

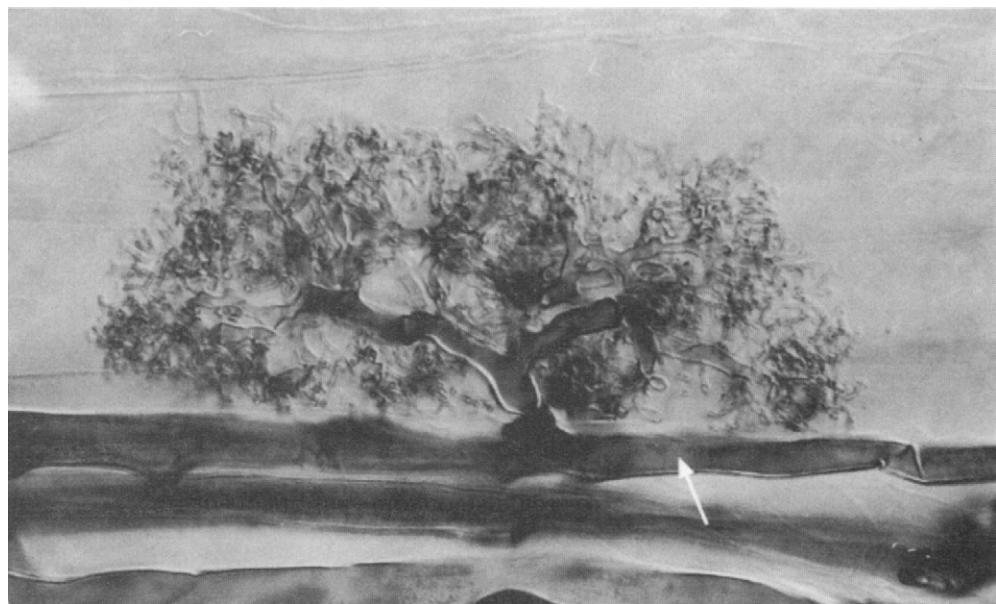
# The symbionts forming arbuscular mycorrhizas

## Introduction

Arbuscular mycorrhizas (AM) are the most common mycorrhizal type. They are formed in an enormously wide variety of host plants by obligately symbiotic fungi which have recently been reclassified on the basis of DNA sequences into a separate fungal phylum, the Glomeromycota (Schüßler *et al.*, 2001). The plants include angiosperms, gymnosperms and the sporophytes of pteridophytes, all of which have roots, as well as the gametophytes of some hepatic and pteridophytes which do not (Read *et al.*, 2000; see Chapter 14). It seems highly likely that the fungi had their origins possibly over 1000 million years ago (predating current estimates of colonization of land) and that arbuscular mycorrhizal (AM) symbioses are also extremely ancient. Through their roles in nutrient uptake, AM fungi were probably important in the colonization of land by plants (Simon *et al.*, 1993; Remy *et al.*, 1994; Taylor *et al.*, 1995; Redecker *et al.*, 2000; Heckman *et al.*, 2001); they remain major determinants of plant interactions in ecosystems to the present day.

The name 'arbuscular' is derived from characteristic structures, the arbuscules (Figure 1.1a) which occur within the cortical cells of many plant roots and also some mycothalli colonized by AM fungi. Together with storage vesicles located within or between the cells, these structures have been considered diagnostic for AM symbioses. However, a rather wide range of intraradical structures formed by AM fungi is recognized (see Chapter 2 and Dickson, 2004), including well-developed intracellular hyphal coils, which sometimes occur in the absence of any arbuscules (Figure 1.1b). The variations in developmental pattern are determined by both plant and fungal partners, adding to the complexities of identifying a symbiosis as 'AM' on the basis of intraradical fungal morphology. The term vesicular-arbuscular mycorrhiza (VAM), which was in use for many decades, has been dropped in recognition that vesicles are formed by only 80% of AM fungi, but the name 'arbuscular' is currently retained, regardless of the structural diversity which is more and more widely appreciated.

An arbuscular mycorrhiza has three important components: the root itself, the fungal structures within and between the cells of the root and an extraradical mycelium in the soil. The last may be very extensive but does not form complex mycelial strands or rhizomorphs, nor any vegetative pseudoparenchymatous structures comparable to



(a)



(b)

**Figure 1.1** (a) A mature Arum-type arbuscule of *Glomus mosseae* within a cortical cell of *Allium porrum* (leek). The arbuscule has grown from a well-developed intercellular hypha (arrow). From Brundrett et al. (1984), with permission. (b) Paris-type intracellular coils of *Glomus intraradices* in cortical cells of a root of *Panax quinquefolius*, viewed by confocal microscopy. Note absence of intercellular hyphae. Reproduced from Peterson et al. (2004), with permission. See Chapter 2 for discussion of Arum- and Paris-type AM.

the fungal sheath typical of ectomycorrhizas (see Chapter 6). A few of the fungi do form sporocarps or clusters of spores with limited amounts of sterile mycelium. Because the characteristic fungal structures develop within the root and because changes in the rates of root growth and branching are discernible only by detailed comparison with non-mycorrhizal plants, it is not usually possible to tell if a root system is colonized without staining and microscopic examination or molecular probing for diagnostic DNA sequences.

