen in swellings in the slime tracks.

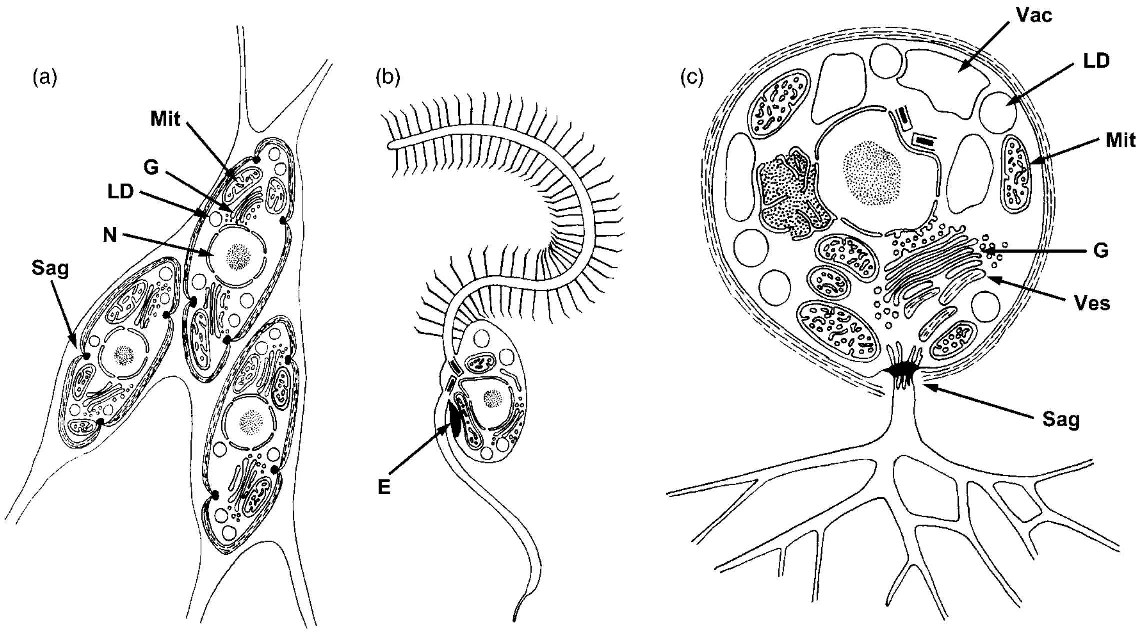

Fig 4.7 Ultrastructural features of Labyrinthulomycota. (a) Spindle-shaped cells of *Labyrinthula* within their slime net. Each cell has mitochondria with tubular cristae (Mit), Golgi stacks (G), a single nucleus (N), and cortical lipid droplets (LD). The slime net is produced by several sagenogens (Sag) in each cell. The plasma membrane is continuous with the inner membrane of the slime net. Wall scales are released at the sagenogen point and accumulate between the plasma membrane and the inner membrane of the slime net. (b) Biflagellate heterokont zoospore of *Labyrinthula* showing an eyespot (E) close to the base of the whiplash flagellum. Note that each TTH of the *Labyrinthula* zoospore produces only one terminal fibre. (c) Young thallus of *Thraustochytrium*. Mitochondria with tubular cristae, a Golgi stack, lipid droplets and larger vacuoles (Vac) are seen. The wall consists of scales pre-formed in Golgi-derived vesicles (Ves). The slime net is produced at the base of the thallus by a single sagenogen. All images schematic and not to scale; redrawn and modified from Porter (1990).

turf-grass on golf courses, infection presumably being brought about by irrigation with contaminated water of unusually high salinity (Bigelow *et al.*, 2005).

4.4.2 Thraustochytriales

Thraustochytrids are probably ubiquitous in marine environments, occurring on organic debris as well as calcareous shells of invertebrates (Porter & Lingle, 1992). Like the labyrinthulids, they feed on organic matter, algae and bacteria (Raghukumar, 2002). Thraustochytrids can be baited by sprinkling pine pollen grains onto water samples or organic debris immersed in water. Within one to several days, the pollen grains become colonized by one or several thalli, the main bodies of which protrude beyond the grain surface (Figs. 4.8a,b). If colonized pollen grains are transferred to a suitable agar medium containing sea salts, yeast extract and sugar (Yokochi *et al.*, 1998), thalli will grow on the agar surface and may be induced to release zoospores by mounting them in water. Thraustochytrids can be stored in pollen grain suspensions or on agar overlaid with sea water. They also possess the ability to survive in a dry state at room temperature for a year or longer (Porter, 1990).

The thallus of thraustochytrids superficially resembles that of an epibiotic monocentric chytrid in having a roughly spherical shape with ‘rhizoids’ at its base (Fig. 4.8c). These ‘rhizoids’ are, in fact, the slime net produced by one basal sagenogen (Fig. 4.7c). The thallus is surrounded by Golgi-derived scales forming a wall, but the slime net does not extend over the thallus. Sexual reproduction is unknown, but asexual biflagellate heterokont zoospores are released from the main body of the thallus,

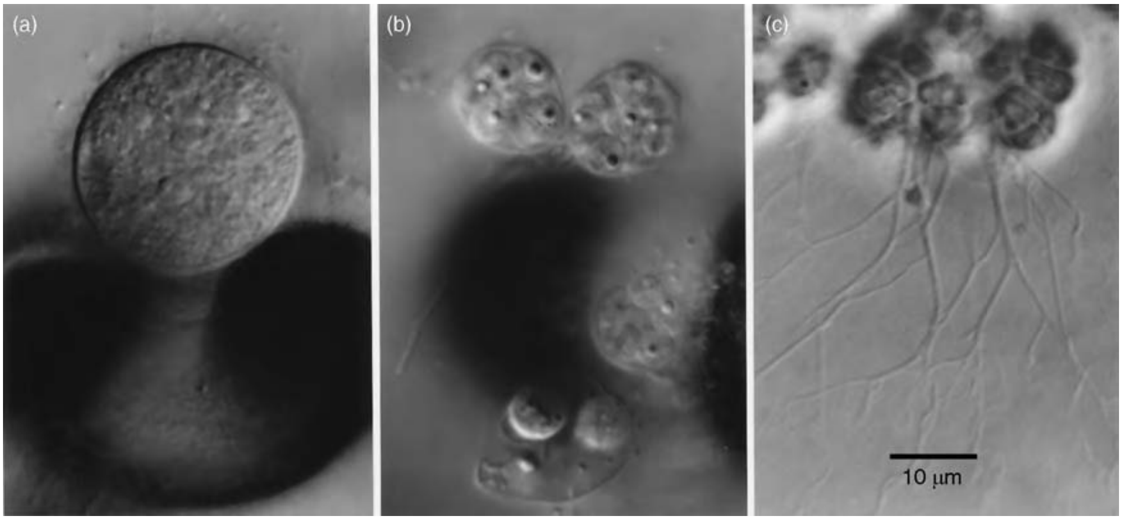

Fig 4.8 Thraustochytriales. (a) Thallus of *Thraustochytrium* sp. growing on a pollen grain sprinkled onto seawater. (b) Thalli of *Schizochytrium* sp. growing on a pollen grain. (c) Thalli of *Schizochytrium* sp. growing on agar medium. Note the slime net extending away from the thalli.

and these can settle onto a suitable substratum, giving rise to new thalli (Porter, 1990). Thus, these zoospores of Thraustochytriales are mitospores formed following mitosis, in contrast with those of Labyrinthulales which are meiospores, i.e. formed by meiosis. Although thraustochytrid zoospores lack a recognizable eye-spot, they are phototropic, reacting to light of blue wavelengths such as that produced by bioluminescent bacteria (Amon & French, 2004). Chemotropism has also been described for thraustochytrid zoospores (Fan *et al*., 2002), and both sensual responses may enable zoospores to locate potential food sources.

Thraustochytrids, and especially the genera *Thraustochytrium* and *Schizochytrium*, have recently attracted attention as producers of polyunsaturated fatty acids (PUFAs). These are important as nutrient supplements, and thraustochytrid oils might eventually be able to compete with fish oils on the market (Yokochi *et al*., 1998; Lewis *et al*., 1999).

5

Straminipila: Oomycota

5.1 Introduction

The phylum Oomycota, alternatively called Peronosporomycetes (Dick, 2001a), currently comprises some 800–1000 species (Kirk *et al.*, 2001). The Oomycota as a whole have been resolved as a monophyletic group within the kingdom Straminipila in recent phylogenetic studies (e.g. Riethmüller *et al.*, 1999; Hudspeth *et al.*, 2000; see Fig. 4.2), although considerable rearrangements are still being performed at the level of orders and families. A scholarly treatment of the Oomycota has been published by Dick (2001a) and will remain the reference work for many years to come. Because of the outstanding significance of Oomycota, especially in plant pathology, we give an extended treatment of this group.

5.1.1 The vegetative hypha

Although some members of the Oomycota grow as sac-like or branched thalli, most of them produce hyphae forming a mycelium. Oomycota are now known to be the result of convergent evolution with the true fungi (Eumycota), and their hyphae differ in certain details. However, the overall functional similarities are so great that they provide a persuasive argument for the fundamental importance of the hypha in the lifestyle of fungi (Barr, 1992; Carlile, 1995; Bartnicki-Garcia, 1996). Much physiological work has been carried out on hyphae of Oomycota (see Chapter 1), and the results have a direct bearing on our understanding of the biology of the Eumycota. Like them, the hyphae of Oomycota display apical growth and enzyme secretion, ramify throughout the substratum by branching to form a mycelium, and can show morphogenetic plasticity by differentiation into specialized structures such as appressoria or haustoria.

The hyphae of Oomycota are **coenocytic**, i.e. they generally do not form cross-walls (septa) except in old compartments or at the base of reproductive structures. The cytoplasm is generally coarsely granular and contains vacuoles, Golgi stacks, mitochondria and diploid nuclei. The apex is devoid of organelles other than numerous secretory vesicles. These are not, as in the Eumycota, arranged into a Spitzenkörper because the microvesicles which contain chitin synthase and make up the Spitzenkörper core are lacking. This is in line with the general absence, with a few exceptions, of chitin from the walls of Oomycota; instead, cellulose, a crystalline β-(1,4)-glucan, contributes the main fibrous component. As in the Eumycota, these structural fibres are cross-linked by branched β-(1,3)- and β-(1,6)-glucans, although the biochemical properties of the glucan synthases seem to differ fundamentally between those of Eumycota on the one hand and those of Oomycota and plants on the other (Antelo *et al.*, 1998). Other biochemical differences include the lysine synthetic pathway (DAP in plants and Oomycota; AAA in true Fungi; see p. 67) and details of sterol metabolism (Nes, 1990; Dick, 2001a).

The mitochondria of Oomycota are indistinguishable by light microscopy from those of the Eumycota, but when viewed with the transmission electron microscope they have tubular

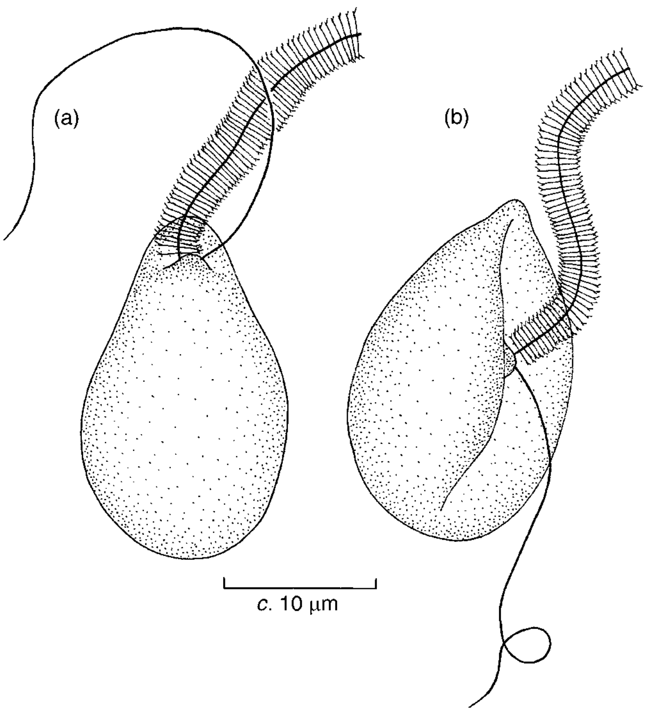

Fig 5.1 Asexual reproductive stages in *Saprolegnia*. (a) Auxiliary (primary) zoospore. (b) Principal (secondary) zoospore. Schematic drawings, based partly on Dick (2001a).

rather than lamellate cristae (see Fig. 4.1). The vacuolar system of Oomycota is also unusual in containing **dense-body vesicles** or '**fingerprint vacuoles**' (see Fig. 5.24b) which consist of deposits of a phosphorylated β-(1,3)-glucan polymer, mycolaminarin. Mycolaminarin may serve as a storage compound for carbohydrates as well as phosphate (Hemmes, 1983), and the polyphosphate storage deposits which are typically found within vacuoles of true Fungi are absent from vacuoles of Oomycota (Chilvers *et al.*, 1985). Apart from that, however, vacuoles of Oomycota share many features with those of true Fungi, including the membranous continuities which often link adjacent vacuoles and provide a means of transport by peristalsis (Rees *et al.*, 1994; see Fig. 1.9). Cytoplasmic glycogen granules, which are one of the major carbohydrate storage sites in Eumycota, are absent from hyphae of Oomycota (Bartnicki-Garcia & Wang, 1983).

5.1.2 The zoospore

The Oomycota are characterized by motile asexual spores (zoospores) which are produced in spherical or elongated zoosporangia. They are heterokont, possessing one straminipilous and one whiplash-type flagellum. Two types of zoospore may be produced and, if so, the **auxiliary zoospore** is the first formed. It is grapeseed-shaped, with both flagella inserted apically (Fig. 5.1a), and it encysts soon after its formation. Encystment is by withdrawal of the flagella, so that a tuft of tripartite tubular hairs (TTHs; see p. 68) is left behind on the surface of the developing cyst (Dick, 2001b). The cyst germinates to give rise to the **principal zoospore**, which is by far the more common type and also the more vigorous swimmer. This typical and readily recognized oomycete zoospore is uniform in appearance across the phylum (Lange & Olson, 1983; Dick, 2001a). In species lacking auxiliary zoospores, the principal zoospore is usually produced directly from a sporangium. It is kidney-shaped, with the flagella inserted laterally in a kinetosome boss which in turn is located within the lateral groove (Fig. 5.1b). Encystment is initiated by the shedding, rather than withdrawal, of the flagella; no tufts of TTHs are left on the cyst surface (Dick, 2001a). Fascinating insights into the cytology of zoospore encystment have been

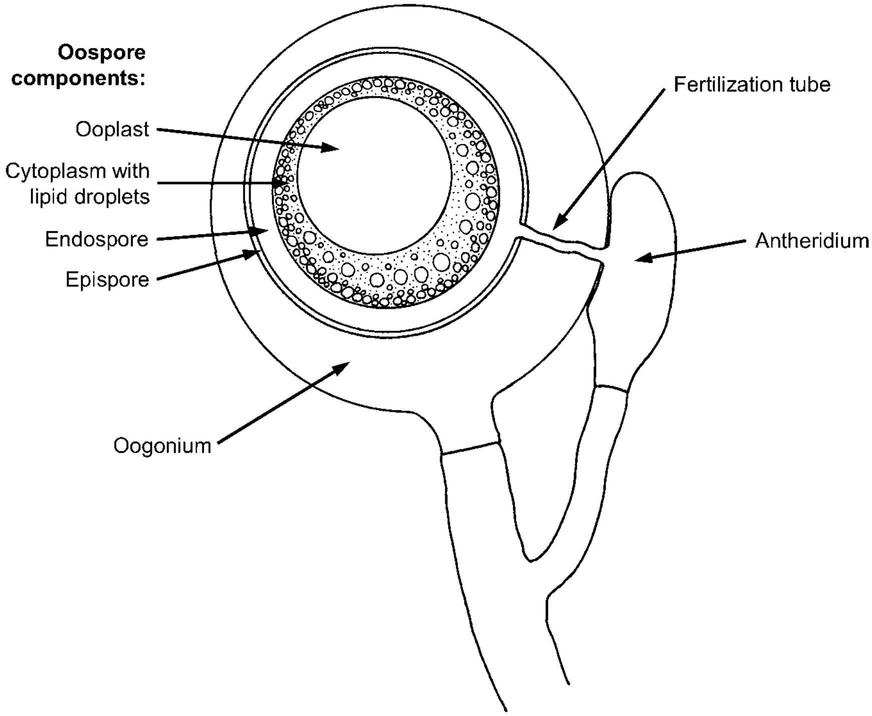

Fig 5.2 Schematic drawing and terminology of sexual reproductive organs in the Oomycota. Modified from Dick (1995).

obtained from several species (see Fig. 5.24). At the onset of encystment, adhesive and cell wall material is secreted by the synchronized fusion of pre-formed storage vesicles with the zoospore plasma membrane (Hardham *et al.*, 1991; Hardham, 1995), thereby providing a rare example of regulated secretion in fungi. Constitutive secretion by growing hyphal tips is more commonly associated with their mode of life.

Some members of the Oomycota have no motile spore stages but can be readily related to groups still producing them.

5.1.3 Sexual reproduction

The life cycle of the Oomycota is of the haplomitotic B type, i.e. mitosis occurs only between karyogamy and meiosis. All vegetative structures of Oomycota are therefore diploid (see Figs. 5.3 and 5.19). This is in contrast to the Eumycota in which vegetative nuclei are usually haploid, the first division after karyogamy being meiotic. Sexual reproduction in Oomycota is **oogamous**, i.e. male and female gametangia are of different size and shape (Fig. 5.2). Meiosis occurs in the male **antheridia** and in the female **oogonia**, and is followed by plasmogamy (fusion between the protoplasts) and karyogamy (fusion of haploid nuclei). Numerous meioses can occur synchronously, so that true sexual reproduction can actually happen within the same protoplast (Dick, 1990a). Heterothallic species of Oomycota display **relative sexuality**, i.e. a strain can produce antheridia in combination with a second strain but oogonia when paired against a third (see pp. 86 and 95). Steroid hormones play an important role in sexual reproduction (see Fig. 5.11).

The mature oospore contains three major pools of storage compounds (Fig. 5.2; Dick, 1995). The oospore wall often appears stratified, and this is due in part to a polysaccharide reserve compartment, the **endospore**, which is located

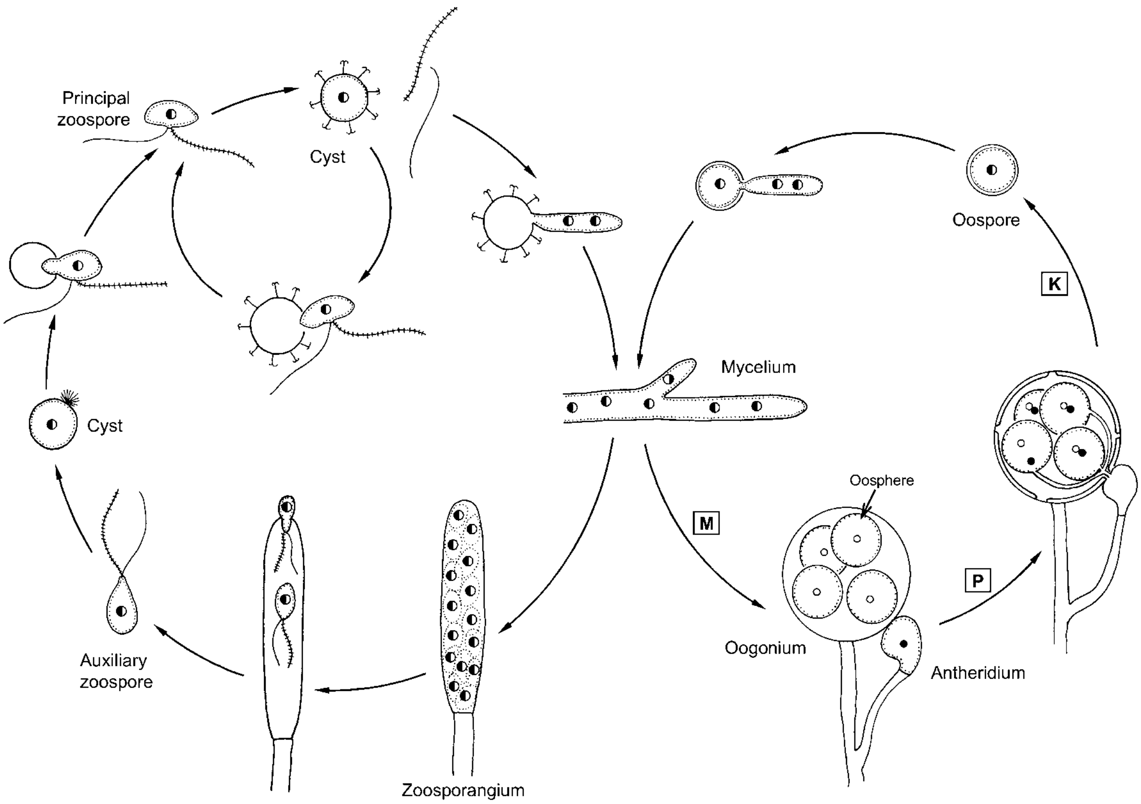

Fig 5.3 Life cycle of *Saprolegnia*. Vegetative hyphae are diploid and coenocytic. Asexual reproduction is by means of diplanetic (auxiliary and principal) zoospores. The principal zoospore state is polyplanetic. *Saprolegnia* is homothallic, and sexual reproduction is initiated by the formation of antheridia and oogonia. For simplicity, only a single nucleus is shown in each of the oospheres and in the antheridium. Each oogonium contains several oospheres. Karyogamy occurs soon after fertilization of an oosphere by an antheridial nucleus. The oospore may germinate by means of a germ sporangium (not shown) or a hyphal tip. Open and closed circles represent haploid nuclei of opposite mating type; diploid nuclei are larger and half-filled. Key events in the life cycle are meiosis (M), plasmogamy (P) and karyogamy (K).

between the plasma membrane and the outer spore wall (**epispore**). Upon germination, the endospore is thought to coat the emerging germ tube with wall material, and some material may also be taken up by endocytosis. A large storage vacuole inside the oospore protoplast is called the **ooplast**. It arises by fusion of dense-body vesicles and, like them, contains mycolaminarin and phosphate. Dick (1995, 2001a) speculated that the ooplast contributes membrane precursor material during the process of oospore germination. The third storage compartment consists of one or several lipid droplets which provide the endogenous energy supply required for germination. Ultrastructural changes during oospore germination have been described by Beakes (1981).

5.1.4 Ecology and significance

Oomycota have a major impact on mankind as pathogens causing plant diseases of epidemic proportions. Two events have had particularly far-reaching political and social consequences, and have shaped and interlinked the young disciplines of mycology and plant pathology in the nineteenth century. These were the great Irish potato famine of 1845–1848 caused by *Phytophthora infestans* (Bourke, 1991), and the occurrence of downy mildew of grapes caused by *Plasmopara viticola* (Large, 1940). The former prepared the way for the then revolutionary theory that fungal infections can be the cause rather than the consequence of disease, whereas the latter stimulated research into chemical control of diseases which directly gave rise to the first fungicide, Bordeaux mixture (p. 119; Large, 1940).

Although all members of Oomycota depend on moist conditions for the dispersal of their zoospores, they are cosmopolitan and ubiquitous even in terrestrial situations. In species adapted to drier habitats, the sporangia often germinate directly to produce a germ tube, with zoospores released as an alternative germination method only in the presence of moisture, or lacking altogether. Oomycota occur in freshwater, the sea, in the soil and on above-ground plant organs. Most are obligate aerobes, although some tolerate anaerobic conditions (Emerson & Natvig, 1981; Voglmayr *et al.*, 1999), and one species (*Aqualinderella fermentans*) is obligately anaerobic and lacks mitochondria (Emerson & Weston, 1967). Oomycota live either saprotrophically on organic material, or they may be obligate (biotrophic) or facultative (necrotrophic) parasites of plants. Some can also cause diseases of animals, such as *Aphanomyces astaci* which has all but eliminated European crayfish from many rivers (p. 94), *Saprolegnia* spp. which cause serious infections of farmed fish, especially salmon (Plate 2a; Dick, 2003), or *Pythium insidiosum* causing equine phycomycosis (de Cock *et al.*, 1987). Yet other Oomycota, notably *Lagenidium giganteum*, parasitize insects and may prove valuable in the biological control of mosquito larvae (Dick, 1998).

5.1.5 Classification

As indicated above, the classification of Oomycota at the level below the phylum is still an ongoing process, and it is difficult at present to reconcile the different classification schemes that are being proposed. Kirk *et al.* (2001) listed eight orders in the phylum Oomycota, of which Dick (2001b) treated six within the class Peronosporomycetes, his equivalent to the Oomycota, considering the other two of uncertain affinity (*incertae sedes*). These groups are summarized in Table 5.1.

5.2 Saprolegniales

The order Saprolegniales is currently divided up into two families, the Saprolegniaceae (e.g. *Achlya*, *Brevilegnia*, *Dictyuchus*, *Saprolegnia*, *Thraustotheca*) and Leptolegniaceae (*Aphanomyces*, *Leptolegnia*, *Plectospira*), totalling 132 species in about 20 genera (Dick, 2001a; Kirk *et al.*, 2001). The Saprolegniales are the best-known group of aquatic fungi, often termed the water moulds. Members of this group are abundant in wet soils, lake margins and freshwater, mainly as saprotrophs on plant and animal debris. Whilst some Saprolegniales occur in brackish water, most are intolerant of it and thrive best in freshwater. A few species of *Saprolegnia* and *Achlya* are economically important as parasites of fish and their eggs (Willoughby, 1994). *Aphanomyces euteiches* causes a root rot of peas and some other plants, whilst *A. astaci* is a serious parasite of the European crayfish *Astacus* (Alderman *et al.*, 1990). Algae, fungi, rotifers and copepods may also be parasitized by members of the group, and occasional epidemics of disease among zooplankton have been reported.

Members of the Saprolegniales are characterized by coarse, stiff hyphae which branch to produce a typically fast-growing mycelium. The hyphae of Saprolegniales are coenocytic, containing a peripheral layer of cytoplasm surrounding a continuous central vacuole. Cytoplasmic streaming is readily observed in the peripheral cytoplasm. Numerous nuclei are present. Mitotic division is associated with the replication of paired centrioles and the development of an intranuclear mitotic spindle; the nuclear membrane remains intact throughout division (Dick, 1995). Filamentous mitochondria and lipid droplets can also be observed in vegetative hyphae. The mitochondria are orientated parallel to the long axis of the hypha and are sufficiently large to be seen in cytoplasmic streaming in living material. Important physiological work has been carried out on the

Table 5.1. Summary of the most important groups of Oomycota and their characteristic features. Only the last four groups are considered further in this book. Based on information provided by Dick (2001a,b) and Kirk *et al.* (2001).

Order	Number of species	Thallus and reproduction	Ecology
Myzocytiopsidales (*incertae sedes*)	74	Holocarpic,* later coralloid or breaking up into segments. Zoospores, oospores.	Parasites of invertebrates or algae.
Olpidiopsidales (*incertae sedes*)	21	Holocarpic,* becoming converted into a sporangium. Zoospores, oospores.	Biotrophic parasites of Oomycota, Chytridiomycota and algae.
Rhipidiales	12	Eucarpic* with rhizoids. Zoospores, oospores.	Freshwater saprotrophs, facultatively or obligately anaerobic.
Leptomitales	25	Constricted hyphae producing sporangia. Zoospores, oospores.	Freshwater saprotrophs or parasites of animals.
Saprolegniales (see Section 5.2)	132	Mycelium of wide stout hyphae. Zoospores, oospores.	Saprotrophs or necrotrophic pathogens of animals, plants and other organisms.
Pythiales (see Section 5.3)	>200	Mycelium of relatively narrow hyphae. Zoospores, oospores.	Saprotrophs or pathogens (often necrotrophic) of plants, fungi and animals.
Peronosporales (see Section 5.4)	252	Intercellular mycelium with haustoria. Differentiated sporangiophores. Zoospores or 'conidia', oospores.	Biotrophic plant pathogens, causing downy mildews and other diseases.
Sclerosporaceae (see Section 5.5)	22	Mycelium of very narrow hyphae. Differentiated sporangiophores. Zoospores or 'conidia', oospores.	Biotrophic pathogens of grasses, causing downy mildews.

*For thallus terminology, see Fig. 6.1.

mechanisms of hyphal polarity and growth regulation in *Achlya* and *Saprolegnia* (see Heath, 1995b; Hyde & Heath, 1997; Heath & Steinberg, 1999). Like other Oomycota but in contrast to the Eumycota (Pfyffer *et al.*, 1986; Rast & Pfyffer, 1989), these fungi are unable to synthesize compatible osmotically active solutes such as glycerol, mannitol and other polyols to maintain their intrahyphal turgor pressure against fluctuating external conditions. Under conditions of water stress, the turgor pressure in hyphae of *Achlya* and *Saprolegnia* approaches zero, yet hyphal growth can still occur at least under laboratory conditions because of the enhanced secretion of cell wall-softening enzymes and the role of the cytoskeleton in pushing forward the growing tip (see pp. 6–9; Money & Harold, 1992, 1993; Money, 1997; Money & Hill, 1997).

The Saprolegniales are the only order within the Oomycota to produce both auxiliary and principal zoospores, although both forms are not produced in all genera. The production of two distinct motile stages is termed **diplanetism**. It has also been called **dimorphism**, but this term has several different meanings and is best avoided in the current context. Depending on environmental conditions, the cysts of principal zoospores may germinate either by means of a germ tube developing into a hypha or by the emergence of a new principal zoospore. The repetition of the same type of motile spore is called **polyplanetism.**

Sexual reproduction in the Saprolegniales is oogamous, with a large, usually spherical oogonium containing one or several oospheres. Antheridial branches apply themselves to the wall of the oogonium and penetrate the wall by fertilization tubes through which a single nucleus is introduced into each oosphere. A feature of many Saprolegniales, especially when grown in culture, is the formation of thick-walled enlarged terminal or intercalary portions of hyphae which become packed with dense cytoplasm and are cut off from the rest of the mycelium by septa. These structures, which may occur singly or in chains (see Fig. 5.6g), are termed gemmae or **chlamydospores**, and their formation can be induced by manipulating the culture conditions. Morphologically less distinct but otherwise similar structures are frequently found in old cultures. Although it is known that chlamydospores cannot survive desiccation or prolonged freezing, they remain viable for long periods in less extreme conditions. They may function as female gametangia or as zoosporangia, but more frequently they germinate by means of a germ tube. Another feature of old cultures is the fragmentation of cylindrical pieces of mycelium cut off at each end by a septum.

Members of the Saprolegniales can be isolated readily from water, mud and soil by floating split boiled hemp seeds or dead house flies in dishes containing pond water, or by covering soil samples or waterlogged twigs with water (Stevens, 1974; Dick, 1990a). Within about 4 days the fungi can be recognized by their stiff, radiating, coarse hyphae bearing terminal sporangia, and cultures can be prepared by transferring hyphal tips or zoospores to cornmeal agar or other suitable media. The most commonly encountered genera are *Achlya*, *Dictyuchus*, *Saprolegnia*, *Thraustotheca* and *Aphanomyces*. With the exception of a few obligately parasitic species, most of the Saprolegniales will grow readily in pure culture even on chemically defined media, and extensive studies of their nutritional physiology have been undertaken (summarized by Cantino, 1955; Gleason, 1976; Jennings, 1995). Most species examined have no requirement for vitamins. Organic forms of sulphur such as cysteine, cystine, glutathione and methionine are preferred, and most species are unable to reduce sulphate. Organic nitrogen sources such as amino acids, peptone and casein are preferred to inorganic sources. Ammonium is widely utilized, but nitrate is not. Glucose is the most widely utilized carbon source, but many species also degrade maltose, starch and glycogen. In liquid culture, *Saprolegnia* can be maintained in the vegetative state indefinitely if supplied with organic nutrients in the form of broth. When the nutrients are replaced by water, the hyphal tips quickly develop into zoosporangia. The formation of sexual organs can similarly be affected by manipulating the external conditions in some species, and the concentration of salts in the medium may play a decisive role (Barksdale, 1962; Davey & Papavizas, 1962).

5.2.1 *Saprolegnia* (Saprolegniaceae)

Species of *Saprolegnia* are common in soil and in freshwater as saprotrophs on plant and animal remains. A few species such as *S. parasitica* and *S. polymorpha* cause disease in fish and their eggs (Plate 2a). Salmonid fish are particularly affected, and the disease can cause significant damage in fish farms around the world (Willoughby, 1994, 1998a). Control by fungicides is difficult but possible (Willoughby & Roberts, 1992). The disease is also seen in wild salmon and other fish (Söderhäll *et al*., 1991; Bly *et al*., 1992). Pathogenic strains or species may be closely related to non-pathogenic ones but can be distinguished by physiological characteristics, DNA sequencing (Yuasa & Hatai, 1996) and the length of the 'boat hook' appendages on the cysts of principal zoospores (Figs. 5.5b,c; Beakes, 1983; Burr & Beakes, 1994).

The life cycle of *Saprolegnia* is summarized in Fig. 5.3. A monographic treatment of the genus has been published by Seymour (1970).

Asexual reproduction in *Saprolegnia*

Sporangia of *Saprolegnia* develop when a hyphal tip, which is pointed in the vegetative condition, swells, rounds off and becomes club-shaped. It accumulates denser cytoplasm around the vacuole which remains clearly visible. A septum develops at the sporangial base and it is at first straight or convex with respect to the sporangium, i.e. it bulges into it (Figs. 5.4c,d). The sporangium contains numerous nuclei, and

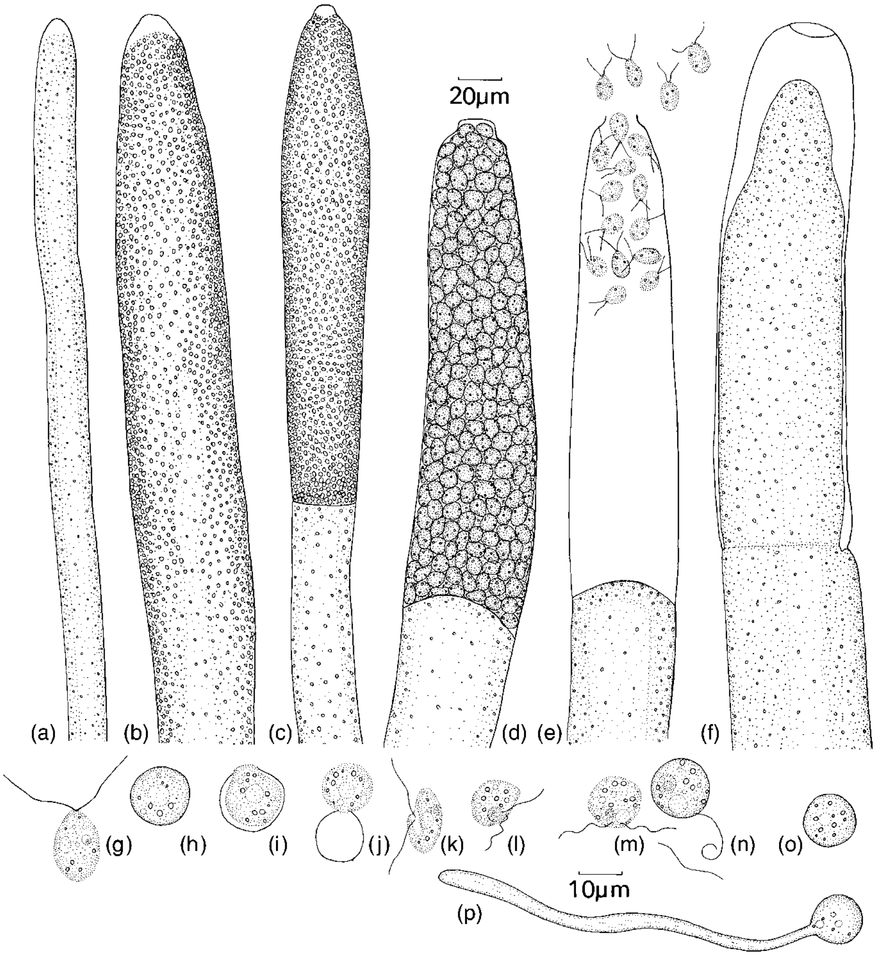

Fig 5.4 *Saprolegnia*. (a) Apex of vegetative hypha. (b–d) Stages in the development of zoosporangia. (e) Release of zoospores. (f) Proliferation of zoosporangium. A second zoosporangium is developing within the empty one. (g) Auxiliary zoospore (first motile stage). (h) Cyst formed at the end of the first motile stage (auxiliary cyst). (i,j) Germination of auxiliary cyst to release a second motile stage (principal zoospores). These have the typical reniform shape. (k–m) Principal zoospores. (n) Principal zoospore at the moment of encystment. Note the shed flagellum. (o) Principal cyst. (p) Principal cyst germinating by means of a germ tube. (a–f) to same scale; (g–p) to same scale. Note that the straminipilous flagellum cannot be distinguished from the whiplash flagellum at the magnification chosen.

cleavage furrows separate the cytoplasm into uninucleate pieces, each of which differentiates into an auxiliary zoospore. As the zoospores are cleaved, the central vacuole disappears. The tip of the cylindrical sporangium contains clearer cytoplasm and a flattened protuberance, the **papilla**, develops at the apex. As the sporangium ripens and the zoospores become fully differentiated, they show limited movement and change of shape (Figs. 5.4b–d). Shortly before discharge, there is evidence of a build-up of turgor pressure within the sporangium because the basal septum becomes concave, i.e. it is bent towards the lumen of the hypha beneath the sporangium. After cleavage, the positive turgor pressure is lost concomitantly with the loss of the sporangial plasma membrane which becomes part of the zoospore membranes, and the septum again bulges into the sporangium while the zoospores become fully differentiated. The sporangium undergoes a slight change of shape at this time and the sporangium wall breaks down at the papilla. The spores are released quickly, many zoospores escaping in a few seconds and moving as a column through the opening. Osmotic phenomena have been invoked to explain the rapidity of discharge, and the osmotically active substances must be large enough to be contained by the sporangial wall. Mycolaminarin, released from the central vacuole during zoospore differentiation, is the likely solute (Money & Webster, 1989). The whole process of sporangium differentiation takes about 90 min. The zoospores leave the sporangium backwards, with the blunt posterior end emerging first. The size of the zoospore is sometimes greater than the diameter of the sporangial opening so that the zoospores are squeezed through it. An occasional zoospore may be left behind, swimming about in the empty zoosporangium for a while before making its exit. Zoospores in partially empty sporangia orientate themselves in a linear fashion along the central axis of the sporangium.

A characteristic feature of *Saprolegnia* is that, following the discharge of a zoosporangium, growth is renewed from the septum at its base so that a new apex develops inside the old sporangial wall by **internal proliferation**. This in turn may develop into a zoosporangium, discharging its spores through the old pore (Fig. 5.4f). The process may be repeated so that several empty zoosporangial walls may be found inside, or partially inside, each other.

Upon release, the auxiliary zoospores slowly revolve and eventually swim somewhat sluggishly with the pointed end directed forwards. They are grapeseed- or pear-shaped ('Conference' pear; Dick, 2001a) and bear two apically attached flagella (see Figs. 5.1a, 5.4g). Each zoospore also contains a diploid nucleus, mitochondria, a contractile vacuole and numerous vesicles (Holloway & Heath, 1977a,b). The zoospores from a single sporangium show variation in their period of motility, the majority encysting within about a minute, but some remaining motile for over an hour. The zoospore then withdraws its flagella and encysts, i.e. the cytoplasm becomes surrounded by a distinct wall which is produced from pre-formed material stored in the cytoplasmic vesicles. Only the axonemes of the flagella are withdrawn, leaving the TTHs of the straminipilous flagellum at the surface of the cyst (see Fig. 5.5a). Following a period of rest (2–3 h in *S. dioica*), the cyst germinates to release a further zoospore, the principal zoospore (Figs. 5.4i,j). This differs in shape from the auxiliary zoospore in being bean-shaped, with the two flagella inserted laterally in a shallow groove running down one side of the zoospore (Fig. 5.1b). The principal zoospore may swim vigorously for several hours before encysting. Salvin (1941) compared the rates of movement of auxiliary and principal zoospores in *Saprolegnia* and found that the latter swam about three times more rapidly. The probable reason for this is that the lateral insertion of both flagella allows the straminipilous flagellum to point forward and the whiplash one to point backward, thereby improving the propulsion relative to the apical insertion in which both flagella point forward.

Movement of principal zoospores is chemotactic and zoospores can be stimulated to aggregate on parts of animal bodies such as the leg of a fly, or the surface of a fish (Fischer & Werner, 1958; Willoughby & Pickering, 1977). When principal zoospores encyst, they shed

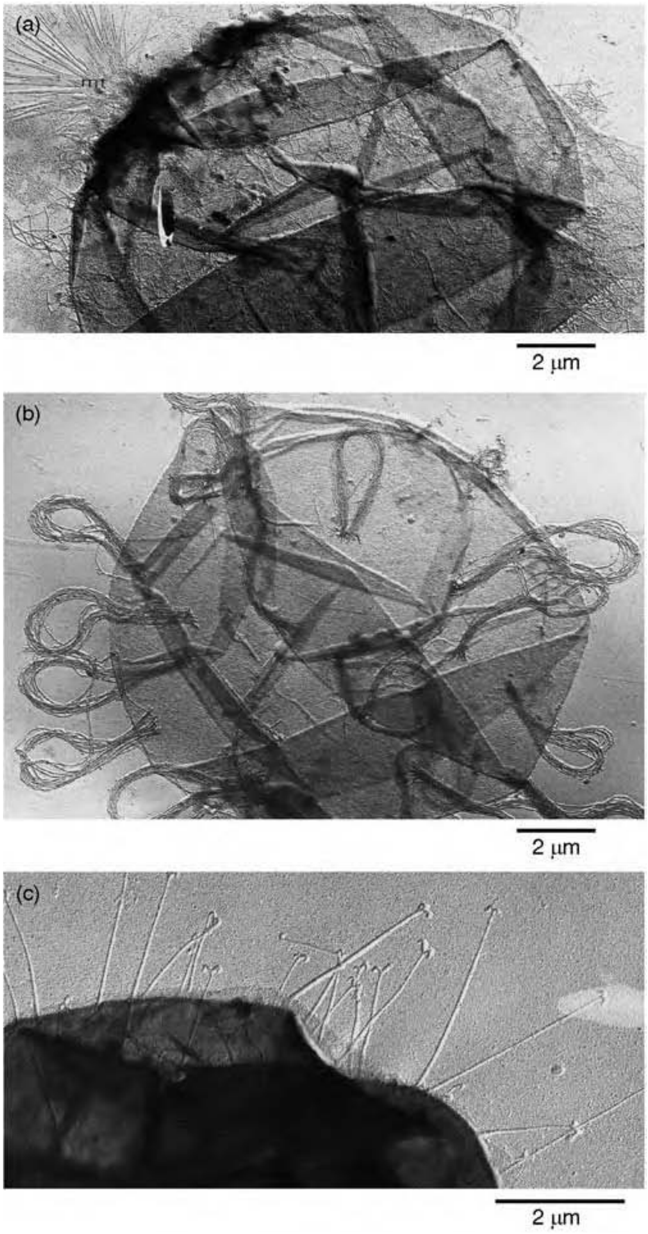

Fig 5.5 Surface features of *Saprolegnia*. (a) Detail of an auxiliary zoospore cyst of *S. parasitica* showing the tuft of TTHs (mt) at the point where the straminipilous flagellum was withdrawn. (b) Surface of a principal zoospore cyst of *S. parasitica*; the long boat hook spines are arranged in fascicles. (c) Surface of a principal zoospore cyst of *S. hypogyna* with discrete boat hooks of intermediate length. All bars = 2 μm. All images kindly provided by M.W. Dick and I.C. Hallett; (b) reprinted from Hallett and Dick (1986), with permission from Elsevier.

rather than withdraw their flagella. The first step in encystment is the fusion of vesicles called K-bodies with the plasma membrane. These are so called because they are located near the kinetosome. The material they secrete is involved in attachment of the zoospore to a substratum, which occurs in the region of the groove near the flagellar bases, designated the ventral region (Lehnen & Powell, 1989). The cyst wall and preformed boat hook spines are secreted by fusion of encystment vesicles with the plasma membrane (Beakes, 1987; Burr & Beakes, 1994). The length and arrangement of spines on the surface of a mature principal cyst are characteristic features of individual species (Figs. 5.5b,c). They probably mediate attachment of the cyst to the host, and pathogenic isolates of *Saprolegnia* have much longer spines than saprotrophic ones (Burr & Beakes, 1994). Alternatively, the boat hooks may mediate attachment to the water meniscus.

Either way, attachment must be very effective because trout or char, placed in a water bath with principal zoospores of *S. parasitica* for 10 min and followed by 1 h in clean water, had an extremely high concentration of cysts attached to the skin (Willoughby & Pickering, 1977).

Principal zoospore cysts can germinate either by means of a germ tube (Fig. 5.4p) or by releasing a further principal zoospore which in turn may germinate directly or by releasing yet another motile stage. *Saprolegnia* is therefore polyplanetic. The auxiliary and principal zoospores, as well as the cysts they form, differ morphologically from each other, i.e. they are diplanetic.

Sexual reproduction in *Saprolegnia*

All members of the genus *Saprolegnia* characterized to date are homothallic, i.e. a culture derived from a single zoospore will give rise to a mycelium forming both oogonia and antheridia. In contrast, *Achlya* also contains heterothallic species in which sexual reproduction occurs only when two different strains are juxtaposed, one forming oogonia, the other antheridia (see Fig. 5.10).

Sexual reproduction follows a similar course in all members of the Saprolegniales. Oogonia containing one or several eggs are fertilized by antheridial branches. Fertilization is accomplished by the penetration of fertilization tubes into the oogonium. In some species, ripe oogonia are found without antheridia associated with them (Fig. 5.6f); this could be due either to the fusion of two haploid nuclei from adjacent meiotic events in a single oogonium (**apomixis**) or the formation of an oospore around a diploid nucleus that never underwent meiosis (**parthenogenesis**). Both processes are impossible to distinguish without detailed cytological evidence (Dick, 2001a). The typical arrangement of oogonia and antheridia in Saprolegnia is shown in Fig. 5.6. Antheridial branches arising from the stalk of the oogonium or the same hypha as the oogonium are said to be **monoclinous** whereas they are **diclinous** if they originate from different hyphae.

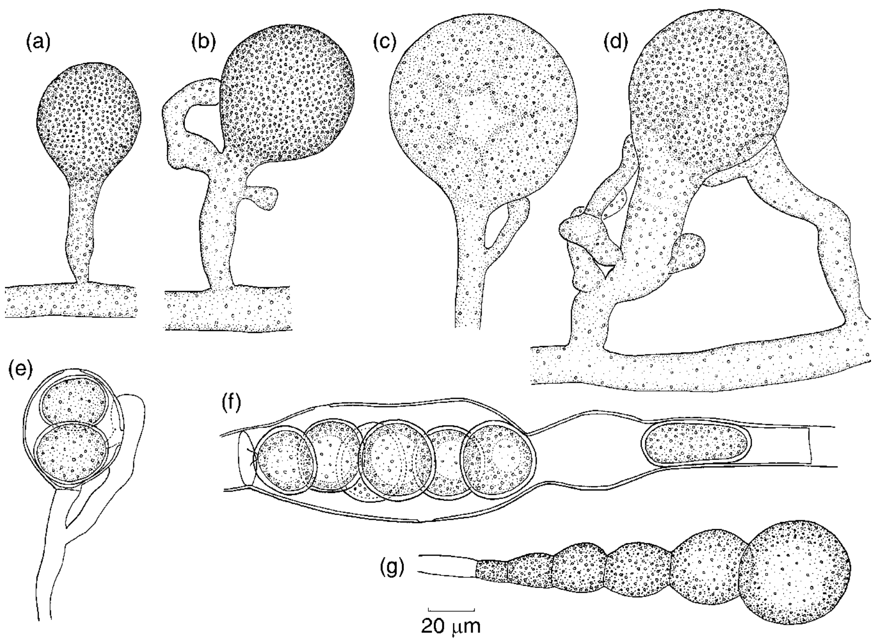

Fig 5.6 *Saprolegnia litoralis*. (a–d) Stages in the development of oogonia. (c) Oogonium showing furrowed cytoplasm indicative of centrifugal cleavage. (d) Outlines of two oospheres become visible. (e) Oogonium with two mature oospores. (f) Intercalary oogonium lacking antheridia. The oospores have developed by apomixis or parthogenesis. (g) Chain of chlamydospores.

The oogonial initial is multinucleate, and nuclear divisions continue as it enlarges. Eventually some of the nuclei degenerate, leaving only those nuclei which are included in the oospheres. From the central vacuole within the oogonium, cleavage furrows radiate outwards to divide the cytoplasm into uninucleate portions which round off to form oospheres. Oogonium differentiation is thus centrifugal, which is typical of the Saprolegniales. Cleavage of the oospheres from the cytoplasm is brought about by the coalescence of dense body vesicles which finally fuse with the plasma membrane of the oogonium so that the oospheres tumble into the centre of the oogonium (Dick, 2001a). The entire mass of cytoplasm within the oogonium is used up in the formation of oospheres and there is no residual cytoplasm (**periplasm**) as in the Peronosporales. The wall of the oogonium is often uniformly thick, but in some species it shows thin areas or pits through which fertilization tubes may enter (Fig. 5.6e). A septum at the base of the oogonium cuts it off from the subtending hypha.

The antheridia are also multinucleate. The antheridial branch grows towards the oogonium and attaches itself to the oogonial wall. The tip of the antheridial branch is cut off by a septum, and the resulting antheridium puts out a fertilization tube which penetrates the oogonial wall and may branch within the oogonium. After the tube has penetrated an oosphere wall, a male nucleus eventually fuses with the single oosphere nucleus. The fertilized oosphere (oospore) undergoes a series of changes described by Beakes and Gay (1978a,b). The wall of the oospore thickens and oil globules become obvious. Mature oospores contain a membrane-bound vacuole-like body, the ooplast, surrounded by cytoplasm containing various organelles, with lipid droplets particularly prominently visible. In *Saprolegnia*, the ooplast contains particles in Brownian motion. The position of the ooplast in the oospore is used for species identification, and four types of oospore have been distinguished (Fig. 5.7; Seymour, 1970; Howard, 1971). **Centric** oospores have a central ooplast surrounded by one or two peripheral layers of small lipid droplets (e.g. *S. hypogyna*, *S. ferax*). **Subcentric** oospores have several layers of small lipid droplets on one side of the ooplast and only one layer or none at all on the other (e.g. *S. unispora*, *S. terrestris*). In **subeccentric** oospores, the small lipid droplets have fused into several large ones all grouped to one side, with the ooplast contacting the plasma membrane on the opposite side (e.g. *S. eccentrica*). The **eccentric** type (found, for example, in *S. anisospora*) is similar to the subeccentric type except that there is only one very large lipid drop. These descriptive terms are also used for many other species of Oomycota.