Arbuscular mycorrhizas were first recognized and described in the last decades of the nineteenth century. Their widespread occurrence and common presence in plants of many phyla in most parts of the world, especially in the tropics, was realized very soon (Janse, 1897; Gallaud, 1905), but very little functional information was learnt about them until the mid-1950s. Almost all writings about the identity of the fungi until 1953 may be ignored, except for those of Peyronel (1923) who showed that hyphae of the endophyte could be traced to the sporocarps of species of fungi, then classified in the Endogonaceae, in the surrounding soil. Later, Butler (1939) in an influential review, agreed that the fungi called *Rhizophagus* were almost certainly imperfect members of the Endogonaceae, which then included the majority of fungi now transferred to the Glomeromycota. The work of Mosse (1953), which showed convincingly that mycorrhizal strawberry plants were colonized by a species of *Endogone* (later transferred to *Glomus*), may be said to have heralded the modern period. Soon Mosse, Baylis, Gerdemann, Nicolson and Daft and Nicolson greatly extended these early observations and demonstrated by inoculation that fungi in the Endogonaceae were symbiotic with many kinds of plants. Further information about the history of AM research is outlined in the highly readable 'A history of research on arbuscular mycorrhiza' by Koide and Mosse (2004).

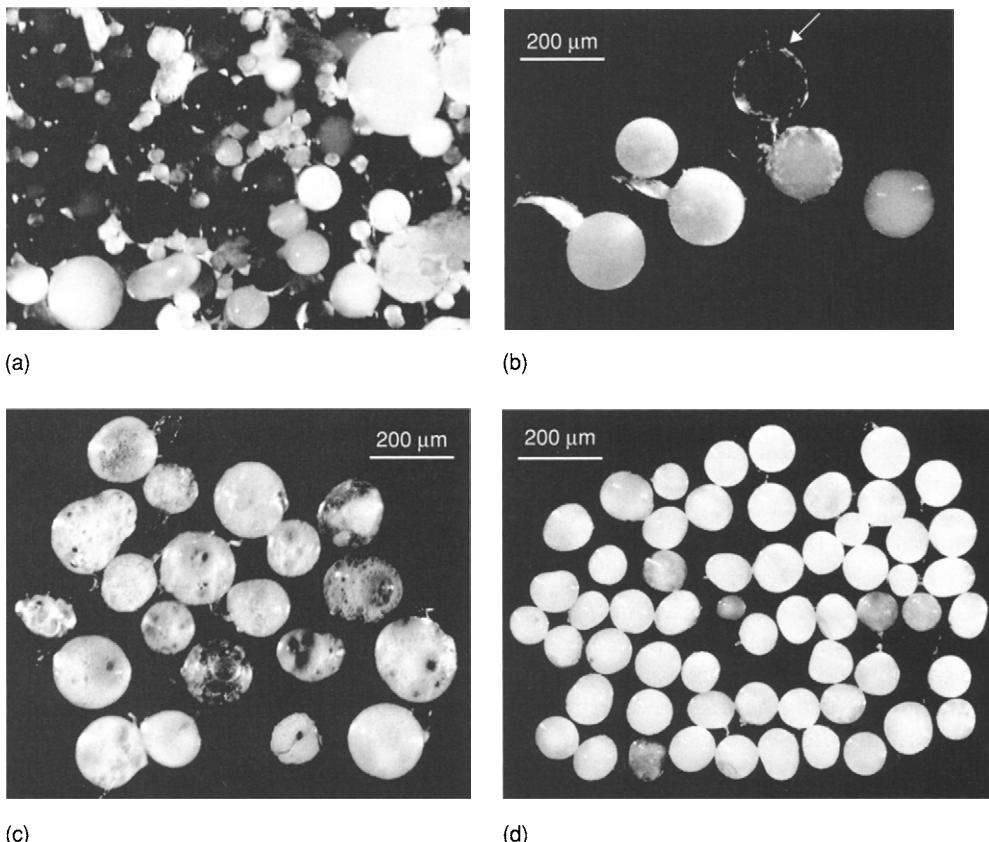
A major milestone was reached in 1974 with a successful symposium on endomycorrhizas at which a number of key ideas were developed for the first time. Many of the papers presented at that meeting remain classics (Sanders *et al.*, 1975) and, together with the two previous editions of this book (Harley and Smith, 1983; Smith and Read, 1997), provide a general introduction to AM symbioses. While recent research has extended and confirmed the generalizations established by the early work, there has also been an increased appreciation of the genetic, structural and functional diversity to be found in AM fungi and the symbioses that they form and an important new emphasis on the cellular and molecular interactions between the symbionts and their roles in ecosystems.