5.2.2 *Achlya* (Saprolegniaceae)

Phylogenetic analyses have shown that the genera *Achlya* and *Saprolegnia* as well as minor genera of the Saprolegniales are closely related to each other, with possible overlaps which may necessitate the re-assignment of some species in future (Riethmüller *et al.*, 1999; Leclerc *et al.*, 2000; Dick, 2001a). Morphologically and ecologically, *Achlya* and *Saprolegnia* also share several key features. Both are common in soil and in waterlogged plant debris such as twigs, and certain species are pathogens of fish (Willoughby, 1994; Kitancharoen *et al.*, 1995). Unlike *Saprolegnia*, some species of *Achlya* are heterothallic, but their life cycle is otherwise similar to that of *Saprolegnia* given in Fig. 5.3. Heterothallic strains of *Achlya* have been the subject of classical

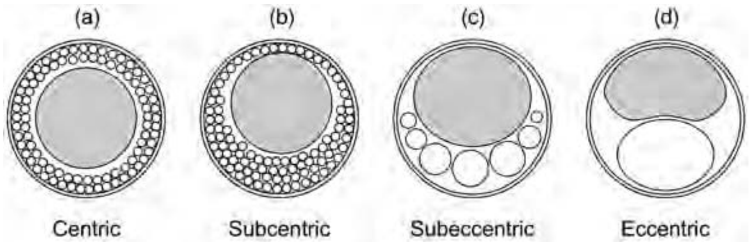

Fig 5.7 Possible arrangements of the ooplast (shaded organelle) and lipid droplets (empty circles or ellipses) in oospores of *Saprolegnia*. (a) Centric. (b) Subcentric. (c) Subeccentric. (d) Eccentric.

studies on the nature of mating hormones (pheromones); additionally, more recent work has focused on zoospore release. Both aspects are described below.

Asexual reproduction in *Achlya*

The development of zoosporangia in *Achlya* is similar in all aspects to that in *Saprolegnia* but has been better researched. The central vacuole in the developing cylindrical sporangium is typical of the Saprolegniales and originates from the fusion of dense body vesicles containing mycolaminarin. The centrifugal cleavage of cytoplasm from the vacuole towards the plasma membrane, and the partitioning of individual spores, are controlled mainly by the actin cytoskeleton (Heath & Harold, 1992). In the Pythiales, vital roles of microtubules in the organization of differentiating cytoplasm have been described (see p. 102), and microtubules may have similar but as yet undescribed functions in the Saprolegniales. As the plasma membrane of the *Achlya* zoosporangium is breached, the zoosporangial volume decreases by about 10% due to the loss of turgor pressure. Since the membranes of the vacuole contribute to the zoospore plasma membrane, the vacuolar contents of water-soluble mycolaminarins (β-1,3-glucans) are released into the sporangium. These molecules are osmotically active but are too large to diffuse through the sporangial wall, thus causing the osmotic inward movement of water into the sporangium, which in turn pressurizes the sporangium and drives the rapid discharge of the auxiliary zoospores (Money & Webster, 1985, 1988; Money *et al*., 1988).

On discharge, the zoospores do not swim away but cluster in a hollow ball at the mouth of the zoosporangium and encyst there (Fig. 5.8a). In fact, it is doubtful whether the term 'zoospore' is altogether appropriate as functional flagella are probably not formed. Partial fragmentation of the cyst ball frequently occurs and may have ecological significance in the dispersal of cysts prior to the release of principal zoospores. Unlike certain species of *Saprolegnia*, *Achlya* cysts are normally found at the bottom of culture dishes, and presumably also at the water/bottom sediment interface in natural environments. Cysts of

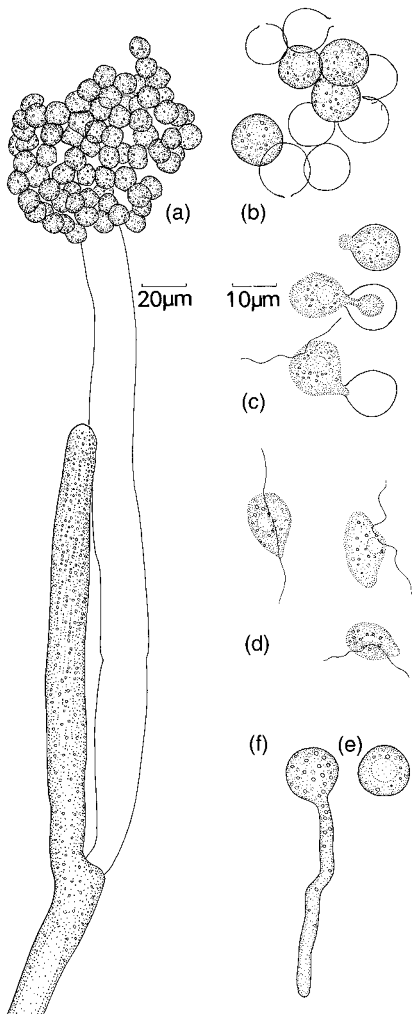

Fig 5.8 *Achlya colorata*. (a) Zoosporangium showing a clump of primary cysts at the mouth. Note the lateral proliferation of the hypha from beneath the old sporangium. (b) Full and empty auxiliary cysts. (c) Stages in the release of principal zoospores from an auxiliary cyst. (d) Principal zoospores. (e) Principal cyst. (f) Principal cyst germinating by means of a germ tube.

A. klebsiana may remain viable for at least two months when stored aseptically at 5°C (Reischer, 1951). However, most auxiliary cysts remain at the mouth of the sporangium for a few hours and then each cyst releases a principal zoospore through a small pore (Figs. 5.8b,c). After a period of swimming, principal zoospores encyst, and principal cysts germinate either by a germ tube or by releasing another principal zoospore. When the zoosporangium of *Achlya* has released its

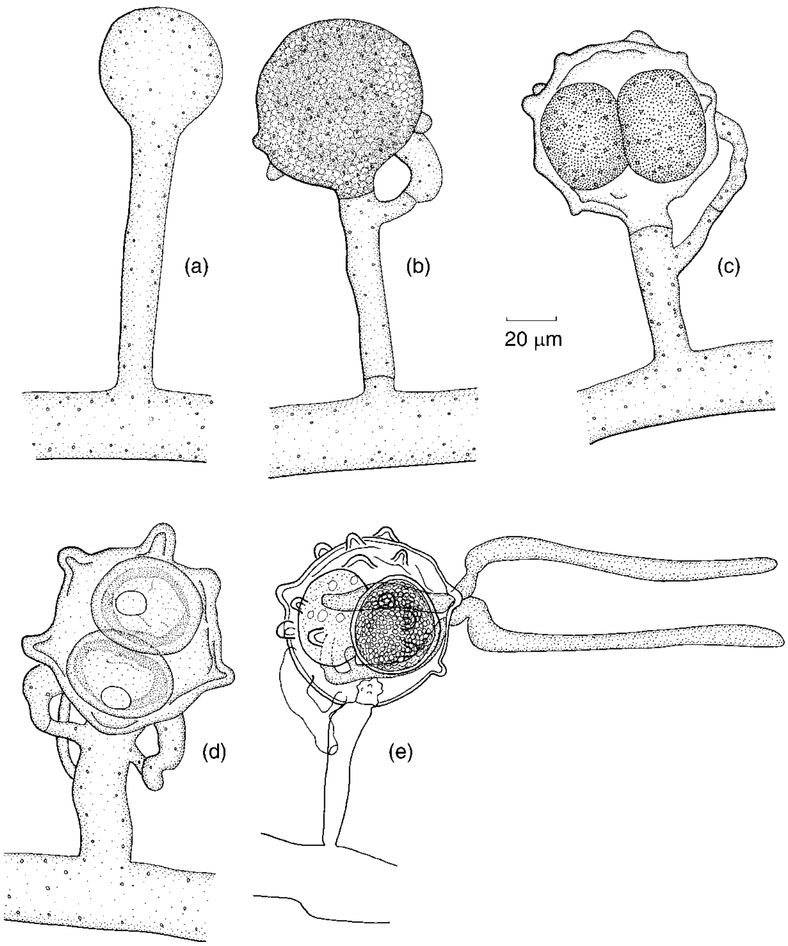

Fig 5.9 *Achlya colorata*. (a–d) Stages in the development of oogonia. (e) Six-month-old oospores germinating after 40 h in charcoal water.

zoospores, growth is usually renewed laterally by the outgrowth of a new hyphal apex just beneath the first sporangium (Fig. 5.8a), rather than by internal proliferation.

Sexual reproduction in *Achlya*

Some species of *Achlya* are homothallic (Fig. 5.9) whereas others are heterothallic (Fig. 5.10). *Achlya colorata*, a homothallic species common in Britain, has oogonial walls which develop blunt, rounded projections so that the oogonium appears somewhat spiny (Fig. 5.9d). Otherwise, the process of sexual reproduction is similar to that of *Saprolegnia litoralis* (Fig. 5.6). Germination of oospores is often difficult to achieve with Oomycota, but can be stimulated in *A. colorata* by transferring mature oospores to freshly distilled water (preferably after shaking with charcoal and filtering). Germination occurs by means of a germ tube which grows out from the oospore through the oogonial wall. Here it may continue growth as a mycelium (Fig. 5.9e) or may give rise to a sporangium.

The study of heterothallic species of *Achlya* by John R. Raper quickly revealed that the formation of oogonia and antheridia by compatible strains must be under hormonal control (Raper, 1939, 1957). A particularly readable account of the classical series of experiments leading to the discovery of the steroid sex hormone, antheridiol (Fig. 5.11b), has been given by Carlile (1996b). Several reviews of the broader role of hormones in fungal reproduction have appeared recently (Gooday & Adams, 1992; Elliott, 1994). If isolates of *Achlya bisexualis*, *A. ambisexualis* or *A. heterosexualis* made from water or mud are grown singly

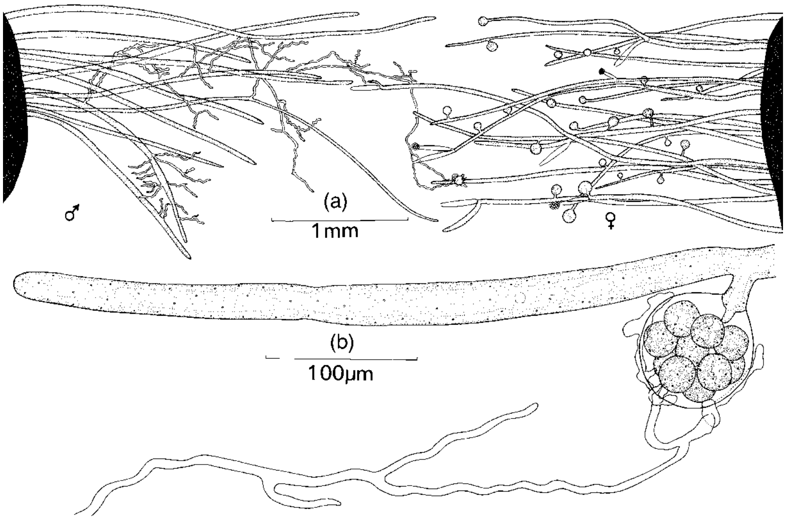

Fig 5.10 *Achlya ambisexualis*. (a) Male and female mycelia grown on hemp seeds and placed together in water for 4 days. Note the formation of antheridial branches on the male and oogonial branches on the female. (b) Fertilization, showing the diclinous origin of the antheridial branch.

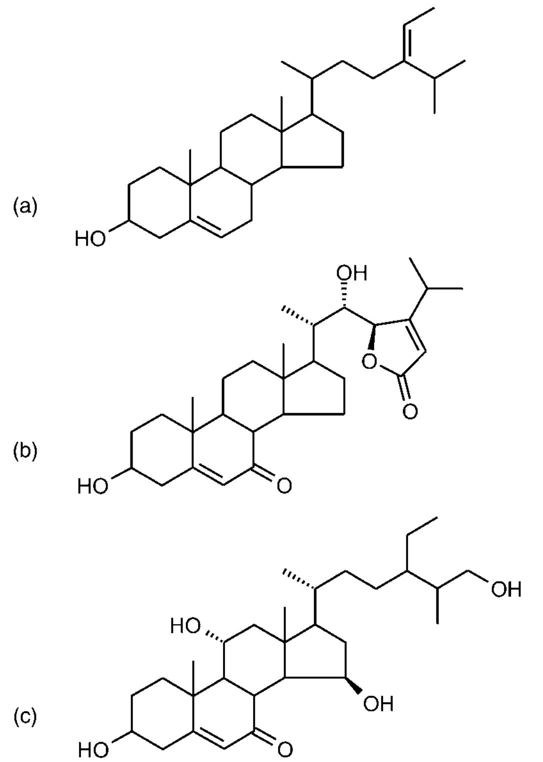

Fig 5.11 Sterols from *Achlya* spp. Fucosterol (a) is one of the most abundant sterols in Oomycota and precursor to the sex hormones antheridiol (b) and oogoniol (c).

on hemp seed in water, reproduction is entirely asexual, but when certain of the isolates are grown together in the same dish, it becomes apparent within 2–3 days that one strain is forming oogonia, and the other antheridia. The development of oogonia and antheridia occurs even when the two strains are held apart in the water or separated by a cellophane membrane or by agar. This suggests that one or more diffusible substances are responsible for the phenomenon. As compatible colonies approach each other, the first observable reaction is the production of fine lateral branches behind the advancing tips of the male hyphae. These are antheridial branches.

By growing male (antheridial) strains in water in which a female (oogonial) strain had been grown previously, Raper (1939) showed that the vegetative female mycelium was capable of initiating the development of antheridial branches on the male. The reverse experiment showed no effect on female colonies in medium in which undifferentiated male colonies had been grown. The role of the vegetative female colony as initiator of the sequence of events leading to sexual reproduction was confirmed by ingenious experiments in micro-aquaria consisting of several consecutive chambers through which water flowed by means of small siphons. Male and female colonies were placed alternately in successive chambers, so that water from a male colony would flow over a female colony and so on. If a female colony was placed in the first chamber, the male colony in the second chamber reacted by developing antheridial hyphae. If, however, a male colony was placed in the first chamber, the male colony in the third chamber was the first to react. Raper (1939) postulated that the development of the antheridial branches was in response to a hormone, termed Hormone A, secreted by vegetative female colonies. By further experiments of this kind, he showed that the later steps in the sexual process were also regulated by means of diffusible substances. He postulated that the antheridial branches secreted a second substance, Hormone B, which resulted in the formation of oogonial initials on the female colony. The oogonial initials in their turn secreted a further substance called Hormone C, which stimulated the antheridial initials to grow towards the oogonial initials and also resulted in the antheridia being delimited. Having made contact with the oogonial initials, the antheridial branches secreted Hormone D which resulted in the formation of a septum cutting off the oogonium from its stalk, and in the formation of oospheres. The original scheme (Table 5.2) therefore implicated four hormones, but confusion arose subsequently because the effect of Hormone A can be modulated by amino acids and other metabolites released from the hemp seeds (Barksdale, 1970; Schreurs *et al*., 1989).

Since Hormone A is active at extremely low concentrations of 2×10^{-11} M (Barksdale, 1969), purification of this substance was extremely challenging, and 6000 l of culture fluid had to be extracted to obtain 20 mg crystalline Hormone A (Barksdale, 1967). It was eventually identified as the steroid antheridiol (Fig. 5.11b). Soon after, the structure of Hormone B was elucidated and found also to be a steroid, oogoniol (Fig. 5.11c), which is, in fact, present as three chemically closely related forms (McMorris *et al*., 1975). The effect postulated by Raper (1939) to be due to Hormone C is now thought to be mediated by antheridiol activity, whereas Hormone D may not exist (Carlile, 1996b). Both antheridiol and the oogoniols are derived from fucosterol (Fig. 5.11a), the principal sterol in *Achlya* (see Elliott, 1994).

The physiological roles of antheridiol and the oogoniols are several-fold and include induction or suppression of sexuality (Thomas & McMorris, 1987), directional growth of gametangial tips (McMorris, 1978), and stimulation of the production of cell wall-softening enzymes (especially cellulase) at points of branching and contact between gametangia (Mullins, 1973; Gow & Gooday, 1987). A cytoplasmic receptor protein for antheridiol has been detected (Riehl *et al*., 1984), and the hormone probably acts like its equivalents in mammalian cells, by the receptor–hormone complex moving to the nucleus and binding specifically to DNA, increasing transcription rates of certain genes (Elliott, 1994).

There is evidence that the co-ordination of sexual reproduction by hormonal control is not confined to heterothallic forms of *Achlya*, but also takes place in homothallic species. The fact that it is possible to initiate sexual reactions between homothallic and heterothallic species of *Achlya* shows that some of the hormones are common to more than one species, although

Table 5.2. Postulated effects of hormones on sexual reactions in *Achlya ambisexualis*.

Hormone	Produced by	Affecting	Specific action
A	Vegetative hyphae	Vegetative hyphae	Induces formation of antheridial branches.
B	Antheridial branches	Vegetative hyphae	Initiates formation of oogonial initials.
C	Oogonial initials	Antheridial branches	(1) Attracts antheridial branches. (2) Induces thigmotropic response and delimitation of antheridia.
D	Antheridia	Oogonial initials	Induces delimitation of oogonium by formation of basal septum.

After Raper (1939). So far, only hormones A and C (antheridiol) and B (oogoniol) have been shown to exist.

there is also evidence of some degree of specificity of the hormones of different species (Raper, 1950; Barksdale, 1965).

One further interesting phenomenon which has been discovered in relation to heterothallic *Achlya* spp. is relative sexuality. If isolates of *A. bisexualis* and *A. ambisexualis* from separate sources are paired in all possible combinations, it is found that certain strains show a capacity to react either as male or as female, depending on the particular partner to which they are apposed. Other strains remain invariably male or invariably female, and these are referred to as true or strong males or females. The strains can be arranged in a series with strong males and strong females at the extremes, and intermediate strains whose reaction may be either male or female depending on the strength of their mating partner. Similar interspecific responses between strains of *A. bisexualis* and *A. ambisexualis* are also possible. Further, some of the strains which appear heterothallic at room temperature are homothallic at lower temperatures. Barksdale (1960) has postulated that the heterothallic forms are derived from homothallic ones. She argued that the most notable difference between strong males and strong females lies in their differential antheridiol production and response. Very little of this substance is found in male cultures, and these are much more sensitive in their response to the hormone than female cultures. Another important difference is in the uptake of antheridiol. Certain strains appear capable of absorbing it much more readily than others, and it is the strains with a high ability to absorb antheridiol that produce antheridial branches during conjugation with other thalli (Barksdale, 1963). If one assumes that heterothallic forms have been derived from homothallic ones, this might have occurred by mutations leading to increased sensitivity to antheridiol and hence to maleness. Conversely, mutations leading to enhanced extracellular accumulation of antheridiol should lead to increasing femaleness.

Germination of the oospores of *A. ambisexualis* results in the formation of a multinucleate germ tube which develops into a germ sporangium if transferred to water, or into a coenocytic mycelium in the presence of nutrients. This mycelium can be induced to form zoosporangia when transferred to water. From zoosporangia of either source, single zoospore cultures can be obtained which can be mated with the parental male or female strains. All zoospores or germ tubes derived from a single oospore gave the same result with regard to their sexual interaction. This finding suggests that nuclear division on oospore germination is not meiotic, and is thus consistent with the idea that the life cycle is diploid (Mullins & Raper, 1965). Confirmation of these results, implying meiosis during gamete differentiation, has also been obtained with *A. ambisexualis* (Barksdale, 1966).

5.2.3 *Thraustotheca, Dictyuchus* and *Pythiopsis* (Saprolegniaceae)

In *Thraustotheca clavata* the sporangia are broadly club-shaped, and there is no free-swimming auxiliary zoospore stage. Encystment occurs within the sporangia and the auxiliary cysts are released by irregular rupture of the sporangial wall (Fig. 5.12a). After release, the angular cysts germinate to release bean-shaped principal zoospores with laterally attached flagella (Figs. 5.12c,d). After a period of swimming, further encystment occurs, followed by germination by a germ tube (Figs. 5.12e,f), or by emergence of a further principal zoospore. The zoospores are thus monomorphic and polyplanetic. Sexual reproduction is homothallic, but formation of gametangia is stimulated by *Achlya* sex hormones (Raper, 1950). Oospores germinate either by a germ tube or by a germ sporangium (Fig. 5.12g).

In *Dictyuchus*, there is again no free-swimming auxiliary zoospore stage. Commonly the entire zoosporangium is deciduous, and detached zoosporangia are capable of forming zoospores. Auxiliary zoospore initials are cleaved out but encystment occurs within the cylindrical sporangium. The cysts are tightly packed together and release their principal zoospores independently through separate pores in the sporangial wall (Fig. 5.13a). When zoospore release is complete, a network made up of the polygonal walls of the auxiliary cysts is left behind. After swimming, the laterally biflagellate zoospores

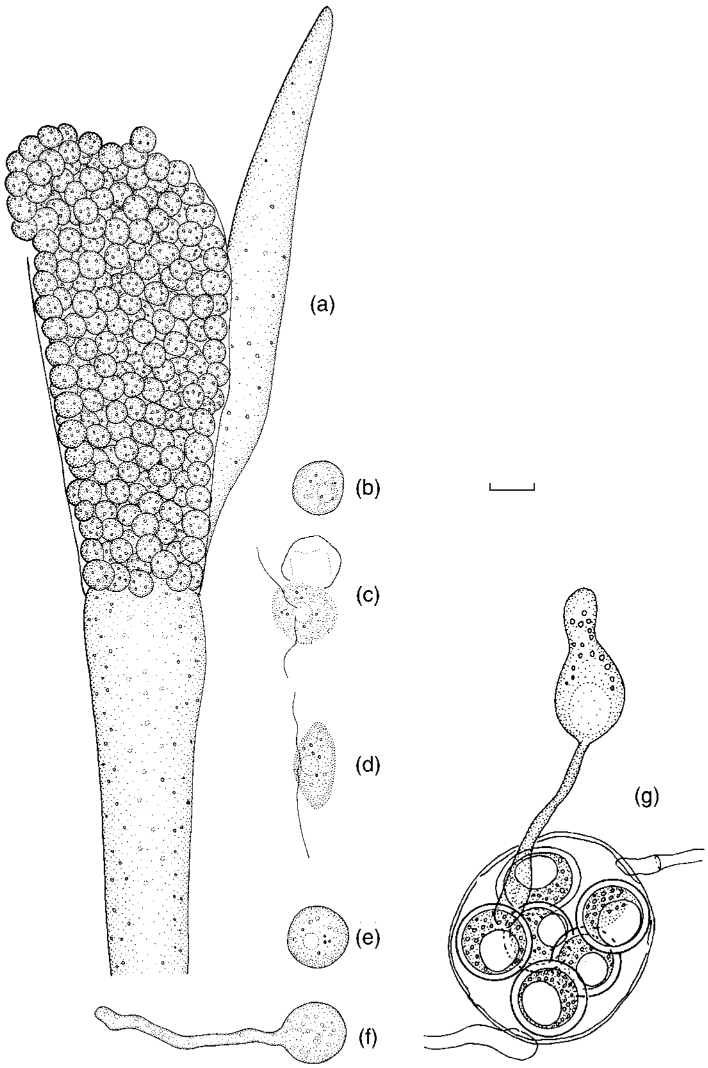

Fig 5.12 *Thraustotheca clavata*.
(a) Zoosporangium showing formation of auxiliary cysts within the sporangium. The auxiliary cysts are being released through breakdown of the sporangial wall.
(b) Auxiliary cyst. (c) Auxiliary cyst germinating to release a principal zoospore, the first motile stage in this species.
(d) Principal zoospore. (e) Principal cyst.
(f) Principal cyst germinating by means of a germ tube. (g) Sexual reproduction. Six-month-old oospore germinating after 17 h in charcoal water. The germ tube is terminated by a germ sporangium. Bar=20 μm (a) or 10 μm (b)–(g).

encyst (Figs. 5.13b,c). Electron micrographs have shown that the wall of the secondary cyst of *D. sterile* bears a series of long spines looking somewhat like the fruit of a horse chestnut (Fig. 5.14; Heath *et al*., 1970). Following the formation of the first zoosporangium, a second may be produced immediately beneath it by the formation of a septum cutting off a subterminal segment of the original hypha, or growth may be renewed laterally to the first sporangium (Fig. 5.13a).

Because there is only one motile stage in *Thraustotheca* and *Dictyuchus* (i.e. a zoospore of the principal type), they are said to be monomorphic. *Pythiopsis cymosa* (Figs. 5.13e–i) is also monomorphic, but in this species the only motile stage is of the auxiliary type and principal zoospores are not formed. After swimming, the zoospore encysts and then germinates directly by means of a germ tube (Figs. 5.13g–i).

5.2.4 Aplanetic forms

In certain cultures of Saprolegniaceae the zoosporangia produce cysts which do not release any motile stage. Instead, germ tubes are put out which penetrate the sporangial wall. Forms without motile spores are said to be **aplanetic**. The aplanetic condition is occasionally found in staling cultures of *Saprolegnia*, *Achlya* and

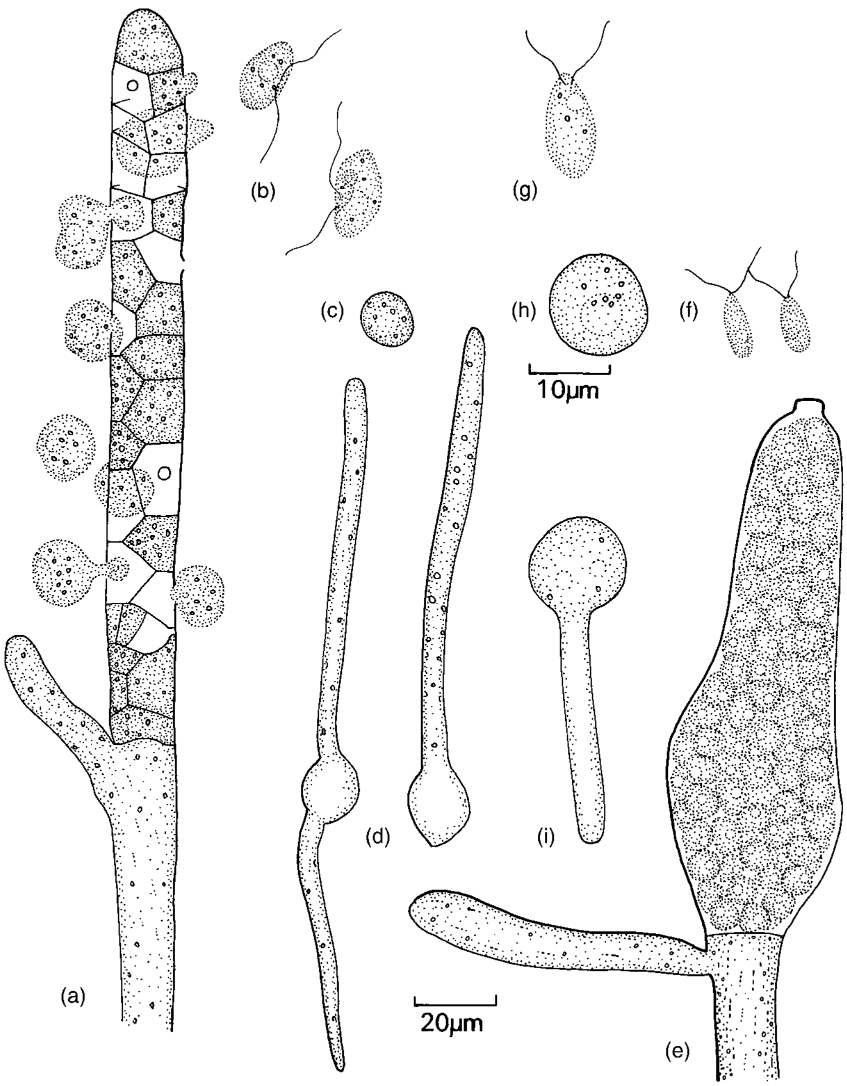

Fig 5.13 (a–d) *Dictyuchus sterile*. (a) Zoosporangium showing cysts within the sporangium and the release of principal zoospores through separate pores in the sporangium wall. Note the network of auxiliary cyst walls. (b) Principal zoospores. (c) Principal cyst. (d) Germination of principal cysts by means of germ tubes. (e–i) *Pythiopsis cymosa*. (e) Zoosporangium. (f,g) Auxiliary zoospores. (h) Auxiliary cyst. (i) Auxiliary cyst germinating by means of a germ tube. Principal zoospores have not been described. (a–c,e,f) to same scale; (g–i) to same scale.

Dictyuchus. Some species produce sporangia only rarely and the genus *Aplanes* has been erected for these forms. However, in very clean cultural conditions, all have been shown to behave as *Achlya*, and they are currently accommodated within that genus (Dick, 2001a). Two species of Saprolegniaceae are not known to form sporangia at all. They are common in soil, and have been placed in a separate genus, *Aplanopsis*. Another genus, *Geolegnia*, forms sporangia containing thick-walled aplanospores which never produce a flagellate stage. The final classification of these small genera of Saprolegniaceae will have to await the results of comparisons of suitable DNA sequences (see M.A. Spencer *et al.*, 2002).

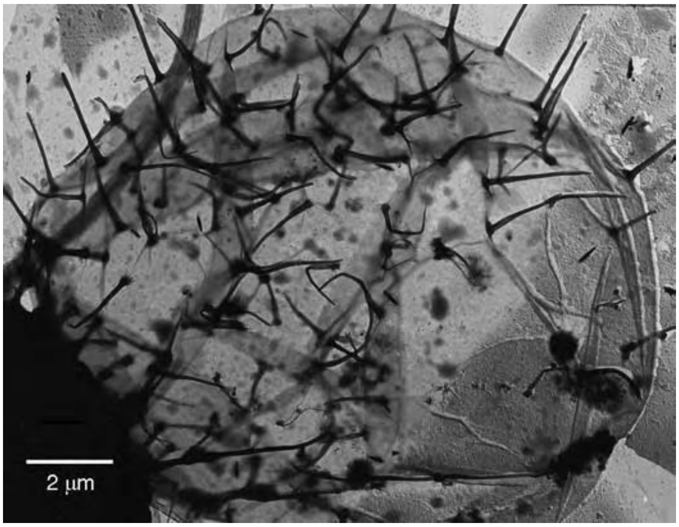

Fig 5.14 Surface of a principal cyst of *Dictyuchus sterile*. Note the spines covering the surface. Image kindly provided by M.W. Dick and I.C. Hallett; reprinted from Hallett and Dick (1986), with permission from Elsevier.

5.2.5 *Aphanomyces* (Leptolegniaceae)

Aphanomyces is distinguished from *Achlya* by its thin, delicate hyphae and its narrow sporangia containing a single row of spores. Based on these morphological differences and DNA sequence analyses, the genus *Aphanomyces* has been removed from the Saprolegniaceae and classified in the family Leptolegniaceae, still within the Saprolegniales (Dick *et al.*, 1999; Hudspeth *et al.*, 2000; Dick, 2001a).

Asexual reproduction in *Aphanomyces* is variable. In *A. euteiches*, flagella do not develop on the first-formed spores. Protoplasts are cleaved out, move to the mouth of the sporangium, and encyst. Principal zoospores develop from the cysts and are the first true motile stage. *Aphanomyces euteiches* is thus monomorphic. In *A. patersonii*, the motility of the first-formed zoospore is controlled by variation in temperature. Below 20°C, encystment of the auxiliary zoospores at the mouth of the sporangium occurs in a manner typical of the genus, but above this temperature the auxiliary zoospores swim away and encyst some distance away from the zoosporangium.

The genus *Aphanomyces* has been monographed by Scott (1961). It has gained notoriety particularly because *A. astaci* is the cause of the plague of European crayfish. Having been introduced probably in the 1860s from America, where the local crayfish populations are fairly resistant to *A. astaci* infections, the fungus has now spread across Europe, severely damaging commercial production of the highly susceptible European crayfish, *Astacus fluviatilis* (Alderman & Polglase, 1986; Cerenius *et al.*, 1988; Alderman *et al.*, 1990). Although it would be possible to introduce resistant stock of American crayfish into European river systems affected by the disease, resistant crayfish still harbour the pathogen, thereby making it impossible to restore the native crayfish populations in the future (Dick, 2001a). The difference in resistance between North American and European crayfish lies in the melanization reaction which arrests hyphal growth from encysted zoospores (Nyhlén & Unestam, 1980; Cerenius *et al.*, 1988). In European crayfish, melanization occurs too slowly to prevent the spread of the fungus into the haemocoel which causes rapid death. *Aphanomyces astaci* can also cause epizootic ulcerative disease in fish, the symptoms often being very similar to those caused by *Saprolegnia* (Lilley & Roberts, 1997).

Aphanomyces euteiches is a significant pathogen of roots of peas and other terrestrial plants (Papavizas & Ayers, 1974; Persson *et al.*, 1997). Recently, methods have been developed to quantify the prevalence of the pathogen in infected plants by measuring the levels of specific fatty acids which are produced by *A. euteiches* but not by plants or pathogens belonging to the Eumycota (Larsen *et al.*, 2000). Other species of *Aphanomyces* are keratinophilic, occurring in the soil or in water on insect remains (Dick, 1970; Seymour & Johnson, 1973).

5.3 Pythiales

The order Pythiales includes two families, the Pythiaceae and Pythiogetonaceae (Dick, 2001a; Kirk *et al*., 2001). The Pythiogetonaceae are a small group of aquatic saprotrophs presently comprising one genus and six species. They occur in anoxic sediments at the bottom of freshwater lakes and are facultatively anaerobic as well as obligately fermentative, i.e. they break down sugars incompletely to give organic acids irrespective of the presence or absence of oxygen (Emerson & Natvig, 1981; Natvig & Gleason, 1983). Another member of the Pythiogetonaceae, *Pythiogeton zeae*, causes root and stalk rot in maize (Jee *et al*., 2000). The Pythiogetonaceae are clearly related to the Pythiaceae by DNA sequence homology (Voglmayr *et al*., 1999).

Only the Pythiaceae will be considered further in this book. This is a large family of over 200 species in approximately 10 genera, of which 2 are of outstanding significance: *Pythium* and *Phytophthora*. *Phytophthora* species are primarily pathogenic to plants from which they can be isolated and grown in pure culture. The genus *Pythium* is best known for its saprotrophic soil-inhabiting members, many of which are opportunistic pathogens especially in young plants. There are also obligately pathogenic *Pythium* spp. Generally, *Pythium* spp. parasitize a wider diversity of hosts than *Phytophthora*, including mammals, fungi and algae.

5.3.1 Life cycle of Pythiaceae

The life cycle of *Phytophthora infestans* is summarized in Fig. 5.19. Asexual reproduction in *Pythium* and *Phytophthora* is by means of sporangia which vary in shape from swollen hyphae or globose structures (*Pythium*) to lemon-shaped (*Phytophthora*). Sporangia are borne on more or less undifferentiated hyphae. In most cases, sporangia germinate to produce zoospores which are of the principal (kidney-shaped) type. In many *Pythium* spp., the final stages of zoospore differentiation take place outside the sporangium in a walled **vesicle**, followed by breakdown of the soft wall and release of the zoospores. In *Phytophthora*, in contrast, zoospores differentiate within the sporangium and are released directly or via a very short-lived vesicle which is surrounded only by a membrane. About 20% of the total respiratory activity within a released zoospore is used up to fuel propulsion (Hölker *et al*., 1993). The forward-directed straminipilous flagellum generates about 10 times more thrust than the posterior whiplash flagellum which acts mainly as a rudder (Erwin & Ribeiro, 1996). Zoospores can swim for several hours before they encyst. The process of encystment has been examined in great detail for *Phytophthora* (see p. 102). Cysts usually germinate by means of a germ tube, only rarely producing a further zoospore stage. In many species, sporangia can germinate either indirectly by releasing zoospores or directly by means of a germ tube, depending on environmental conditions and age of the sporangium.

Sexual reproduction is oogamous. Each oogonium contains a single oosphere (except for *Pythium multisporum* in which there are several). The antheridial and oogonial initials are commonly multinucleate at their inception and further nuclear divisions may occur during development. Meiosis eventually takes place in the gametangia so that karyogamy occurs between haploid antheridial and oogonial nuclei. In many forms, there is only one functional male and female nucleus, but in others multiple fusions occur. Oospores germinate either by producing a single germ sporangium, or by sending out vegetative hyphae.

Most members of the Pythiaceae are homothallic, although heterothallism and relative sexuality have been reported, e.g. for *Phytophthora infestans* (Fig. 5.19) and *Pythium sylvaticum*. Heterothallic species are thought to be derived from homothallic ones (Kroon *et al*., 2004). The situation of mating in heterothallic strains is rather complex and still only incompletely understood. A system of two mating types (A1 and A2) seems to be superimposed on a hormonal control mechanism of mating akin to that described for *Achlya* (p. 86). When two strains of *Pythium* or *Phytophthora* were separated by a membrane preventing hyphal contact but permitting the exchange of diffusible metabolites, oospores were formed by either or both strains (Ko, 1980; Gall & Elliott, 1985). Because the mycelia were separated by a membrane, oospores formed by selfing, whereas in direct contact they may form by hybridization (Shattock *et al*., 1986a,b). Oospore formation can also be induced by non-specific stimuli, such as volatile metabolites of the unrelated fungus *Trichoderma* stimulating reproduction in A2 but not A1 strains of *Phytophthora palmivora* (Brasier, 1975a). This '*Trichoderma* effect' may well have ecological implications, since *Trichoderma* spp. are very common, especially in soil. Oospore formation may be a defence reaction against antibiotics commonly produced by *Trichoderma*, and the '*Trichoderma* effect' may actually enhance the survival of *Phytophthora* spp. in soil, since it stimulates production of the long-lived oospore stage even in the absence of a compatible mating type (Brasier, 1975b). It is not known why *Trichoderma* spp. do not stimulate oosporogenesis in A1 strains.

Like *Achlya*, the Pythiaceae display relative sexuality, i.e. a strain can act as male in one pairing but as female in another. To complicate matters further, a given strain of *Phytophthora parasitica* can switch its mating type from predominantly male to predominantly female or vice versa, e.g. upon fungicide treatment (Ko *et al*., 1986). Clearly, despite substantial research efforts over many years the genetic basis of sexual reproduction in the Pythiaceae still poses numerous unresolved questions!

By analogy with the hormones oogoniol and antheridiol of *Achlya*, a male strain needs to be induced to produce the oogonium-inducing hormone whereas female strains constitutively produce the antheridium-inducing hormone (Elliott, 1994). The ability of homothallic species to stimulate sexual reproduction in heterothallic species (Ko, 1980) indicates that these hormones may also fulfil a morphogenetic role in homothallic sexual reproduction. However, nothing seems to be known as yet about the chemical nature of these hormones.

Sterols are neither synthesized nor strictly required by vegetatively growing *Pythium* or *Phytophthora* spp. (Nes *et al*., 1979). None the less, they are required for the formation of sexual reproductive organs (Elliott, 1994). It seems, therefore, that sterols – especially sitosterol and stigmasterol which are normally taken up from the host plant – are converted into as yet unidentified steroid hormones which initiate sexual morphogenetic events downstream of the action of the diffusible *Achlya*-like hormones (Elliott, 1994). An alternative hypothesis is that sterols interact with an as yet unknown membrane protein to transmit the hormonal signal and trigger the signalling cascade leading to sexual morphogenesis (Nes & Stafford, 1984). In *Lagenidium giganteum*, a member of the Pythiaceae parasitizing mosquito larvae (Cuda *et al*., 1997), this cascade seems to be carried by Ca^{2+} and calmodulin (Kerwin & Washino, 1986).

5.3.2 *Pythium*

Species of *Pythium* grow in water and soil as saprotrophs, but under suitable conditions, e.g. where seedlings are grown crowded together in poorly drained soil, they can become parasitic, causing diseases such as pre-emergence killing, damping off and foot rot. Damping off of cress (*Lepidium sativum*) can be demonstrated by sowing seeds densely on heavy garden soil or garden compost which is kept liberally watered. Within 5–7 days some of the seedlings may show brown zones at the base of the hypocotyl, and the hypocotyl and cotyledons become water-soaked and flaccid. In this condition the seedling collapses. A collapsed seedling coming into contact with other seedlings will spread the disease (Plate 2b). The host cells separate from each other easily due to the breakdown of the middle lamella, probably brought about by pectic and possibly cellulolytic enzymes secreted by the fungus. The enzymes diffuse from their points of secretion at the hyphal tips, so that softening of the host tissue actually occurs ahead of the growing mycelium. Pure culture studies suggest that species of *Pythium* may also secrete heat-stable substances which are toxic to plants. Within the host the mycelium is coarse and

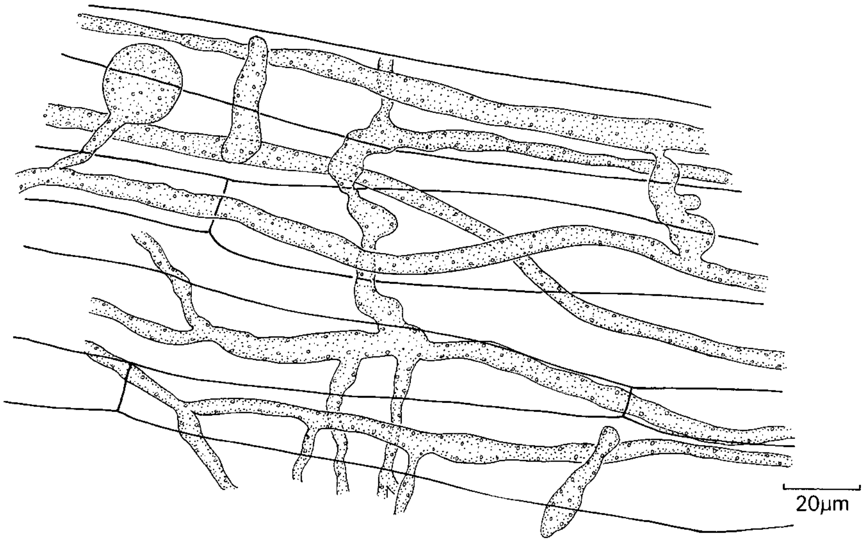

Fig 5.15 *Pythium* mycelium in the rotting tissue of a cress seedling hypocotyl. Note the spherical sporangium initial and the absence of haustoria.

coenocytic, with typically granular cytoplasmic contents (Fig. 5.15). At first there are no septa, but later cross walls may cut off empty portions of hyphae. Thick-walled chlamydospores may also be formed. There are no haustoria.

Several species are known to cause damping off, e.g. *P. debaryanum* and, perhaps more frequently, *P. ultimum*. *Pythium aphanidermatum* is associated with stem rot and damping off of cucumber, and the fungus may also cause rotting of mature cucumbers. *Pythium mamillatum* causes damping off of mustard and beet seedlings and is also associated with root rot in *Viola*. Many *Pythium* spp. have a very wide host range; e.g. *P. ultimum* parasitizes over 150 plant species belonging to many different families (Middleton, 1943; Hendrix & Campbell, 1973). Far from parasitizing only plant roots, several soil-borne species, e.g. *P. oligandrum*, *P. acanthicum* and *P. nunn*, are capable of attacking hyphae of filamentous fungi, including plant-pathogenic species and even other *Pythium* spp. (Foley & Deacon, 1986b; Deacon *et al.*, 1990). Attack may be mediated by the secretion of wall-degrading β-1,3-glucanase, chitinase and cellulase, or by inducing the host to undergo autolysis (Elad *et al.*, 1985; Laing & Deacon, 1991; Fang & Tsao, 1995). In contrast to plant-pathogenic *Pythium* spp., the mycoparasitic species require thiamine for growth and are unable to utilize inorganic nitrogen sources. These deficiencies may explain their mycoparasitic habit (Foley & Deacon, 1986a). Other species of *Pythium* parasitize freshwater and marine algae (Kerwin *et al.*, 1992).

The taxonomy of *Pythium* is somewhat confused at present due to the existence of numerous synonyms. Including a few varieties, Dick (2001a) listed 129 names in current use. Since the morphological characteristics traditionally used for diagnosis can be variable, the delimitation of species and their assignment to the genus *Pythium* will have to await the results of detailed molecular phylogenetic analyses which are in progress (Matsumoto *et al.*, 1999; Lévesque & de Cock, 2004). Keys and descriptions have been published by Waterhouse (1967, 1968), van der Plaats-Niterink (1981) and Dick (1990b).

Asexual reproduction

The mycelium within the host tissue or in culture usually produces sporangia, but their form varies. In some species, e.g. *P. gracile*,

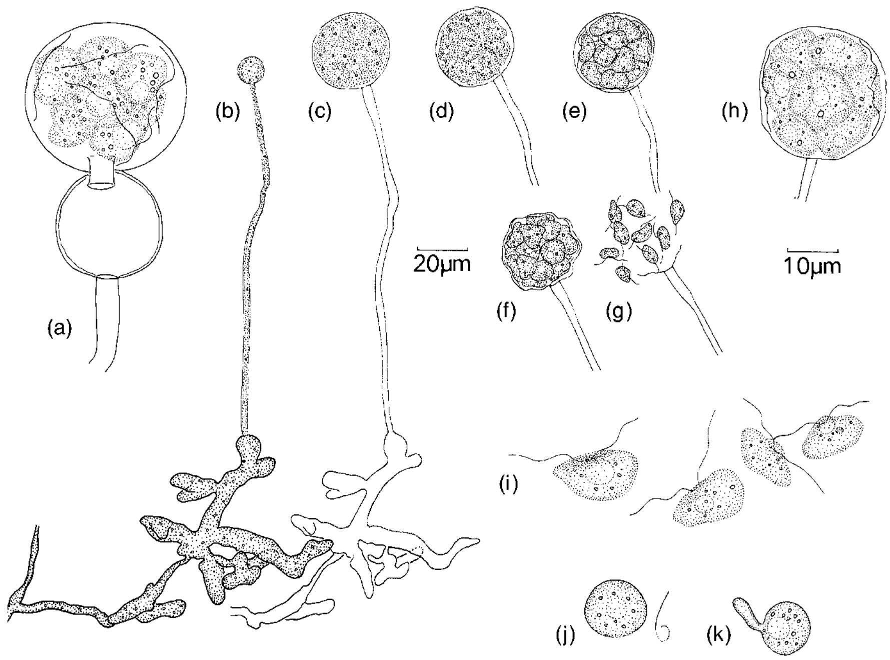

Fig 5.16 Sporangia and zoospores of *Pythium*. (a) *Pythium debaryanum*. Spherical sporangium with short tube and a vesicle containing zoospores. (b–k) *Pythium aphanidermatum*. (b) Lobed sporangium showing a long tube and the vesicle, which is beginning to expand. (c–g) Further stages in the enlargement of the vesicle, and differentiation of zoospores. Note the transfer of protoplasm from the sporangium to the vesicle in (c). The stages illustrated in (b–g) took place in 25 min. (h) Enlarged vesicle showing the zoospores. Flagella are also visible. (i) Zoospores. (j) Encystment of zoospore showing a shed flagellum. (k) Germination of a zoospore cyst. (b–g) to same scale; (a) and (h–k) to same scale.

the sporangia are filamentous and are scarcely distinguishable from vegetative hyphae. In *P. aphanidermatum*, the sporangia are formed from inflated lobed hyphae (Fig. 5.16b). In many species, however, e.g. *P. debaryanum*, the sporangia are globose (Fig. 5.16a). A terminal or intercalary portion of a hypha enlarges and assumes a spherical shape, then becomes cut off from the mycelium by a cross wall. The sporangia contain numerous nuclei. Cleavage of the cytoplasm to form zoospores begins in the sporangium, but is completed within a thin-walled vesicle which is extruded from the sporangium. This is a **homohylic** vesicle because its glucan wall is continuous with one layer of the sporangial wall (Dick, 2001a). Within the sporangium, cleavage vesicles begin to coalesce to separate the cytoplasm into uninucleate portions; membrane-bound packets of TTHs are already present within the cytoplasm of the sporangium. In *P. middletonii* (Fig. 5.17), the fascinating process of differentiation from amorphous cytoplasm to motile zoospores takes about 30–45 min (Webster, 2006a) and is readily demonstrated in the laboratory (Weber *et al.*, 1999). The sporangium is extended into an apical papilla capped by a mass of fibrillar material which is lamellate in ultrastructure (Lunney & Bland, 1976). Shortly before sporangial discharge, there is an accumulation of cleavage vesicles behind the apical cap and at the periphery of the cytoplasm close to the sporangium wall. The cleavage vesicles around the sporangial cytoplasm discharge their contents to form a loose, fibrous interface between the cytoplasm and the sporangial wall.

Discharge of the sporangium occurs by the formation of a thin-walled vesicle at the tip of the papilla from the fibrillar material of the apical cap, and the partially differentiated zoospore mass is extruded into it. The movement of the cytoplasm from the sporangium into the

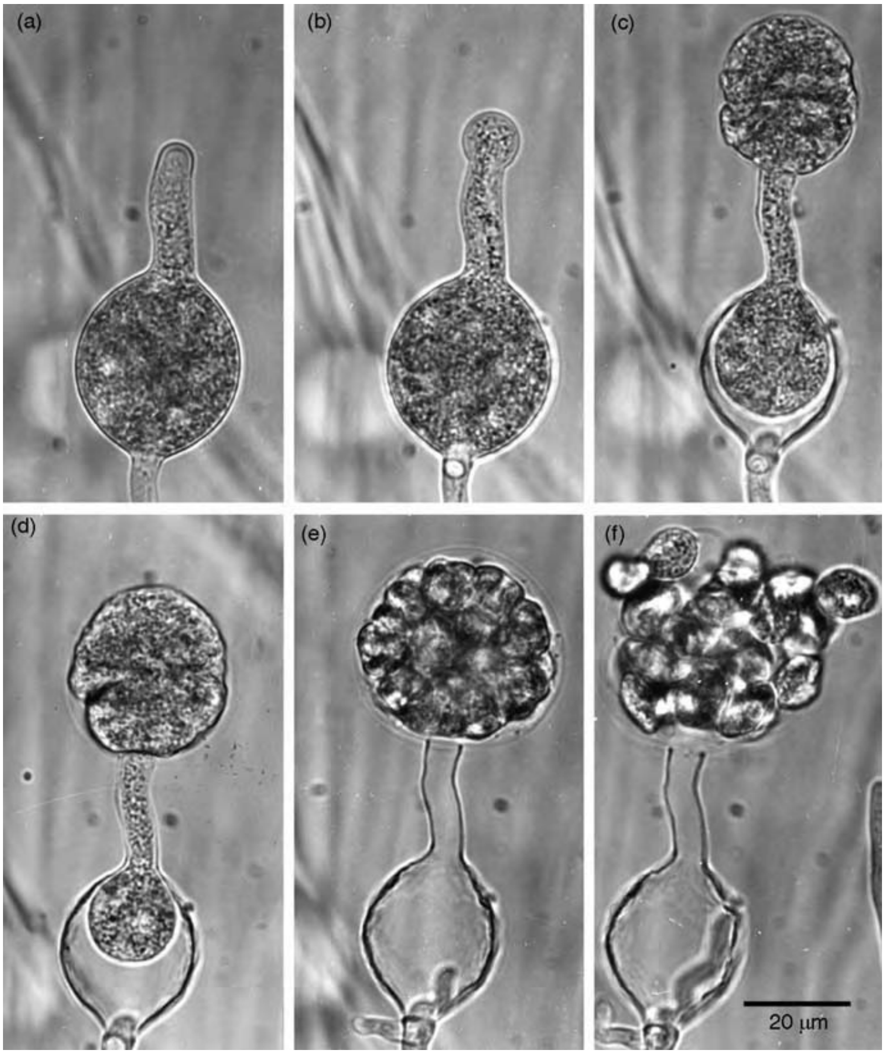

Fig 5.17 *Pythium middletonii*. Stages in zoospore discharge. (a) Sporangium shortly before discharge. Note the thickened tip of the papilla which consists of a cap of cell wall material. (b) Inflation of the vesicle begins. (c,d) Protoplasm is retreating from the sporangium. Note the shrinkage in sporangium diameter as compared with (a). (e) Zoospores have differentiated within the vesicle, with flagella visible between the vesicle wall and the zoospores. (f) Zoospores escape following the rupture of the vesicle wall. The whole process of discharge takes about 20 min.

vesicle is probably the result of several forces including the elastic contraction of the sporangium wall and possibly surface energy (Webster & Dennis, 1967). Lunney and Bland (1976) have also suggested that the fibrillar material extruded from the cleavage vesicles at the zoosporangium periphery may imbibe water, resulting in a build up of turgor pressure. The vesicle enlarges as cytoplasm from the sporangium is transferred to it, and during the next few minutes the cytoplasm cleaves into 8–20 uninucleate zoospores which jostle about inside the sporangium, causing the thin vesicle wall to bulge irregularly (Fig. 5.17). Finally, about 20 min after the inflation of the vesicle, its wall breaks down and the zoospores swim away. Internal sporangial proliferation, i.e. the formation of a new sporangium inside an old discharged one, occurs in certain species, e.g. *P. middletonii* and *P. undulatum*.