In brief, AM fungi have been recognized as obligate symbionts of a very wide range of plant species. The symbioses are biotrophic and normally mutualistic, the long-term compatible interactions being based largely on bidirectional nutrient transfer between the symbionts, sometimes supplemented by other benefits such as drought and disease tolerance (see Chapters 5 and 6).

## Arbuscular mycorrhizal fungi

### General biology and development

The spores (Figure 1.2; see Colour Plate 1.1) formed by AM fungi are very large (up to 500 µm diameter), with abundant storage lipid, some carbohydrate and thick, resistant



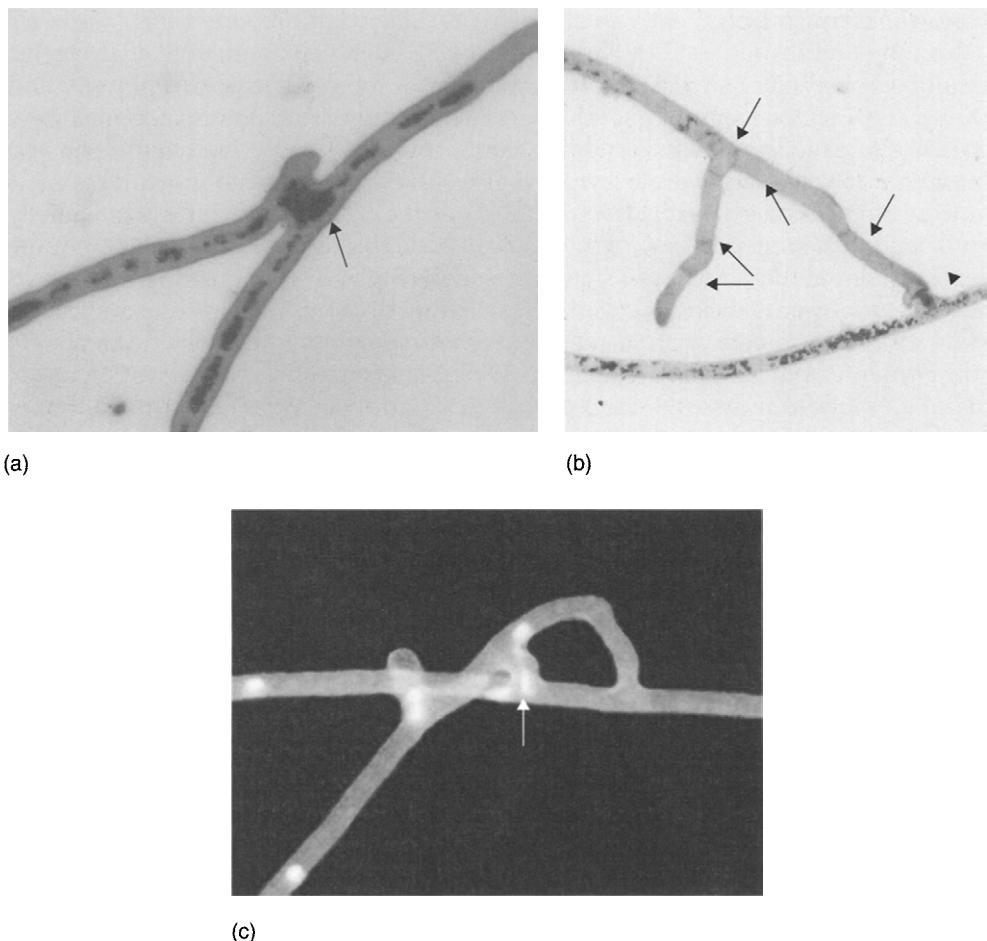
**Figure 1.2** (a) Collection of spores of nine species of AM fungi isolated from a grassland that had developed from an abandoned agricultural site. Reproduced from Bever *et al.* (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *Bioscience* **51**, 923–931, Copyright, American Institute of Biological Sciences, with permission. (b) Spores of *Acaulospora laevis*. A translucent sporiferous saccule can be seen attached to one of the spores (arrowed). (c) A group of spores of *Glomus mosseae*. (d) A group of spores of *Gigaspora gigantea*. Images (b), (c) and (d) are reproduced courtesy of Joe Morton. See also Colour Plate I.1.