In some forms, e.g. *P. ultimum* var. *ultimum*, sporangia do not release zoospores but germinate directly by producing a germ tube. Sporangia of *P. ultimum* var. *ultimum* may survive in soil, whether moist or air-dry, for several months, and are stimulated to germinate within a few hours by sugar-containing exudates from seed coats. The germ tubes grow very rapidly so that a host in the vicinity may be penetrated within 24 h (Stanghellini & Hancock, 1971). The oospore of *P. ultimum* var. *ultimum* can germinate either by means of a germ tube or by forming a zoosporangium which releases zoospores (Figs. 5.18d,e).

The zoospore

Zoospores of *Pythium* spp. are always of the principal type. They can swim for several hours in a readily recognizable manner of helical forward movement. Donaldson and Deacon (1993) have provided evidence that the zoospore swimming pattern is regulated by Ca^{2+} and calmodulin; manipulations of Ca^{2+} concentrations cause aberrations such as circular, straight, spirally skidding or irregular movement. Zoospores of *Pythium* are attracted towards host surfaces, usually roots. The Ca^{2+}/calmodulin system may be the means by which the sensing of attractants is translated into directed movement. It is this *directed* movement (**taxis**), i.e. the ability to aim precisely at a suitable encystment site, rather than the ability to move per se, which represents the main benefit of zoospores to their producer (Deacon & Donaldson, 1993). Chemotaxis to root exudates is often non-host-specific, being mediated by amino acids and other common metabolites (Jones *et al.*, 1991). Other tactic movements also occur, such as phototaxis, electrotaxis or negative geotaxis (Dick, 2001a). In general, zoospores of *Pythium* spp. accumulate around the root cap, root elongation zone or sites of injury.

Once the zoospore has alighted on a suitable surface, it encysts by shedding rather than withdrawing its flagella, and secreting a wall from pre-formed material. Much valuable ultrastructural work has been carried out on the encystment process of *Phytophthora* and is discussed on pp. 102–111. The cyst of *Pythium* spp. can germinate almost immediately, usually by emitting a germ tube which can directly penetrate the relatively soft root tissue. In *P. marinum*, which is parasitic on marine red algae, the germ tube forms a specialized infection structure termed an **appressorium** (Kerwin *et al.*, 1992); this is also commonly formed by leaf-infecting *Phytophthora* spp. The entire process from zoospore encystment to successful penetration is called **homing sequence** and may take place in as little as 30 min (Deacon & Donaldson, 1993). If a zoospore encysts on a non-host surface, the cyst may germinate by producing a further principal zoospore.

Sexual reproduction

Most species of *Pythium* are homothallic, i.e. oogonia and antheridia are readily formed in cultures derived from single zoospores. However,

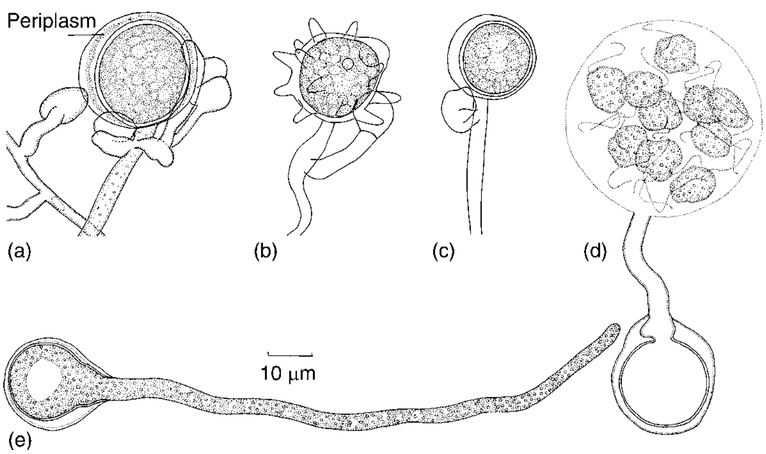

Fig 5.18 Oogonia and oospores of *Pythium*. (a) *Pythium debaryanum*. Note that there are several antheridia. (b) *Pythium mamillatum*. Oogonium showing spiny outgrowths of oogonial wall. (c) *Pythium ultimum*. (d, e) Germination of oospores of *P. ultimum* (after Drechsler, 1960).

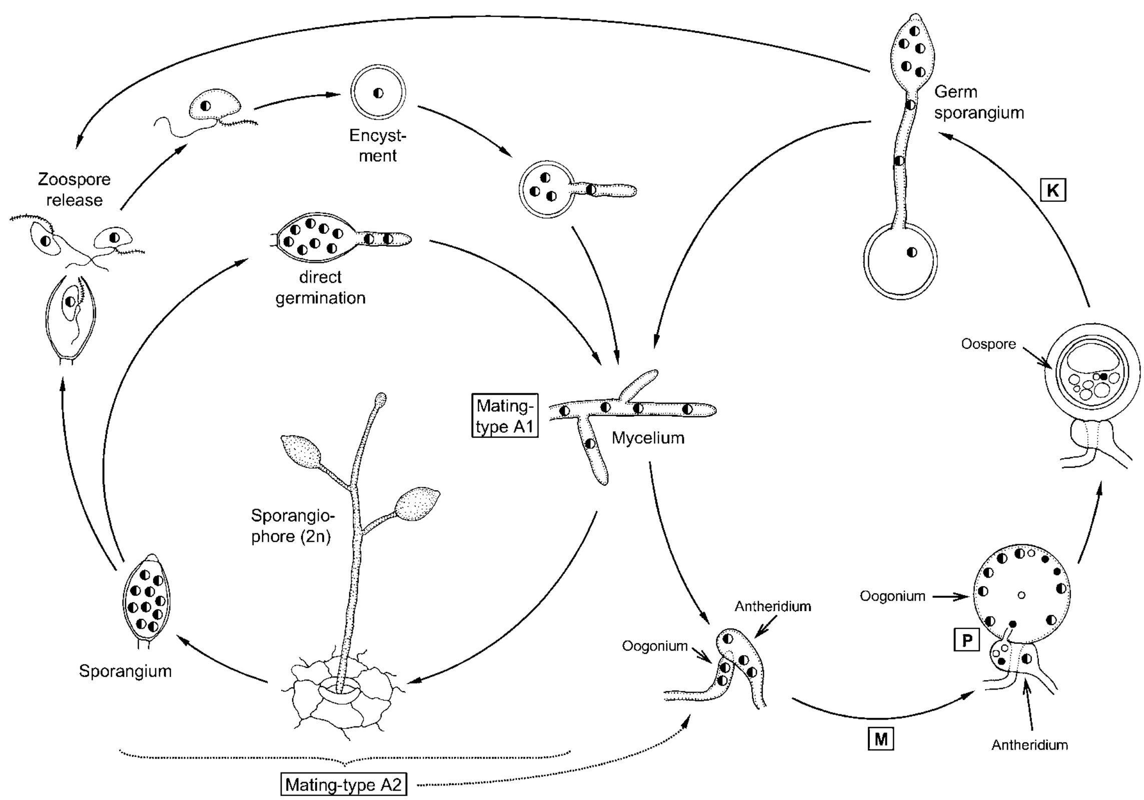

Fig 5.19 Life cycle of *Phytophthora infestans*. This fungus is heterothallic, and the asexual part of the life cycle (left of diagram) is shown only for one mating type (A1). Nuclei in vegetative states are diploid. When two compatible mycelia meet, multinucleate oogonia and antheridia are differentiated, and one meiotic event in each results in the transfer of one haploid nucleus from the gametangium to the oogonium. Karyogamy is delayed until shortly before oospore germination. Open and closed circles represent haploid nuclei of opposite mating type; diploid nuclei are larger and half-filled. Key events in the life cycle are meiosis (M), plasmogamy (P) and karyogamy (K).

some heterothallic species are known, e.g. *P. sylvaticum*, *P. heterothallicum* and *P. splendens*. In these cases, mating is a complicated affair under hormonal control, and with relative sexuality (see p. 95).

Oogonia arise as terminal or intercalary spherical swellings which become cut off from the adjacent mycelium by cross-wall formation. In some species, e.g. *P. mamillatum*, the oogonial wall is folded into long projections (Fig. 5.18b). The antheridia arise as club-shaped swollen hyphal tips, often as branches of the oogonial stalk (monoclinous) or sometimes from separate hyphae (diclinous). In some species, e.g. *P. ultimum*, there is typically only a single antheridium to each oogonium, whilst in others, e.g. *P. debaryanum*, there may be several (Fig. 5.18a). The young oogonium is multinucleate and the cytoplasm within it differentiates into a multinucleate central mass, the ooplasm from which the oosphere develops, and a peripheral mass, the periplasm, also containing several nuclei. The periplasm does not contribute to the formation of the oosphere.

As soon as the gametangia have become delimited by the basal septum, mitotic divisions cease. Nuclei may be aborted at this stage, and in oogonia of *P. debaryanum* 1–8 nuclei undergo meiosis (Sansome, 1963). Meiotic divisions are synchronous in the antheridium and the oogonium, although no protoplasmic continuities exist at this stage (Dick, 1995). In the antheridium of *P. debaryanum* and *P. ultimum*, all nuclei but one degenerate prior to meiosis, so that four haploid nuclei are present in each antheridium just prior to plasmogamy (Sansome, 1963; Win-Tin & Dick, 1975). The antheridium then attaches itself to the oogonial wall and penetrates it by means of a fertilization tube. Following penetration, only three nuclei were counted in the antheridium, suggesting that one had entered the oogonium. Later still, empty antheridia were found, and it is presumed that the three remaining nuclei enter the oogonium and join the oogonial nuclei degenerating in the periplasm. Fusion between a single antheridial and oosphere nucleus has been described. The fertilized oosphere secretes a double wall, and the ooplast appears in the protoplasm. Material derived from the periplasm may also be deposited on the outside of the developing oospore. Such oospores may need a period of rest (after-ripening) of several weeks before they are capable of germinating. Germination may be by means of a germ tube, or by the formation of a vesicle in which zoospores are differentiated (Figs. 5.18d,e), or in some forms the germinating oospore produces a short germ tube terminating in a sporangium.

Ecological considerations

Pythium spp. can live saprotrophically and may survive in air-dry soil for several years. They are more common in cultivated than in natural soils (Foley & Deacon, 1985), and appear to be intolerant of highly acidic soils. As saprotrophs, species of *Pythium* are important primary colonizers, probably gaining initial advantage by virtue of their rapid growth rate. They do not, however, compete well with other fungi which have already colonized a substrate, and they appear to be rather intolerant of antibiotics.

The control of diseases caused by *Pythium* is obviously rendered difficult by its ability to survive saprotrophically and as oospores in soil. Its wide host range means that it is not possible to control diseases by means of crop rotation. The effects of disease can be reduced by improving drainage and avoiding overcrowding of seedlings. *Pythium* infections are particularly severe in greenhouses and nurseries, where some measure of control can be achieved by partial steam sterilization of soil. Recolonization of the treated soil by *Pythium* is slow. The use of certain types of compost instead of peat in nurseries can provide good control (Craft & Nelson, 1996; Zhang *et al*., 1996). The fungicide metalaxyl (see Fig. 5.27) also gives good control of seedling blight.

Pythium insidiosum

This species is associated with algae in stagnant freshwater in tropical and subtropical regions. When horses or cattle come into contact with *P. insidiosum*-contaminated water, zoospores are attracted to wounds and can infect them, causing severe open lesions of skin and subcutaneous tissues known as *pythiosis insidiosi* (Meireles *et al*., 1993; Mendoza *et al*., 1993). If contaminated water is consumed, gastrointestinal or systemic infections may also arise. In addition to grazing animals, infections in dogs and humans have been reported. *Pythium insidiosum* is keratinophilic and survives well at 37°C. Infections can be treated successfully by immunotherapy in which horses are injected with killed fungal material, the immune response leading to healing of infections (Mendoza *et al*., 1992). *Pythium insidiosum* used to be known under different names, but its taxonomy has been clarified by de Cock *et al*. (1987).

5.3.3 *Phytophthora*

The name *Phytophthora* (Gr.: 'plant destroyer') is apt, most species being highly destructive plant pathogens. The best known is *P. infestans*, cause of late blight of potatoes (Plate 2e). This fungus is confined to solanaceous hosts (especially tomato and potato), but others have a much wider host range. For example, *P. cactorum* has been recorded from over 200 species belonging to 60 families of flowering plants, causing a variety of diseases such as damping off or rots of roots, fruits and shoots (Erwin & Ribeiro, 1996). *Phytophthora cinnamomi* has the widest host range of all species, being capable of infecting over 1000 plants and causing serious diseases especially on woody hosts, including conifers and *Eucalyptus* (Zentmyer, 1980). Several other *Phytophthora* spp. and related *Pythium* spp. can also cause die-backs and sudden-death symptoms of trees, with roots severely rotted by the time above-ground symptoms become apparent (Plate 2c,d). Other important pathogens are *P. erythroseptica* associated with pink rot of potato tubers (Plate 2f), *P. fragariae* causing red core of strawberries, and *P. palmivora* causing pod rot and canker of cocoa. The genus is cosmopolitan, although there are differences in the geographic distribution of individual species; for instance, *P. cactorum*, *P. nicotianae*, *P. cinnamomi* and *P. drechsleri* occur worldwide whereas *P. fragariae* and *P. erythroseptica* are found predominantly in Northern Europe and North America (Erwin & Ribeiro, 1996). Many *Phytophthora* spp. are spreading actively at present, e.g. *P. infestans* which has been spread worldwide by human activity (Fry & Goodwin, 1997) or *P. ramorum*, a serious pathogen of oak trees and other woody plants (Henricot & Prior, 2004). To make matters worse, different *Phytophthora* species may hybridize in nature, producing strains with new host spectra. An example is the recent outbreak of wilt of *Alnus glutinosa* in Europe caused by *P. alni*, a tetraploid hybrid of species resembling *P. cambivora* and *P. fragariae* (Brasier *et al*., 2004).

In accordance with the great importance of the genus *Phytophthora* in mycology and plant pathology, a vast amount of literature has been published, and some of it has been summarized by Erwin & Ribeiro (1996) and Dick (2001a). Several books on the genus have appeared, including those edited by Erwin *et al*. (1983), Ingram and Williams (1991) and Lucas *et al*. (1991), and the masterly compendium by Erwin and Ribeiro (1996). Keys to the genus have been produced by Waterhouse (1963, 1970) and Stamps *et al*. (1990). Including *formae speciales*, Dick (2001a) listed 84 names in current use.

Phytophthora is closely related to *Pythium* and there are transitional species which may need to be re-assigned as more DNA sequences and other data become available (Panabières *et al*., 1997). In general, the two genera can be distinguished morphologically in that the sporangia of *Phytophthora* spp. are typically pear- or lemon-shaped with an apical papilla (Fig. 5.20b), and ecologically by the predominantly saprotrophic existence of *Pythium* and the predominantly parasitic mode-of-life of *Phytophthora*. Probably all *Phytophthora* spp. are pathogenic on plants in some form, and they differ merely in the extent to which they have a free-living saprotrophic phase. All may survive in the soil at least in the form of oospores, or in infected host tissue. However, in contrast to the downy mildews (Peronosporales; Section 5.4), almost all pathogenic forms can be isolated from their hosts and can be grown in pure culture. Selective media, often incorporating antibiotics or fungicides such as pimaricin or benomyl, have been devised for the isolation of *Phytophthora* (Tsao, 1983; Erwin & Ribeiro, 1996).

Vegetative growth

Most species form an aseptate mycelium producing branches at right angles, often constricted at their point of origin. Septa may be present in older cultures. Within the host, the mycelium is intercellular, but **haustoria** may be formed. These are specialized hyphal branches which penetrate the wall of the host cell and invaginate its plasmalemma, thereby establishing a point of contact between pathogen and host membranes. Haustoria are typical of biotrophic pathogens such as the Peronosporales (see Fig. 5.29) but may also be formed during initial biotrophic phases of infections which subsequently turn necrotrophic. In *P. infestans* within potato tubers, the haustoria appear as finger-like protuberances (Fig. 5.20c). Electron micrographs of infected potato leaves show that the haustoria are not surrounded by host cell wall material, but by an encapsulation called the **extrahaustorial matrix** which is probably of fungal origin. This is delimited on the outside by the host plasma membrane, and on the inside by the wall and then the plasma membrane of the pathogen (Fig. 5.21; Coffey & Wilson, 1983; Coffey & Gees, 1991). Haustoria of *Phytophthora* do not normally contain nuclei, although one may be situated near the branching point within the intercellular hypha (Fig. 5.21a).

Asexual reproduction

The sporangia of *Phytophthora* spp. are usually pear-shaped or lemon-shaped (Fig. 5.22a) and arise on simple or branched sporangiophores which are more clearly differentiated than

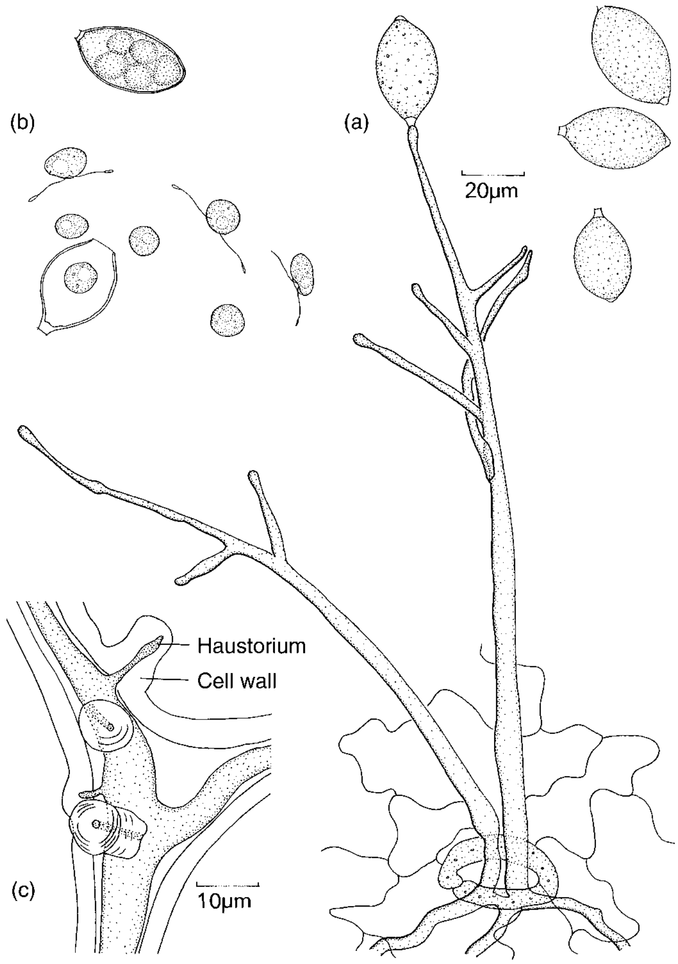

Fig 5.20 *Phytophthora infestans*.
(a) Sporangiophores penetrating a stoma of a potato leaf. (b) Zoospores and zoospore cysts, one formed inside a zoosporangium. (c) Intercellular mycelium from a potato tuber showing the finger-like haustoria penetrating the cell walls. Note the thickening of the cell walls around the haustorium.

those of *Pythium*. On the host plant, the sporangiophores may emerge through the stomata, as in *P. infestans* (Fig. 5.20a). The first sporangium is terminal, but the hypha bearing it may push it to one side and form further sporangia by sympodial growth. Mature sporangia of most species have a terminal papilla which appears as a plug because it consists of material different from the sporangial wall (Coffey & Gees, 1991).

In species of *Phytophthora* infecting aerial plant organs, the sporangia are detached, possibly aided by hygroscopic twisting of the sporangiophore on drying, and are dispersed by wind before germinating. In aquatic or soil-borne forms, zoospore release commonly occurs whilst the sporangia are still attached; internal proliferation of attached sporangia may occur. Whether deciduous or not, sporangia may germinate either directly by means of a germ tube, or by releasing zoospores. The latter seems to be the original route because undifferentiated sporangia contain pre-formed flagella within their cytoplasm, and these are degraded under unfavourable conditions leading to direct germination (Hemmes, 1983; Erwin & Ribeiro, 1996). The mode of germination is dependent on environmental parameters. For example, in *P. infestans*, uninucleate zoospores are produced below 15°C whilst above 20°C multinucleate germ tubes arise. Further, with increasing age sporangia lose their capacity to produce zoospores and tend to germinate directly. In *P. cactorum*, sporangia have been preserved for several months under moderately dry conditions.

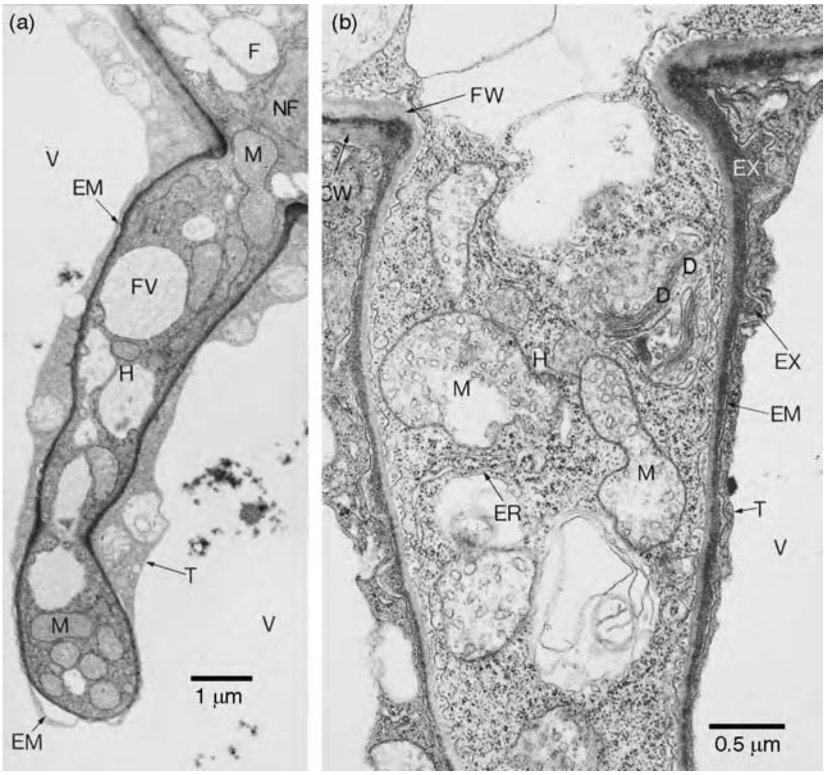

Fig 5.21 TEM images of haustoria of *P. infestans*. (a) Mature haustorium within a leaf cell of potato. (b) The basal region of a haustorium. The haustorium contains fungal vacuoles (FV) and mitochondria (M) but no nuclei. However, a nucleus (NF) is located within the intercellular hypha close to the branch point. The plant tonoplast (T), plant extrahaustorial membrane (EM), extrahaustorial matrix (EX) and fungal wall (FW) are visible. The seemingly empty space surrounding the haustorium is the plant vacuole (V). Both images reprinted from Coffey and Wilson (1983) by copyright permission of the National Research Council of Canada. Original prints kindly provided by M. D. Coffey.

When water becomes available again, such sporangia may germinate by the formation of a vegetative hypha, or a further sporangium.

Thick-walled asexual spherical chlamydospores have also been described for many *Phytophthora* spp. and can survive in soil for several years (Ribeiro, 1983; Erwin & Ribeiro, 1996). The morphological differences between sporangia, chlamydospores and oospores are illustrated in Fig. 5.22.

Once formed, mature sporangia may remain undifferentiated for several hours or even days, but zoospore differentiation can be induced by suspending mature sporangia in chilled water or soil extract. Detailed methods to trigger zoospore release have been established for many species (Erwin & Ribeiro, 1996). Once cold-shock has been received, differentiation can be completed in less than 60 min and probably involves cAMP-mediated signalling cascades (Yoshikawa & Masago, 1977). The processes of differentiation of sporangial protoplasm into zoospores differ in certain details between *Phytophthora* and the Saprolegniales (see Hardham & Hyde, 1997). For instance, in *Saprolegnia* the central vacuole is prominent and its membrane as well as the plasma membrane contribute to the plasma membranes of the developing zoospores (p. 81). In contrast, in *Phytophthora* the central vacuole disappears from the young sporangium before cleavage of the cytoplasm begins, and the plasma membrane remains intact even after zoospores have become fully differentiated. The zoospore plasma membranes therefore mostly originate from Golgi-derived cleavage cisternae (Hyde *et al*., 1991). Detailed cytological studies

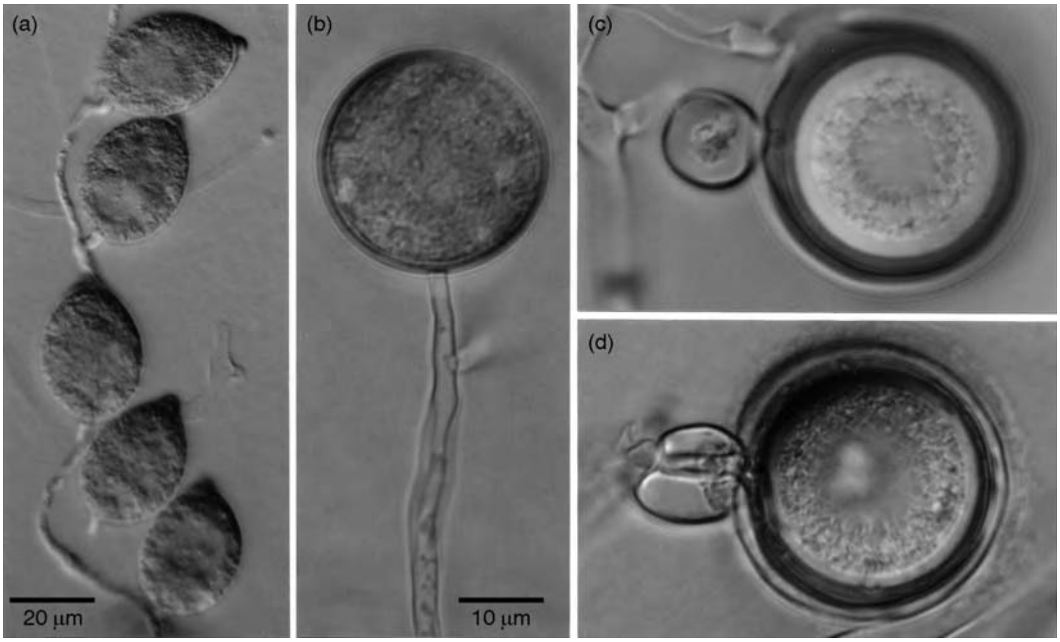

Fig 5.22 Reproductive structures in *Phytophthora cactorum*. (a) Sporangia. (b) Chlamydospore. (c) Oospore showing the paragynous mode of fertilization. (d) Oospore with amphigynous fertilization. (b–d) to same scale.

have revealed an important role of microtubules in organizing the distribution of nuclei during zoospore formation (Hyde & Hardham, 1992, 1993). Cleavage of the cytoplasm of a zoospore begins close to that end of the nucleus which subsequently points towards the ventral groove. At this stage, three types of vesicle which become important during zoospore encystment also move into their positions: large peripheral vesicles, dorsal vesicles, and small ventral vesicles. When the pre-formed flagella have been inserted, the zoospores acquire their mobility (Hardham, 1995). Zoospores are either discharged directly through the plug after this has dissolved, or they are transferred into a very transient membranous vesicle which forms outside the opened plug upon discharge and bursts one or a few seconds later (Gisi, 1983). Since the plasma membrane of the sporangium has not become part of the zoospore membranes, the membranous vesicle is probably continuous with the plasma membrane.

Encystment of zoospores

Zoospores of *Phytophthora* swim for several hours, travelling distances of a few centimetres in water or wet soil, although they can be spread much further by passive movement within water currents (Newhook *et al*., 1981). They are attracted chemotactically to plant roots by non-specific root exudates such as amino acids, host-specific substances, or the electrical field generated by plant roots (Carlile, 1983; Deacon & Donaldson, 1993; Tyler, 2002). No equivalent studies seem to have been carried out for zoospores of *Phytophthora* infecting leaves. The process of zoospore encystment described below for *Phytophthora* seems to apply also to *Pythium* (Hardham, 1995). It is an act of regulated secretion, i.e. the release of pre-formed contents by synchronous fusion of vesicles with the plasma membrane. Regulated secretion is common in animal cells, e.g. in epithelial or neuronal systems, but in fungi it is probably confined to encysting zoospores.

Zoospores of *Phytophthora* are kidney-shaped; both flagella arise from the kinetosome boss protruding from within the longitudinal groove at the ventral surface. The anterior end of the spore is indicated externally by the straminipilous flagellum and internally by the water expulsion vacuole; the nucleus is located in

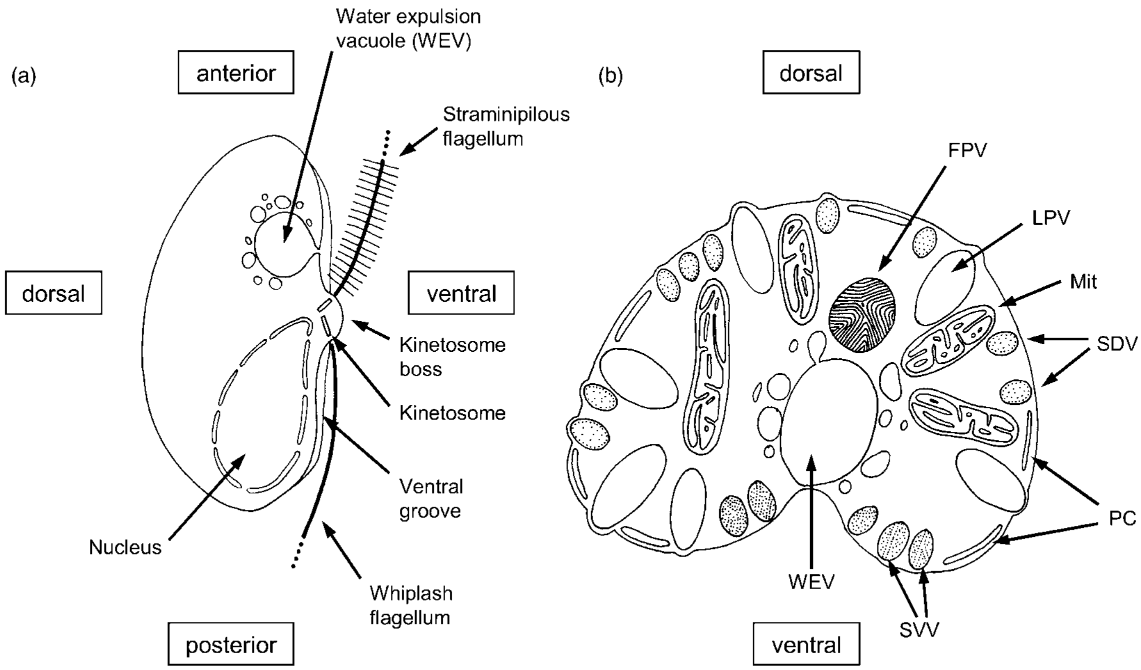

Fig 5.23 Schematic drawings of a zoospore of *Phytophthora* (not to scale). (a) Longitudinal section. (b) Transverse section of the anterior region showing several types of vesicle, namely the water-expulsion vacuole (WEV), fingerprint vacuole (FPV), large peripheral vesicles (LPV), small ventral vesicles (SVV), small dorsal vesicles (SDV) and peripheral cisternae (PC). Mitochondria (Mit) with unusually lamellate cristae are also indicated. a modified from Dick (2001b); b based on the ultrastructural work of Hardham *et al*. (1991).

the posterior half of the spore (Fig. 5.23a). The nucleus is associated with the microtubular roots of the flagella which force it into a somewhat conical shape, the pointed end pointing towards the kinetosome boss. Zoospores contain several vesicular compartments. Their positions are drawn schematically in Fig. 5.23, and electron micrographs are provided in Fig. 5.24. Fingerprint vacuoles, equivalent to the dense-body vesicles of *Saprolegnia* and *Achlya*, are defined by the lamellate structure of their contents, presumably deposits of β-1,3-glucan (mycolaminarin) and phosphate. Fingerprint vacuoles are located mainly in the interior of the zoospore and play no part in the encystment process but are thought to provide carbon and energy reserves during subsequent germination of the cyst (Gubler & Hardham, 1990). In zoospores of *Phytophthora cinnamomi*, there are several kinds of peripheral vesicle which have been distinguished morphologically (Fig. 5.23) and by labelling with specific antibodies. When zoospores approach a root, the groove of the ventral surface faces the root surface, initial contact presumably being made by the flagella. Attachment of the zoospore is achieved by means of a glue discharged by the synchronous fusion of the small ventral vesicles with the ventral plasma membrane (Hardham & Gubler, 1990). At the same time, the small dorsal vesicles also secrete their contents, leading to the deposition of the first cyst wall (Figs. 5.24c,d; Gubler & Hardham, 1988). The process of exocytosis is complete within 2 min of receiving the encystment trigger. In contrast, the large peripheral vesicles do not fuse with the plasma membrane but withdraw to the centre of the cyst. Their contents are proteinaceous and probably serve as reserves for the germination process. Peripheral cisternae, ultrastructurally distinct from the ER, line the inside of the zoospore plasma membrane and disappear during encystment (Hardham *et al*., 1991; Hardham, 1995).

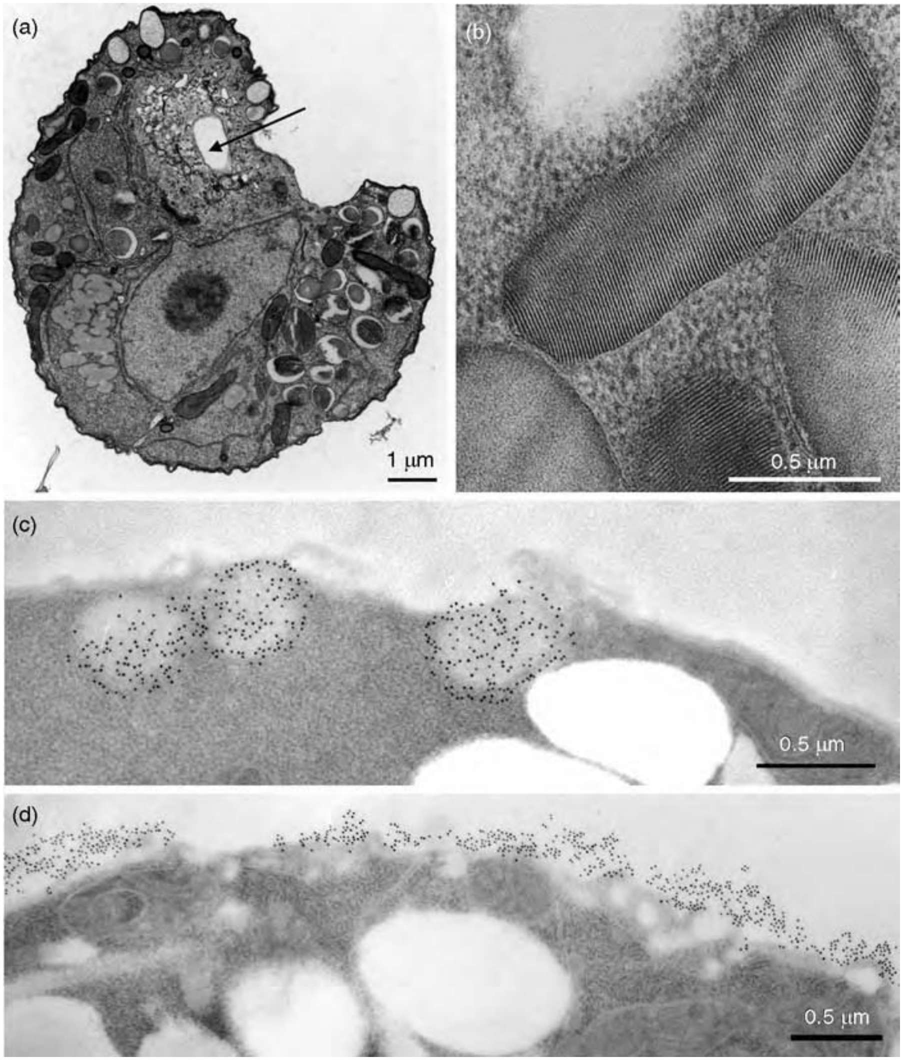

Fig 5.24 Ultrastructure of *Phytophthora cinnamomi* zoospores as seen with the TEM. (a) Oblique section through a zoospore. Several kinds of vesicle are visible, as are mitochondria, the water-expulsion vacuole (arrow) and the conical nucleus with its prominent nucleolus. (b) Fingerprint vacuoles. (c,d) Immunogold labelling of wall material located within dorsal vesicles before (c) and in the cyst wall 1 min after (d) encystment of the zoospore. (a,b) reproduced from Hardham and Hyde (1997), with permission from Elsevier; (c,d) previously unpublished work. All images kindly provided by F. Gubler and A. R. Hardham.

Zoospore encystment can be triggered by several stimuli, e.g. contact with host cell surface polysaccharides, change in medium composition, or presence of root exudates. Commitment to encystment occurs within 20–30 s of receiving the stimulus (Paktitis *et al.*, 1986). Complex signalling cascades involving Ca^{2+} and phospholipase D are involved (Zhang *et al.*, 1992), and commitment to several future developmental processes is made before the onset of encystment, including the point of germ tube emergence (Hardham & Gubler, 1990).

Zoospore cysts germinate quite rapidly after their formation, usually by means of a germ tube which infects the plant roots directly. In the case of hard surfaces such as leaves, the germ tube may form an appressorium which mediates infection (see pp. 378–381).

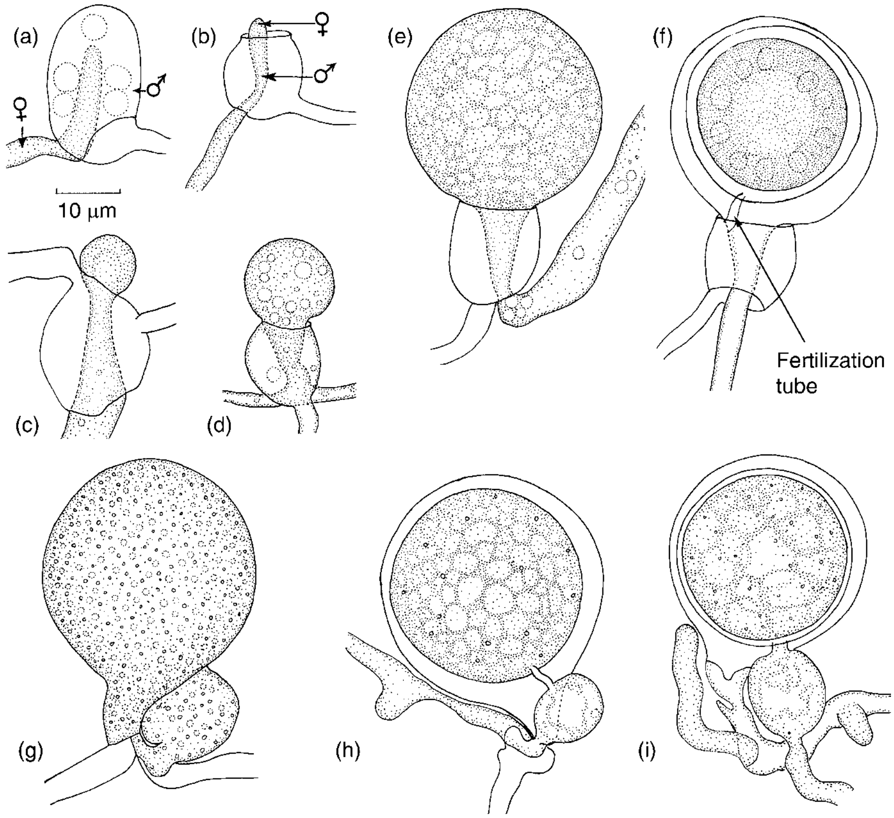

Fig 5.25 Oogonial development in *Phytophthora*. (a–f) Stages of development in *P. erythroseptica*. (g–i). Stages of development in *P. cactorum*.

Sexual reproduction

Oospore formation is dependent on sterols and mating hormones (p. 95) and may be homo- or heterothallic. Phylogenetic studies have indicated that the former is ancestral, heterothallism having arisen repeatedly within the genus *Phytophthora* (Kroon *et al*., 2004). Two distinct types of antheridial arrangement are found. In *P. fragariae*, *P. megasperma* and a number of other species, antheridia are attached laterally to the oogonium and are described as **paragynous** meaning 'beside the female' (Figs. 5.22c, 5.25g–i). In other *Phytophthora* species such as *P. infestans*, *P. cinnamomi* and *P. erythroseptica*, the oogonium, during its development, penetrates and grows through the antheridium (Hemmes, 1983). The oogonial hypha emerges above the antheridium and inflates to form a spherical oogonium, with the antheridium persisting as a collar around its base (Figs. 5.25a–f). This arrangement of the antheridium is termed **amphigynous** ('around the female'). In some species (e.g. *P. cactorum*, *P. clandestina*, *P. medicaginis*), both types of arrangement may be found (Figs. 5.22c,d); one or the other may predominate, depending on strain and culture conditions (Erwin & Ribeiro, 1996).

Both the oogonia and antheridia contain several diploid nuclei, but as the oosphere matures only a single nucleus remains at the centre while the remaining nuclei are included in the periplasm, i.e. the space between the oosphere and the oogonial walls (see Fig. 5.2). Meiosis occurs in the antheridium and oogonium (Shaw, 1983). Fertilization tubes have been observed and a single haploid nucleus is introduced from the antheridium into the oosphere (Fig. 5.26). Fusion between the oosphere nucleus and the antheridial nucleus is delayed. Even mature, dormant oospores may still be binucleate, karyogamy usually occurring after breakage of dormancy as a first step towards germination (Jiang *et al*., 1989).

Following fertilization, the physiology and ultrastructure of the oospore change to

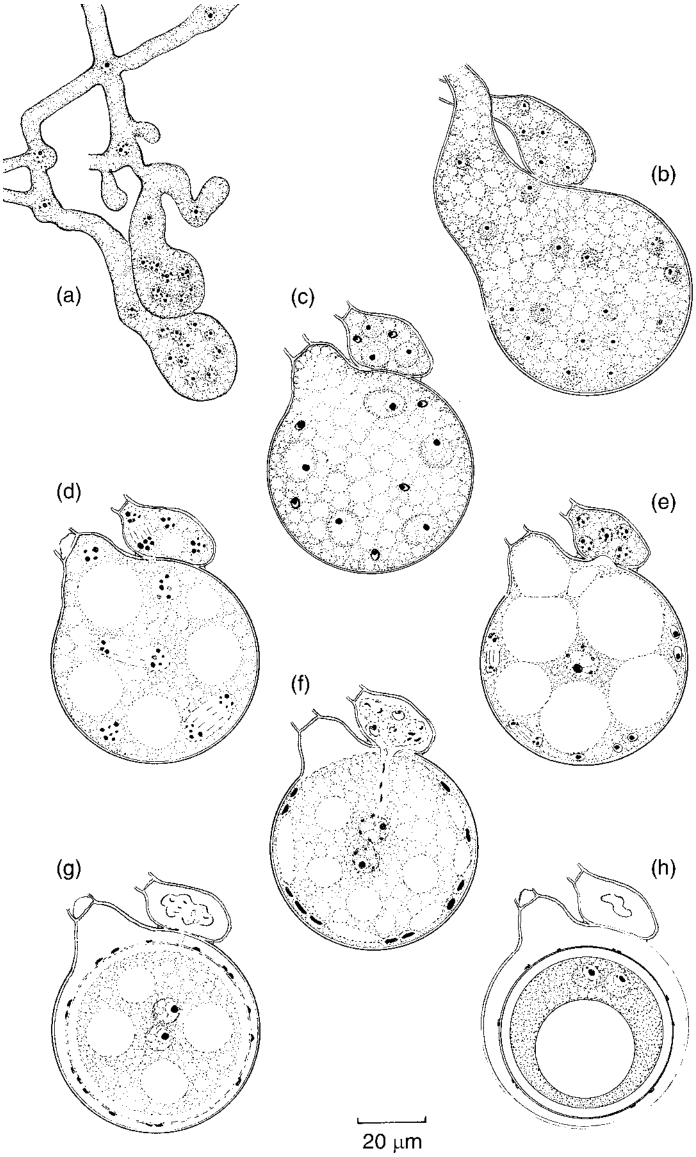

Fig 5.26 *Phytophthora cactorum*. Development of oogonium, antheridium and oospore. (a) Initials of oogonium and antheridium. (b) Oogonium and antheridium grown to full size: the oogonium has about 24 nuclei and the antheridium about 9. (c) Development of a septum at the base of each, and degeneration of some nuclei in each until the oogonium has 8 or 9 nuclei and the antheridium 4 or 5. (d) A simultaneous division of the surviving nuclei in oogonium and antheridium. The protoplast has large vacuoles. (e) Separation of oosphere from periplasm. Nuclei divide in the periplasm prior to degeneration. The oogonium presses into the antheridium. (f) Entry of one antheridial nucleus by a fertilization tube. The protoplasm and remaining nuclei of the antheridium degenerate. (g) Development of oospore wall. (h) The oospore enters its dormant period with exospore formed from dead periplasm, endospore deposited inside it, and paired nuclei in association but not yet fused. (a–h) are composite drawings of eight stages in sequence (after Blackwell, 1943).

a resting state. Oospore differentiation proceeds from the outside inwards (centripetal development). The oospore has a thin outer wall (epispore) which is derived from the periplasm and appears to consist of pectic substances. The inner oospore wall (endospore) is rich in β-1,3-glucans which form a major storage reserve and are mobilized by glucanases just prior to germination (Erwin & Ribeiro, 1996). Within the developing oospore, the numerous small lipid droplets coalesce into a few large ones. Lipids are undoubtedly the major endogenous storage reserve in the spores of Oomycota (Dick, 1995) and many other fungi. Later, the dense body vesicles which are rich in mycolaminarin and phosphate fuse together, giving one large structure, the ooplast. Like the endospore, the ooplast is consumed during germination whereas some lipid droplets are saved and are translocated into the germ tube (Hemmes, 1983). Considering their thick walls and abundant storage reserves, it is not surprising that oospores are the longest-lived propagule of *Phytophthora*, being capable of surviving in soil for many years.

5.3.4 *Phytophthora infestans*, cause of potato late blight

Late blight of potato caused by *P. infestans* is a notorious disease. In the period between 1845 and 1848 it resulted in famine across much of Europe, and especially in Ireland where most people had come to depend on the potato as their major source of food. In Ireland alone, the population size dropped from over 8 million in 1841 to 6.5 million in 1851 (Salaman, 1949). The history of the Great Famine has been ably documented by Large (1940), Woodham-Smith (1962) and Schumann (1991). The social and political repercussions of this tragedy have been immense and still reverberate today.

An enormous amount of literature about *P. infestans* has been published over the past 150 years, including several books (Ingram & Williams, 1991; Lucas *et al*., 1991; Dowley *et al*., 1995). It has been estimated that about 10% of the entire phytopathological literature is concerned just with this one species. None the less, there are many uncomfortable gaps in our knowledge, and the fungus continues to provide unpleasant surprises to this day.

Origin and spread

The probable centre of evolution of most *Solanum* spp. and hence also their pathogens, notably *P. infestans*, lies in Mexico (Niederhauser, 1991), although the potato (*S. tuberosum*) was first cultivated in South America. There are several theories accounting for the spread of *P. infestans* round the world (Ristaino, 2002). In the early 1840s *P. infestans* rapidly spread to North America, and it is generally assumed that it was introduced to Europe (Belgium) in June 1845 with a shipment of contaminated potatoes (Bourke, 1991). *Phytophthora infestans* is heterothallic, and there is good evidence that in the first wave of migration in 1845 only the A1 mating type reached Europe (Goodwin *et al*., 1994a). Over the next century or more, the fungus probably survived entirely on an asexual life cycle, overwintering in tubers infected during the previous season and discarded together with shoots and other debris in the field. Despite the absence of sexual reproduction, *P. infestans* showed a considerable genetic adaptability, as documented by its ability to break the resistance bred into new potato cultivars (p. 114), and also the rapid emergence of strains resistant against newly introduced fungicides (p. 112).

A second wave of *P. infestans* migration brought the A2 mating type from central Mexico to North America and Europe where it was first isolated in 1981 (Hohl & Iselin, 1984). It is now established worldwide (Spielman *et al*., 1991; Fry *et al*., 1993; Gillis, 1993; Goodwin *et al*., 1994b). The enhanced genetic recombination brought about by sexual reproduction is catalysing a change in the genetic make up of *P. infestans*, which may be leading to an explosive evolution of new *P. infestans* strains (Fry *et al*., 1993; Goodwin *et al*., 1995). This situation is seen as the biggest threat posed by *P. infestans* since the 1840s (Fry & Goodwin, 1997).

Epidemiology

There is clear genetic evidence of sexual reproduction taking place in the field, and it is also possible that oospores contribute to the survival of *P. infestans* in soil during the winter (Andrivon, 1995). Additionally, the fungus has a good capacity to survive the winter without oospores. A very low proportion of infected tubers left on the field gives rise to infected 'volunteer' plants in the following spring. In experimental plots, the proportion of infected plants developing from naturally or artificially infected tubers was found to be less than 1% (Hirst & Stedman, 1960). Nevertheless, such infected shoots form foci within the crop from which the disease spreads. The sporangia of *P. infestans* are deciduous, and they are blown from diseased shoots to healthy leaves where they germinate either by the formation of germ tubes or zoospores. Zoospore production is favoured by lower temperatures (9–15°C). After swimming for a time, the zoospores encyst and then form germ tubes which usually penetrate the epidermal walls of the potato leaf, or occasionally enter the stomata. An appressorium is formed at the tip of the germ tube, attaching the zoospore cyst firmly to the leaf. Penetration of the cell wall is probably achieved by a combination of mechanical and enzymatic action and can occur within 2 h. Within the leaf tissue, an intercellular mycelium develops and haustoria are formed where hyphae contact host cell walls (Fig. 5.21). The resulting lesion acquires a dark green water-soaked appearance associated with tissue disintegration (Plate 2e). Such lesions are visible within 3–5 days of infection under suitable conditions of temperature and humidity. Around the margin of the advancing lesion on the lower surface of the leaf, a zone of sporulation is found in which sporangiophores emerge through the stomata (Fig. 5.20a). Sporulation is most prolific during periods of high humidity and commonly occurs at night following the deposition of dew. In potato crops, as the leaf canopy closes over between the rows to cover the soil, a humid microclimate is established which may result in extensive sporulation. As the foliage dries during the morning, the sporangiophore undergoes hygroscopic twisting which results in the flicking-off of sporangia. Thus the concentration of sporangia in the air usually shows a characteristic diurnal fluctuation, with a peak around 10 a.m. Although sporangia can survive drying if they are rehydrated slowly (Minogue & Fry, 1981), in practice the long-range spread of inoculum is probably by sporangia in contact with water drops (Warren & Colhoun, 1975).

The destructive action of *P. infestans* is directly associated with the killing of photosynthetically active foliage. When about 75% of the leaf tissue has been destroyed, further increase in the weight of the crop ceases (Cox & Large, 1960). Thus, the earlier the onset of the epidemic, the more serious the consequences. To a certain extent, the crop reduction may be offset by the fact that epidemics are more common in rainy cool seasons which are conducive to higher crop yields.

Phytophthora infestans can also cause severe post-harvest crop losses because tubers can be infected by sporangia falling onto them, either during growth or lifting. Such infected tubers may rot in storage, and the diseased tissue is susceptible to secondary bacterial and fungal infections.

Chemical control

By spraying with suitable fungicides, epidemic spread of the disease can be delayed. This results in a prolongation of photosynthetic activity of the potato foliage and hence an increase in yield. Fungicides developed against the Eumycota are often ineffective against Oomycota such as *Phytophthora* because the latter differ in fundamental biochemical principles, including many of the molecular targets of fungicides active against Eumycota (Bruin & Edgington, 1983; Griffith *et al*., 1992). In 1991, about 20% of the total amount of money spent on chemicals for controlling plant diseases worldwide was used for the control of Oomycota (Schwinn & Staub, 1995).

The first of all fungicides was Bordeaux mixture, an inorganic formulation containing copper sulphate and calcium oxide which was found to be effective against downy mildew of vines caused by *Plasmopara viticola*, another member of the Oomycota (see p. 119; Large, 1940; Erwin & Ribeiro, 1996). Oomycota in general are extremely sensitive to copper ions, and Bordeaux mixture is still widely used (Agrios, 2005).

The **dithiocarbamates** such as zineb or maneb (Fig. 5.27a) were among the first organic fungicides to be developed. They act against a wide range of fungi, including Oomycota, because of their non-selective mode of action. The molecule is sufficiently apolar to diffuse across the fungal plasma membrane; once inside, it is metabolized, and the released isothiocyanate radical (Fig. 5.27b) reacts with the sulphydryl groups of amino acids (Agrios, 2005).

The most important agrochemicals against Oomycota are the **phenylamides** such as metalaxyl (Fig. 5.27c) which are **systemic fungicides**, i.e. they can enter the plant and are translocated throughout it. Metalaxyl appears to inhibit the transcription of ribosomal RNA in Oomycota but not Eumycota (Davidse *et al*., 1983). This is an inhibition of a specific biochemical target, and the immense genetic variability of *P. infestans* enabled it to develop resistance against metalaxyl in the early 1980s shortly after this was released for agricultural use (Davidse *et al*., 1991). Resistance is now widespread and has serious implications for future control of

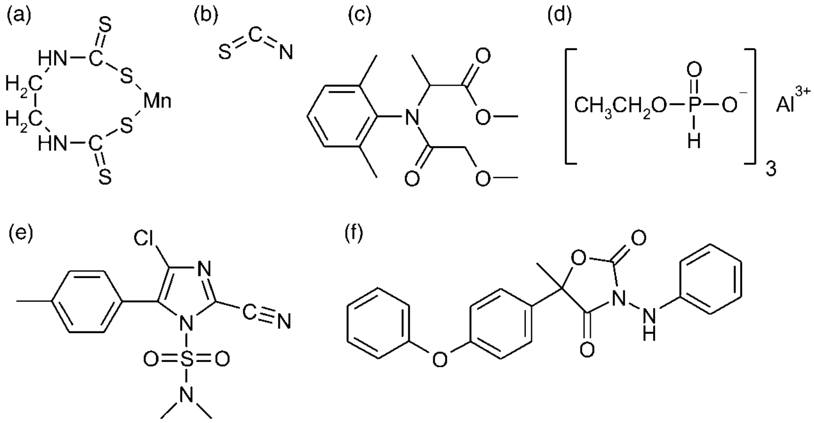

Fig 5.27 Fungicides against *P. infestans*. (a) The dithiocarbamate maneb which is active against Oomycota and Eumycota. (b) The isothiocyanate radical released by metabolism of dithiocarbamates by fungal hyphae. (c) The phenylamide metalaxyl which is active only against Oomycota. (d) Aluminium ethyl phosphonate (fosetyl-Al). (e) Cyazofamid, a new fungicide specific against Oomycota. (f) Famoxadone, a new fungicide active against Oomycota and Eumycota.

Phytophthora spp. (Erwin & Ribeiro, 1996). Phenylamides are now protected by being used in a cocktail, e.g. with the less-specific dithiocarbamates, and tailor-made application regimes are recommended for each year and each region (Staub, 1991).

The **phosphonates** are a different type of fungicide against *Phytophthora* spp. Fosetyl–Al (aluminium ethyl phosphonate; Fig. 5.27d) is readily taken up by plants in which it is broken down to release phosphorous acid (= phosphonate), which seems to be the active principle (Griffith *et al.*, 1992). Fosetyl–Al as well as phosphorous acid can move downwards through the phloem and upwards in the xylem, showing similar transport characteristics as sucrose (Ouimette & Coffey, 1990; Erwin & Ribeiro, 1996). The mode of action of phosphonates is not known but is likely to be complex, with a stimulatory effect also on the host plant immune system (Molina *et al.*, 1998). Although active only against potato tuber blight but not foliar blight caused by *P. infestans* (L.R. Cooke & Little, 2002), phosphonates are effective against a wide range of root-infecting *Phytophthora* spp. and even show good curative properties (Erwin & Ribeiro, 1996).

A useful introduction to current fungicides and their modes of action has been provided by Uesugi (1998). Because of the enormous economic significance of *P. infestans* and other Oomycota, new fungicide candidates are continually being developed and introduced into the market. Two recent examples are cyazofamid (Fig. 5.27e) and famoxadone (Fig. 5.27f). Both inhibit mitochondrial respiration. However, whilst the former is specific against Oomycota (Sternberg *et al.*, 2001), famoxadone inhibits both Oomycota and Eumycota (Mitani *et al.*, 2002). Its molecular target is different from that of cyazofamid but probably the same as that of the strobilurins (see Figs. 13.15e,f), as indicated by the development of cross-resistance in fungal pathogens against famoxadone and strobilurins.

Disease forecast

To avoid unnecessary spraying and to ensure that timely spray applications are made, it has proven possible to provide forecasts of the incidence of potato blight epidemics for certain countries. Beaumont (1947) analysed the incidence of blight epidemics in south Devon (England) and established that a ‘temperature–humidity rule’ controls the relationship between blight epidemics and weather. After a certain date (which varies with the locality) and assuming that inoculum on volunteer plants is always present, Beaumont (1947) predicted that blight would follow within 15–22 days of a period of at least 48 h during which the minimum temperature was not less than 10°C and the relative humidity was over 75%. The warm humid weather during this **Beaumont period** provides conditions suitable for sporulation and the initiation of new infections. Modified in the light of experience and adapted to regional climates, computerized forecasting systems are now used worldwide, limiting fungicide applications to situations in which they are necessary (Doster & Fry, 1991; Erwin & Ribeiro, 1996). After receipt of a blight warning, fungicide sprays are applied prophylactically by the farmer, irrespective of whether *P. infestans* is actually present in his field or not.

Haulm destruction

The danger of infection of tubers by sporangia falling onto them from foliage at lifting time can be minimized by ensuring that all the foliage is destroyed before lifting. This is achieved by spraying the foliage with herbicides 2–3 weeks before harvest time. The ridging of potato tubers also helps to protect the tubers from infection. Although sporangia may survive in the soil for several weeks, they do not penetrate deeply into it.

Crop sanitation

In principle, one infected volunteer plant per hectare is sufficent to initiate an epidemic. This is because late blight is a typical multicyclic disease, with numerous cycles of reproduction occurring in a single growing season under favourable conditions, leading to the rapid build up of inoculum. Crop sanitation, which is effective against single-cycle diseases, therefore has only limited value in the control of *P. infestans* (van der Plank, 1963).

Breeding for major gene resistance

A worldwide screening of *Solanum* spp. showed that a number of them have natural resistance to *P. infestans*. One species which has proven to be an important source of resistance is *S. demissum* which grows in Mexico, the presumed centre of origin of *P. infestans*. Although this species is valueless in itself for commercial cultivation, it is possible to cross it with *S. tuberosum*, and some of the progeny are resistant to the disease. *Solanum demissum* contains at least four major genes for resistance (R_1, R_2, R_3 and R_4), together with a number of minor genes which determine the degree of susceptibility in susceptible varieties (Black, 1952). The four genes may be absent from a particular host strain, or they may be present singly (e.g. R_1), in pairs, in threes, or all together, so that 16 host genotypes are possible representing different combinations of *R* genes. The identification of the *R* gene complex was dependent on the discovery that the fungus itself exists in a number of strains or **physiological races**. For each host *R* gene, the pathogen was assumed to carry a gene which enables it to overcome the effect of the *R* gene. This is the basis of the **gene-for-gene** hypothesis, and gene-for-gene interactions are common in many host–pathogen interactions (Flor, 1971). Assuming a gene-for-gene situation for the interaction of *P. infestans* with *S. tuberosum*, 16 races of *P. infestans* should theoretically be demonstrable. If the corresponding genes of the fungus are termed 1, 2, 3 and 4, then the different races can be labelled (0), (1), (2), etc., (1.1), (1.2), etc., (1.2.3), (1.2.4), etc., and (1.2.3.4). By 1953, 13 of the 16 races had been identified, the prevalent race being Race 4. By 1969, 11 *R* genes had been recognized in Britain (Malcolmson, 1969). Resistance based on a small number of defined genes of major effect has been termed **major gene resistance** or **race-specific resistance**. Because of the uncanny ability of *P. infestans* to break major gene resistance even before the arrival of the A2 mating type in Europe and North America, attempts at breeding fully resistant potato cultivars have now been abandoned (Wastie, 1991).

The origin of physiological races is difficult to determine. The occurrence and spread of resistance genes before the arrival of the A2 mating type may have been due to mutation followed by selection imposed by the monoculture of a resistant host. Another possibility is that the mycelium of *P. infestans* is heterokaryotic, carrying nuclei of more than one race. Yet another scenario is vegetative hybridization followed by parasexual recombination (see p. 230); by mixing sporangia of two different races, new races with a different pattern of virulence towards potato varieties have been obtained after several cycles of inoculation (Malcolmson, 1970). The parasexual cycle has been experimentally demonstrated for *P. parasitica* using fungicide resistance as a genetic marker (Gu & Ko, 1998).

Within 1–2 days of infection, tissues of resistant hosts undergo necrosis so rapidly that sporulation and further growth of the fungus cannot occur. Such a reaction is sometimes termed **hypersensitivity**, and the function of the *R* genes is to accelerate this host reaction. When potato tubers are inoculated with an avirulent race of *P. infestans*, they respond by secreting antifungal substances called **phytoalexins**. Two of the phytoalexins formed by resistant tubers are rishitin and phytuberin. Rishitin, originally isolated from the potato variety Rishiri, is a bicyclic sesquiterpene. Tomiyama *et al.* (1968) showed that R_1 tuber tissue inoculated with an avirulent race of *P. infestans* produced over 270 times the amount of rishitin than when inoculated with a virulent race. The *R* genes of the potato probably determine the ability of host tissue to recognize and respond to avirulent races of *P. infestans* (Day, 1974). The detailed molecular interactions which determine race specificity are, however, complex and still only incompletely understood at present (Friend, 1991).

Breeding for field resistance

In addition to the major genes for resistance in potato, numerous other genes also exist which, although individually of small effect, may contribute to resistance if present together. Resistance of this kind is known as **general resistance** or **field resistance**, and some potato breeding programmes aim at producing varieties possessing it (Niederhauser, 1991). This is preferable to single-gene resistance because *P. infestans* is less likely to overcome the combined resistance of numerous minor genes simultaneously. Field resistance retards the infection process, e.g. by production of a particularly thick cuticle or by a leaf architecture unfavourable to infection, lowers the number of sporangia produced, and extends the time needed by the pathogen to initiate new infections (Wastie, 1991). Field resistance is equally effective against all physiological races of *P. infestans*, and it reduces the severity of an epidemic and consequently the need to apply fungicides (Erwin & Ribeiro, 1996).

Tomato late blight

P. infestans also causes significant worldwide crop losses of tomato (*Lycopersicon esculentum*) which, like potato, belongs to the Solanaceae. The general principles of control of tomato late blight are similar to those described above for potato, including fungicides used and blight forecasting (Erwin & Ribeiro, 1996). Many strains of *P. infestans* are capable of infecting both tomato and potato. However, since the resistance gene systems are different in these two hosts, correlations between virulence of a given strain on potato and tomato cannot be drawn (Legard *et al.*, 1995).

5.4 Peronosporales

The Peronosporales are obligately biotrophic pathogens of a few groups of higher plants and are responsible for diseases mainly of aerial plant organs known collectively as **downy mildews**. The order currently comprises two families, the Peronosporaceae (*Peronospora*, *Plasmopara*, *Bremia*) and Albuginaceae (*Albugo*). There are about 250 species (Kirk *et al.*, 2001). DNA sequencing data (Cooke *et al.*, 2000; Riethmüller *et al.*, 2002) are confusing at present because species of *Phytophthora* (Pythiales) and *Peronospora* (Peronosporales) seem to intergrade in phylogenetic analyses. *Peronospora* seems more closely related to *Phytophthora* than to other members of the Peronosporales such as *Albugo*, which in turn may have affinity with *Pythium*. Considerable rearrangements between the Peronosporales and Pythiales will therefore have to be carried out at some point in the future. However, we prefer to retain the conventional system for the time being because the downy mildews (Peronosporales) represent a convincing biological entity (Dick, 2001a). The key features distinguishing them from the Pythiales are as follows.

First, they are obligate biotrophs and cannot be grown apart from their living host. The mycelium in the host tissues is coenocytic and intercellular, with haustoria of various types penetrating the cell walls. No member of the Peronosporales has as yet been grown in axenic culture, although some can be propagated in dual culture with callus tissues of their plant hosts. None the less, some species (e.g. *Plasmopara viticola*) can cause cell damage to their hosts which leads to the leakage of cytoplasm (Lafon & Bulit, 1981). This is similar to the rots caused, for example, by *Phytophthora erythroseptica* (Plate 2f) and suggests an incomplete adaptation to the biotrophic habit, tying in with the likely origin of Peronosporales from within the Pythiales (Dick, 2001a).

Second, whereas *Pythium* and *Phytophthora* spp. are typically able to attack a very wide range of host plants, Dick (2001a) has pointed out that Peronosporales parasitize a narrow range of angiosperm families, usually dicotyledons, and especially herbaceous plants which are either highly evolved or accumulate large amounts of secondary metabolites such as essential oils or alkaloids. Any one species of downy mildew is specific to only one or a few related host genera. Dick (2001a, 2002) has speculated that a co-evolution of the downy mildews with herbaceous angiosperms occurred mainly in the Tertiary period, and as several independent events, whereby *Phytophthora* and downy mildews share common ancestors. The Peronosporaceae are relatively recent; *Peronospora*, along with its host plants, may have arisen in the mid to late Tertiary in the vicinity of Armenia and Iran. *Plasmopara* is probably of South American origin and dates back to the early Tertiary, whereas *Bremia lactucae* is a central European species. In contrast, the Albuginaceae (*Albugo*) are more ancient, with a late Cretaceous origin possibly in South America (Dick, 2002).

A third major feature of the Peronosporales is the tendency of their sporangia to germinate directly, rather than by releasing zoospores. Many species have lost the ability to produce zoospores altogether, their sporangia being functional 'conidia' which are disseminated by wind. The sporangiophores are well-differentiated, showing determinate growth and branching patterns which provide characteristic features for identification. The production of directly germinating sporangia on well-defined sporangiophores represents an adaptation to the terrestrial lifestyle and supports the postulated origin of the Peronosporales in the drier Tertiary period (Dick, 2002). The life cycle of Peronosporales is similar to that of *Phytophthora* (see Fig. 5.19). Sporangia infect directly or produce infective zoospores, leading to a new crop of sporangiophores and sporangia, and this asexual cycle spreads the disease during the vegetation period. Sexual reproduction is by means of oospores which are formed within the host tissue and survive adverse conditions after host death.

Peronosporales cause economically significant diseases, and one of them – *Plasmopara viticola* – has had a major impact on agriculture and plant pathology because it led to the discovery of Bordeaux mixture (see p. 119). Overviews of the Peronosporales have been given by Spencer (1981), Smith *et al*. (1988) and Dick (2002).

5.4.1 *Peronospora* (Peronosporaceae)

Peronospora destructor causes a serious disease of onions and shallots whilst *P. farinosa* causes downy mildew of sugar beet, beetroot and spinach, but can also be found on weeds such as *Atriplex* and *Chenopodium*. *Peronospora tabacina* causes blue mould of tobacco. This name refers to the bluish purple colour of the sporangia, which is actually a feature of many species of *Peronospora*. Crop losses associated with *P. tabacina* can be up to 95%. This species was introduced into Europe in 1958 and has spread rapidly since (Smith *et al*., 1988).

Peronospora parasitica attacks members of the Brassicaceae. Although many specific names have been applied to forms of this fungus on different host genera, it is now customary to regard them all as belonging to a single species (Dickinson & Greenhalgh, 1977; Kluczewski & Lucas, 1983). Turnips, swede, cauliflower, Brussels sprouts and wallflowers (*Cheiranthus*)

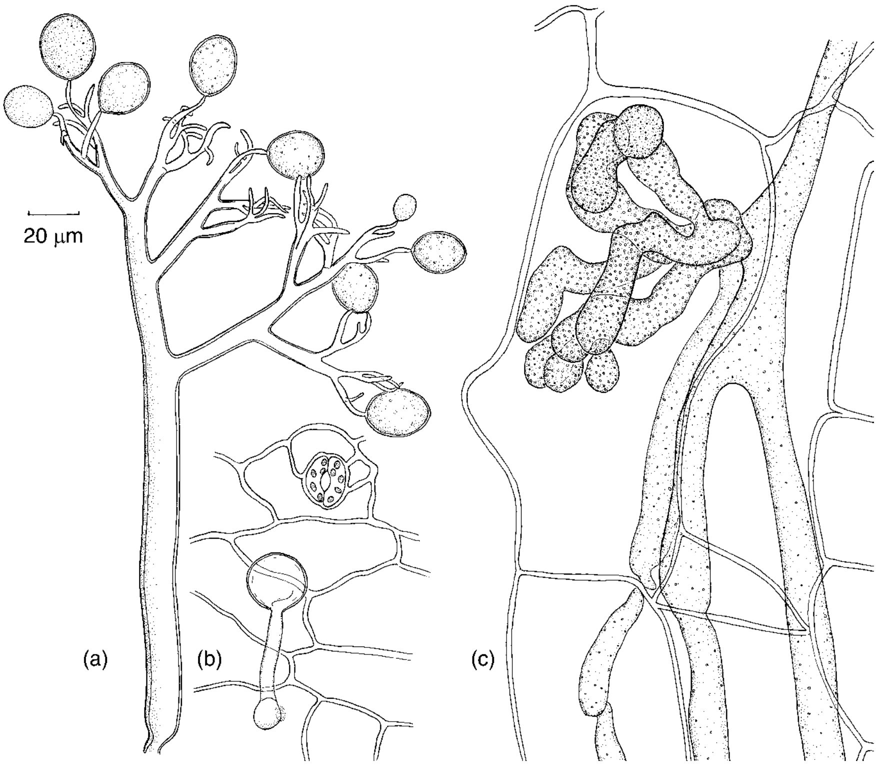

Fig 5.28 *Peronospora parasitica* on *Capsella bursa-pastoris*. (a) Sporangiophore. (b) Sporangium germinating by means of a germ tube. (c) L.S. of host stem showing intercellular mycelium and coarse lobed haustoria.

are commonly attacked, and the fungus is found particularly frequently on shepherd's purse (*Capsella bursa-pastoris*). Diseased plants stand out by their swollen and distorted stems bearing a white 'fur' of sporangiophores (Plate 2g). On leaves the fungus is associated with yellowish patches on the upper surface and the formation of white sporangiophores beneath. Sections of diseased tissue show a coenocytic intercellular mycelium and branched lobed haustoria in certain host cells (Fig. 5.28c; Fraymouth, 1956).

Following penetration of the host cell by *P. parasitica*, reactions are set up between the host protoplasm and the invading fungus. The haustorium becomes ensheathed by a layer of callose which is visible as a thickened collar around the haustorial base in susceptible host plants, whereas the entire haustorium may be coated by thick callose deposits in interactions showing a resistance response (Donofrio & Delaney, 2001). The general appearance of haustoria of *Peronospora* is very similar to that of *Phytophthora* shown in Fig. 5.21; the main body of the haustorium is surrounded by host cytoplasm, the host plasma membrane, an extrahaustorial matrix, the fungus cell wall, and the fungal plasma membrane (Fig. 5.29). Although the haustoria undoubtedly play a major role in the nutrient uptake of the fungus from the host plant, it should be noted that intercellular hyphae are also capable of assimilating nutrients *in planta* (Clark & Spencer-Phillips, 1993; Spencer-Phillips, 1997).

The sporangiophores emerge singly or in groups from stomata. There is a stout main axis which branches dichotomously to bear egg-shaped sporangia at the tips of incurved branches (Fig. 5.28a). Detachment of sporangia is possibly caused by hygroscopic twisting of the sporangiophores related to changes in humidity.

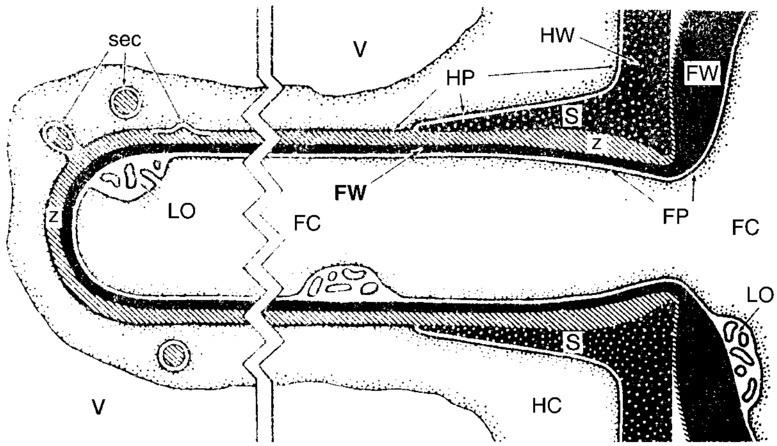

Fig 5.29 *Peronospora manshurica*. Diagram of host–pathogen interface in the haustorial region. Fungal cytoplasm (FC) is bounded by the fungal plasma membrane (FP), lomasomes (LO) and the fungal cell wall (FW) in both the intercellular hyphae (right) and the haustorium (centre). The relative positions of the host cell vacuole (V), host cytoplasm (HC) and host plasmalemma (HP) are indicated. The host cell wall (HW) terminates in a sheath (S). The zone of apposition (Z) separates the haustorium from the host plasmalemma. Invaginations of the host plasmalemma and vesicular host cytoplasm are considered evidence for host secretory activity (sec). After Peyton and Bowen (1963).

In *P. tabacina*, however, it has been suggested that changes in turgor pressure of the sporangiophores occur which parallel changes in the water content of the tobacco leaf. Sporangia may be discharged actively by application of energy at their point of attachment to the sporangiophore. In the Sclerosporaceae (see Section 5.5), violent sporangial discharge also occurs. Upon alighting on a suitable host, sporangia of *P. parasitica* germinate by the formation of a germ tube rather than zoospores. The germ tube penetrates the wall of the epidermis by means of an appressorium (Fig. 5.28b).

Oospores of *P. parasitica*, like those of most other Peronosporales, are embedded in senescent leaf tissues and are found throughout the season. There is evidence that some strains of the fungus are heterothallic whilst others are homothallic (McMeekin, 1960). Both the antheridium and oogonium are at first multinucleate. Nuclear division precedes fertilization, and meiosis occurs in the oogonium and antheridium (Sansome & Sansome, 1974). Fusion between two nuclei is delayed at least until the oospore wall is partly formed.

The wall of the oospore of *P. parasitica* is very tough, and it is difficult to induce germination. In *P. destructor* and some other species, germination occurs by means of a germ tube but in *P. tabacina* zoospores have been described. It is probable that oospores overwinter in soil and give rise to infection in subsequent seasons. Although oospores of *P. destructor* have been germinated after 25 years, it has not proven possible to infect onions from such material. Possibly in this case the disease is carried over by means of systemic infection of volunteer onion bulbs (Smith *et al.*, 1988).

Peronospora parasitica and *Arabidopsis thaliana*

The chance discovery of a *P. parasitica* infection in an *Arabidopsis thaliana* weed population in a Zurich garden showing haustoria, sporangia and oospores (Koch & Slusarenko, 1990) opened up the possibility of using this genetically well-characterized 'model plant' to investigate plant–pathogen interactions involving downy mildews. The interaction between *Arabidopsis* and *Peronospora* is governed by a gene-for-gene relationship, i.e. it is a form of major gene resistance based on specific recognition of a pathogen avirulence gene (*avr*) product by the product of a matching host resistance (*R*) gene (e.g. Botella *et al.*, 1998). Molecular aspects of the *Arabidopsis* immune response to infections by

P. parasitica and other pathogens have been investigated in some detail. Infection of one leaf triggers a localized reaction, the hypersensitive response, leading to death of the plant cells in the vicinity of infection. Additionally, a systemic response is initiated, i.e. plant organs distal to the infected leaf become resistant against further attack. This phenomenon is called **systemic acquired resistance** and is active against attacks by the same as well as many other pathogens. It is triggered at the site of initial infection by various **elicitor** molecules of pathogen origin, e.g. fatty acids such as arachidonic acid, or by other substances. The signal is transmitted by signalling molecules such as salicylic acid (Lawton *et al*., 1995; Ton *et al*., 2002) which itself has no antimicrobial activity. Salicylic acid-independent signalling events are probably also involved (McDowell *et al*., 2000). Salicylic acid is produced at sites of infection, diffuses through the plant and interacts with a signalling chain, leading to the expression of a set of pathogenesis-related (*PR*) genes. A whole subset of *PR* genes involved in resistance to *P. parasitica* (*RPP* genes) is now known (McDowell *et al*., 2000). The function of many *PR* genes is still obscure; those whose functions are known encode chitinases, β-1,3-glucanases, proteinases, peroxidases or enzymes involved in toxin biosynthesis (Kombrink & Somssich, 1997). By creating mutants of *Arabidopsis* or of crop plants which overexpress their own regulatory genes or *PR* genes, or express introduced genes encoding elicitor molecules of pathogen origin, constitutive resistance against pathogen attack may be generated. This is considered to hold great potential for agriculture (Cao *et al*., 1998; Maleck *et al*., 2002).

Control of *Peronospora*

Downy mildew infections caused by *Peronospora* spp. are controlled mainly by fungicide applications. Metalaxyl is very effective against all downy mildews, but resistance has arisen in several species, and thus this fungicide is now applied in a cocktail with dithiocarbamates (Smith *et al*., 1988). Fosetyl—Al is also now widely used as a foliar spray, root dip or soil amendment (Agrios, 2005).

The breeding of cultivars with resistance against *Peronospora* spp. has been successful in certain crops, e.g. in lucerne (*Medicago sativa*) against *P. trifoliorum* (Stuteville, 1981). In tobacco plants attacked by *P. tabacina*, this strategy is a useful component of integrated control but is not sufficient on its own to afford complete control (Schiltz, 1981). In the tobacco—*P. tabacina* system, a disease warning system is also in operation in Europe; subscribing tobacco growers are informed of the occurrence of the pathogen, so that preventative measures can be taken (Smith *et al*., 1988). This is profitable because tobacco is a high-value crop.

Because downy mildews infect aerial plant parts and produce air-borne propagules in large numbers, crop sanitation measures are generally not very effective. However, in the case of *P. destructor* which overwinters systemically in volunteer onion bulbs, removal of volunteers is essential. In *P. viciae* on peas and beans, deep ploughing of the crop residue is important as the pathogen survives on infected haulms (Smith *et al*., 1988).

5.4.2 *Plasmopara* (Peronosporaceae)

Although downy mildews caused by species of *Plasmopara* are rarely serious in temperate climates, *P. viticola* is potentially a very destructive pathogen of the grapevine. The disease, which was endemic in North America and not particularly destructive on the local vines, was introduced into France during the nineteenth century with disastrous results on the French vines which had never been exposed to the disease and were highly susceptible. Large (1940) has vividly recounted the moment when Alexis Millardet, walking past a heavily infected vineyard in 1882, noticed that vines close to the road appeared healthy and had been sprayed with a mixture of lime and copper sulphate to discourage passers-by from pilfering fruit. This led to the discovery of Bordeaux mixture, one of the world's first fungicides and still effective against *P. viticola* and other foliar pathogens belonging to the Oomycota.

Plasmopara nivea is occasionally reported in Britain on umbelliferous crops such as carrot and parsnip, and it is also found on *Aegopodium podagraria. Plasmopara pygmaea* is found on yellowish patches on the leaves of *Anemone nemorosa* (Fig. 5.30b), whilst *P. pusilla* is similarly associated with *Geranium pratense* (Fig. 5.30a). The haustoria of *Plasmopara* are knob-like, the sporangiophores are branched monopodially and the sporangia are hyaline (Fig. 5.30). Two types of sporangial germination have been reported. In *P. pygmaea* there are no zoospores but the entire sporangium detaches and later produces a germ-tube. In other species the sporangia germinate by means of zoospores which encyst and penetrate the host stomata. Oospore germination in *P. viticola* is also by means of zoospores.

Because the grapevine is such a high-value crop, the fungicide market is lucrative. Bordeaux mixtures are still used today, and similar fungicide applications to those described for *Peronospora* are made. Resistance to metalaxyl has been observed in *P. viticola*. Disease forecasting systems are being developed (Lafon & Bulit, 1981; Smith *et al.*, 1988). Breeding for resistant cultivars is being carried out, but because of the long generation times of the crop, this will be a prolonged effort.

5.4.3 *Bremia* (Peronosporaceae)

Bremia lactucae causes downy mildew of lettuce (*Lactuca sativa*) and strains of it can be found on 36 genera of the Asteraceae including *Sonchus* and *Senecio* (Crute & Dixon, 1981). Cross-inoculation experiments using sporangia from these hosts have failed to result in infection of lettuce and it seems that the fungus exists as a number of host-specific strains (*formae speciales*). Although wild species of *Lactuca* can carry strains capable of infecting lettuce, these hosts are not sufficiently common to provide a serious source of infection. The disease can be troublesome both in lettuce grown in the open and under frames,

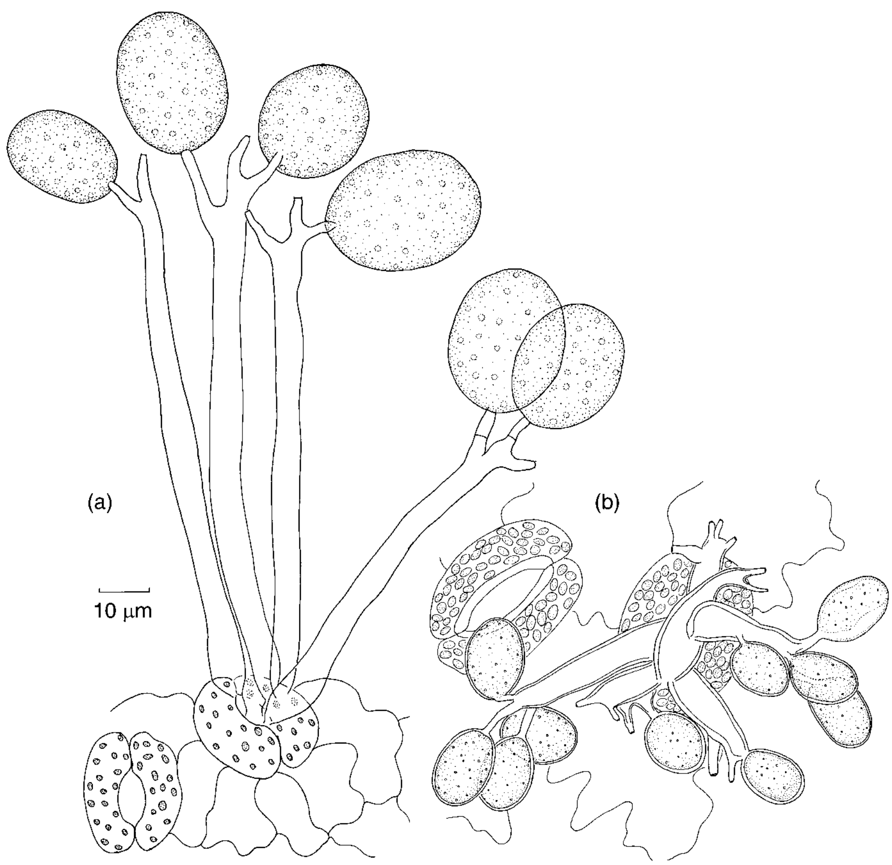

Fig 5.30 *Plasmopara*. (a) Sporangiophores of *P. pusilla* on *Geranium pratense*. (b) Sporangiophores of *P. pygmaea* on *Anemone nemorosa*.

and in market gardens there may be sufficient overlap in the growing of lettuce for the disease to be carried over from one sowing to the next. The damage to the crop caused by *Bremia* may not in itself be severe, but infected plants are prone to secondary infection by the more serious grey mould, *Botrytis cinerea*. Systemic infections can occur. The intercellular mycelium is coarse, and the haustoria are sac-shaped, often several of them being present in each host cell (Fig. 5.31d). The sporangiophores emerge singly or in small groups through the stomata and branch dichotomously. The tip of each branch expands to form a cup-shaped disc bearing short cylindrical

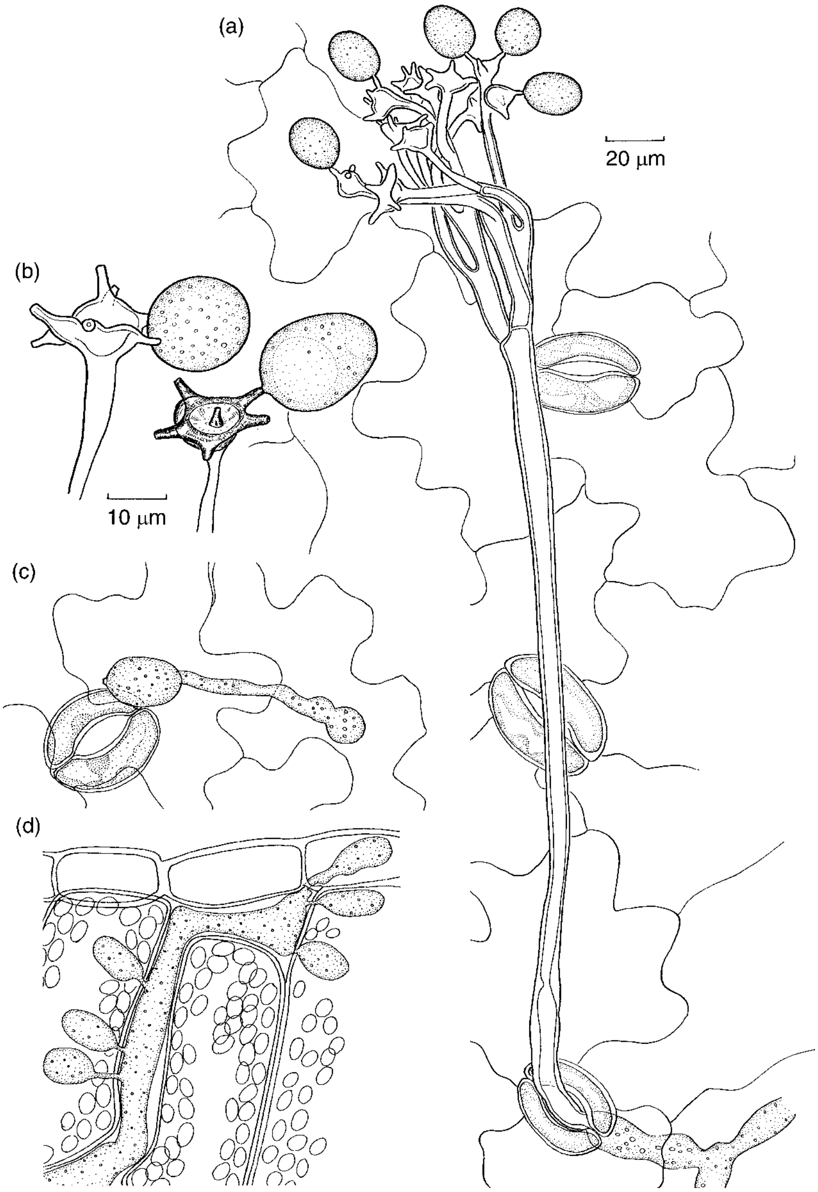

Fig 5.31 *Bremia lactucae* from *Senecio vulgaris*. (a) Sporangiophore protruding through a stoma. (b) Sporangiophore apex. (c) Sporangium germinating by means of a germ tube which has produced an appressorium at its apex. (d) Cells of epidermis and palisade mesophyll, showing intercellular mycelium and haustoria. (a,c,d) to same scale.

sterigmata at the margin and occasionally in the centre, and from these the hyaline sporangia arise (Figs. 5.31a,b). Germination of the sporangia is usually by means of a germ tube which forms an appressorium to penetrate epidermal cells (Fig. 5.31c), or it enters through a stoma. Zoospore formation has been reported but not confirmed. Sexual reproduction is usually heterothallic, although homothallic strains also exist. The oospores are formed in leaf tissue and remain viable for 12 months (Michelmore & Ingram, 1980; Morgan, 1983).

Chemical control of *B. lactucae* on lettuce is certainly possible although not necessarily desirable; hence, intensive efforts for major gene resistance breeding have been made. Integrated control based on resistant cultivars and fungicide applications using metalaxyl and dithiocarbamates is successful (Crute, 1984). However, resistance against metalaxyl arose in Britain as early as 1983. Fosetyl–Al is not as effective as metalaxyl (Smith *et al.*, 1988).

5.4.4 *Albugo* (Albuginaceae)

This family has only a single genus, *Albugo*, with about 40–50 species of biotrophic parasites of flowering plants which cause diseases known as white blisters or white rusts. The commonest British species is *A. candida* causing white blisters of crucifers such as cabbage, turnip, swede, horseradish, etc. (Plate 2h). It is particularly frequent on shepherd's purse (*Capsella bursa-pastoris*). There is some degree of physiological specialization in the races of this fungus on different host genera. *Albugo candida* can infect *Arabidopsis thaliana*, and the host defence response is governed by resistance genes involved in the recognition of the pathogen (Holub *et al.*, 1995). The principle is similar to, although not as well researched as, the *Arabidopsis–Peronospora* interaction described earlier (p. 116). It is also now possible to establish callus cultures of mustard plants (*Brassica juncea*) containing balanced infections of *A. candida* (Nath *et al.*, 2001). This experimental system should facilitate studies of the physiology of host–pathogen interactions. A less common species is *A. tragopogonis*, causing white blisters of salsify (*Tragopogon porrifolius*), goatbeard (*T. pratensis*) and *Senecio squalidus*.

In *A. candida* on shepherd's purse, diseased plants may be detected by the distorted stems and the shining white raised blisters on the stem, leaves and pods before the host epidermis is ruptured (Plate 2h). Later, when the epidermis has burst open, a white powdery pustule is visible. The distortion is possibly associated with altered auxin levels. The host plant may be infected simultaneously with *Peronospora parasitica*, but the two fungi are easily distinguishable microscopically both in the structure of the sporangiophores and by their different haustoria. In *Albugo*, the mycelium in the host tissues is intercellular with only small spherical haustoria (Fig. 5.32) which contrast sharply with the coarsely lobed haustoria of *P. parasitica*. The fine structure of *A. candida* haustoria has been described by Coffey (1975) and Soylu *et al.* (2003). They are spherical or somewhat flattened and about 4 μm in diameter, connected to the intercellular mycelium by a narrow stalk about 0.5 μm wide. Inside the plasma membrane of the haustorium, lomasomes, i.e. tubules and vesicles apparently formed by invagination of the plasma membrane, are more numerous than in the intercellular hyphae. The cytoplasm of the haustorial head is densely packed with mitochondria, ribosomes, endoplasmic reticulum and occasional lipid droplets, but nuclei have not been observed. Since nuclei of *Albugo* are about 2.5 μm in diameter, they may be unable to traverse the constriction which links the haustorium to the intercellular hypha. Nuclei may (e.g. *Peronospora pisi*) or may not be present in the haustoria of other Oomycota. The base of the haustorium of *A. candida* is surrounded by a collar-like sheath which is an extension of the host cell wall, but this wall does not normally extend to the main body of the haustorium. Between the haustorium and the host plasma membrane is an encapsulation. Host cytoplasm reacts to infection by an increase in the number of ribosomes and Golgi complexes. In the vicinity of the haustorium the host cytoplasm contains numerous vesicular and tubular elements not found in uninfected cells. These structures have been interpreted

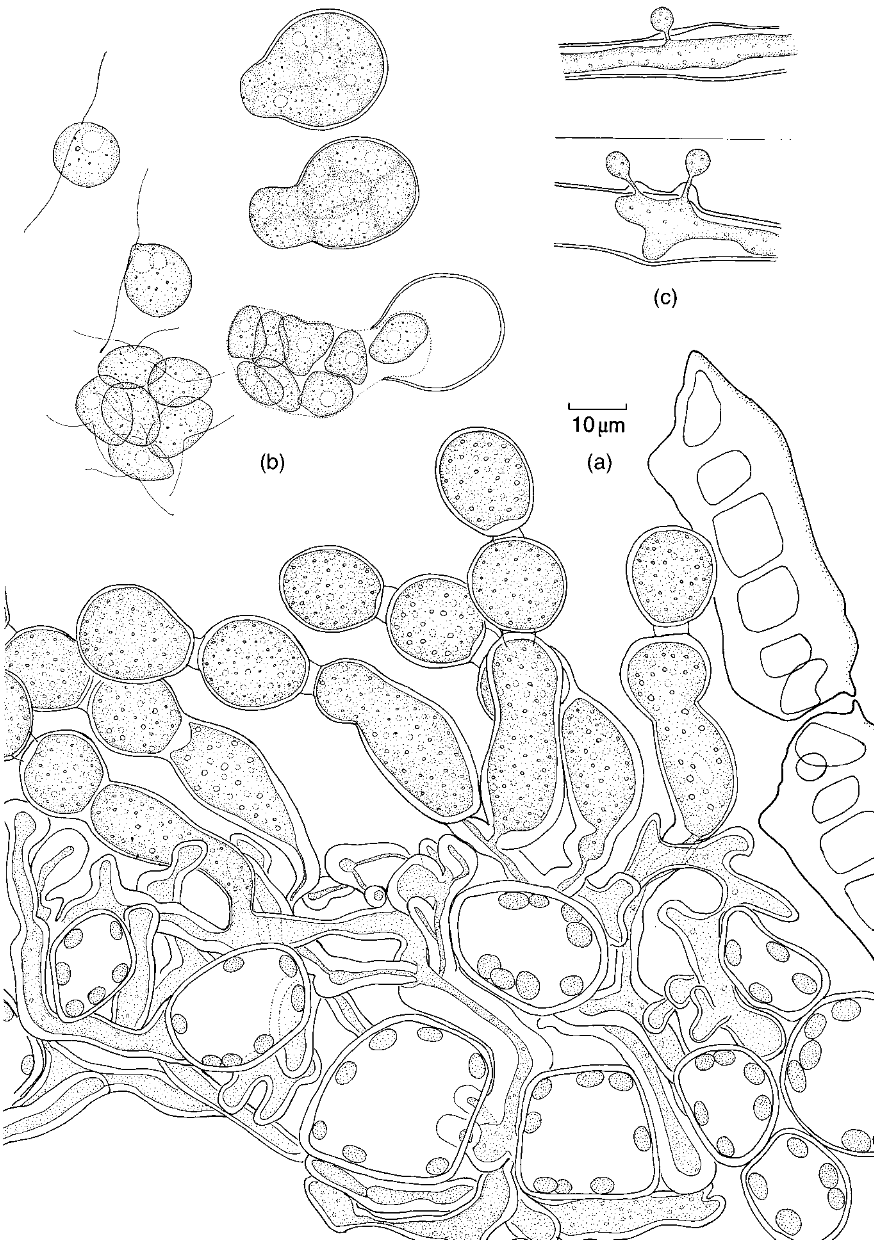

Fig 5.32 *Albugo candida* on *Capsella bursa-pastoris*. (a) Mycelium, sporangiophores and chains of sporangia formed beneath the ruptured epidermis (right). (b) Germination of sporangia showing the release of eight biflagellate zoospores. The stages illustrated took place within 2 min. (c) Haustoria.

as evidence of secretory processes induced in the host cell by the presence of the pathogen.

The intercellular mycelium aggregates beneath the host epidermis to form a palisade of cylindrical or skittle-shaped sporangiophores which give rise to chains of spherical sporangia in basipetal succession – i.e. new sporangia are formed at the base of the chain. The pressure of the developing chains of sporangia raises the host epidermis and finally ruptures it.

The sporangia are then visible externally as a white powdery mass dispersed by the wind. Sporangia reaching a suitable host leaf will germinate within a few hours in films of water to form biflagellate zoospores of the principal type, about eight per sporangium (Fig. 5.32b). After swimming for a time, a zoospore encysts and then forms a germ tube which penetrates the host epidermis. The asexual disease cycle may be completed within 10 days. Infections may be localized or systemic. Gametangia are formed in the intercellular spaces of infected stems and leaves. Both the antheridium and the oogonium are multinucleate at their inception, and during development two further nuclear divisions occur so that the oogonium may contain over 200 nuclei. However, there is only one functional male and one functional female nucleus. In the oogonium all the nuclei except one migrate to the periphery and are included in the periplasm. Following nuclear fusion a thin membrane first develops around the oospore. Division of the zygote nucleus takes place and is repeated, so that at maturity the oospore may contain as many as 32 diploid nuclei. Sansome and Sansome (1974) reported that meiosis occurs within the gametangia. They also suggested that *A. candida* is heterothallic. The high incidence of oospores of *Albugo* in *Capsella* stems simultaneously infected with *Peronospora parasitica* may result from some stimulus towards self-fertilization in *Albugo* produced by *Peronospora*, a situation analogous to the *Trichoderma*-induced sexual reproduction in heterothallic species of *Phytophthora* (see p. 95).

The mature oospore is surrounded by a brown exospore, thrown into warty folds (Fig. 5.33a). Germination of the oospores takes place only after a resting period of several months. Under suitable conditions the outer wall of the oospore bursts and the endospore is extruded as a thin, spherical vesicle, which may be sessile or formed at the end of a wide cylindrical tube. Within the thin vesicle 40–60 zoospores are differentiated and are released on its breakdown (Figs. 5.33b,c).

The cytology of oospore development in some other species of *Albugo* differs from that of *A. candida*. In *A. bliti*, a pathogen of *Portulaca* in North America and Europe, the oogonia and antheridia are also multinucleate and two nuclear divisions take place during their development. Numerous male nuclei fuse with numerous female nuclei and the fusion nuclei

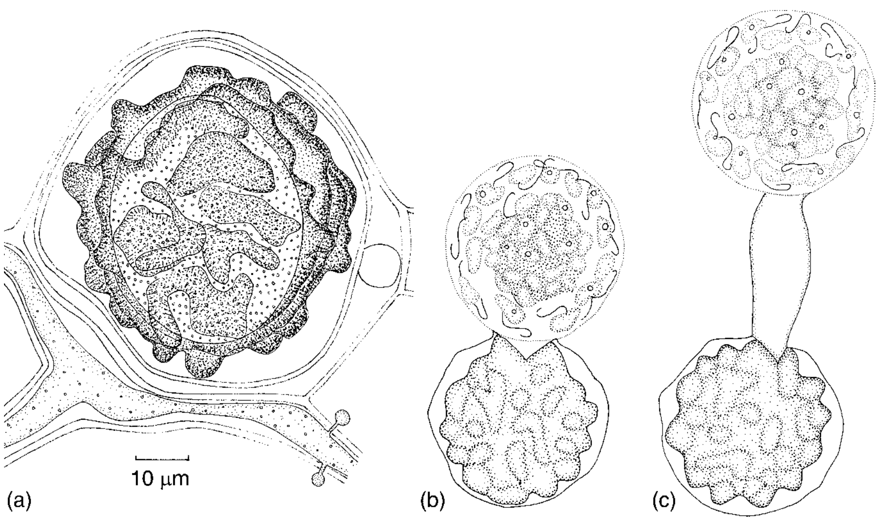

Fig 5.33 *Albugo candida* oospores. (a) Oogonium and oospore from *Capsella* leaf. (b,c) Two methods of oospore germination (after Vanterpool, 1959).