walls which all contain chitin and, in some cases,  $\beta$  (1–3) glucan (Gianinazzi-Pearson *et al.*, 1994b; Lemoine *et al.*, 1995). Each spore contains a huge number of nuclei, with estimates ranging from 800 to about 35 000 in different species (Hosny *et al.*, 1998). Most recent evidence indicates that, in *Glomus intraradices* and *G. etunicatum* and probably also *Scutellospora castanea*, all the nuclei are haploid as in other fungi, with no indication of variations in ploidy (Bianciotto *et al.*, 1995; Hijri and Sanders, 2004, 2005). The presumption is that this is the case with all AM fungi (but see below). DNA content per nucleus appears rather variable; amounts of DNA per spore and estimates of the number of nuclei give values of 1.7 and 3.4 pg for *Glomus versiforme* and *Scutellospora persica* (Viera and Glenn, 1990), whereas fluorimetry of the stained nuclei gives lower values of between 0.26 and 1.65 pg for a range of fungi from four genera (Bianciotto and Bonfante, 1992; Hosny *et al.*, 1998). The genome size of  $\sim$ 16.54 Mb (0.017 pg) in *G. intraradices* is low compared with other fungi but in some other AM

species it is much larger, with a value of 1058.4 Mb (1.1 pg) reported for *S. pellucida* (Bianciotto and Bonfante, 1992; Hosny *et al.*, 1998). High values are unlikely to be the result of polyploidy, but rather of accumulation of repeated sequences (Hosny and Dulieu, 1994). Spores of many species, particularly in the Gigasporaceae, contain bacteria-like organisms (BLOs) as endosymbionts, revealed by electron microscopy and molecular analysis. Many are related to *Burkholderia* and have been shown to possess functional bacterial genes including members of the *nif* operon, which is potentially involved in dinitrogen fixation. The BLOs can be vertically transmitted to new spore generations, but their functional significance in the life of AM fungi or in symbioses is yet to be resolved (Bianciotto *et al.*, 1996, 2004; Minerdi *et al.*, 2002).

As spores germinate, hyphal growth involves some nuclear division (Bianciotto and Bonfante, 1992; Bécard and Pfeffer, 1993; Bianciotto *et al.*, 1995), use of carbohydrate and lipid reserves (Bécard *et al.*, 1991; Bago *et al.*, 1999a) and production of limited amounts of branching, coenocytic mycelium, which is capable of anastomosis. In the absence of a host, plant growth eventually ceases, probably due to lack of signal molecules from the roots which have been shown to stimulate hyphal branching (Giovannetti *et al.*, 1993a; Buee *et al.*, 2000; Tamasloukht *et al.*, 2003; Akiyama *et al.*, 2005; Besserer *et al.*, 2006) (see Chapter 3). The growth arrest appears to be programmed, with controlled retraction of cytoplasm and nuclei and production of septa, allowing the spore and associated mycelium to retain long-term viability and the capacity to regerminate and colonize roots (Koske, 1981; Logi *et al.*, 1998). Once symbiosis with a host has been established, mycelial growth proceeds both within roots and in the soil and ultimately leads to the formation of new multinucleate spores terminally on the hyphae (see Chapters 2 and 3). Despite many efforts, AM fungi have generally proved to be unculturable and are unable to complete their life cycles without forming a symbiosis with an autotrophic plant. Nevertheless, one recent investigation (Hildebrandt *et al.*, 2006) reported the production of a small number of viable and infective spores by a *Glomus intraradices* isolate, co-cultured with two particular bacteria. This encouraging finding may lead to a better understanding of stimuli required for growth and sporulation.

The mycelium originating from a single spore is not likely to remain independent. Anastomosis between hyphae has been known for a century (Gallaud, 1905) and was observed in presymbiotic mycelium by Mosse (1959). Recently, detailed analysis has demonstrated anastomoses between hyphae originating either from spores or colonized roots, resulting in cytoplasmic continuity and nuclear migration (Giovannetti *et al.*, 1999, 2001, 2003, 2004; Giovannetti and Sbrana, 2001; de la Providencia *et al.*, 2005) (Figure 1.3a, b, c). The frequency of anastomosis and, hence, potential genetic exchange, is higher within a mycelium originating from a single spore than between mycelia emanating from different spores of the same isolate. Anastomosis between different geographic isolates of the same species or between different species has not been observed, but more work is needed with a wider range of fungi. Within *G. mosseae*, genetic distance revealed by vegetative incompatibility tests (Figure 1.3b) has been confirmed by total protein profiles and ITS RFLPs (Giovannetti *et al.*, 2003), suggesting that the tests may provide an assay to detect genetic relatedness among AM fungi. The occurrence of anastomoses has significant implications for understanding the genetics of AM fungi, as well as in establishing and maintaining mycelial networks involved in plant colonization and nutrient transfers (Jakobsen, 2004).

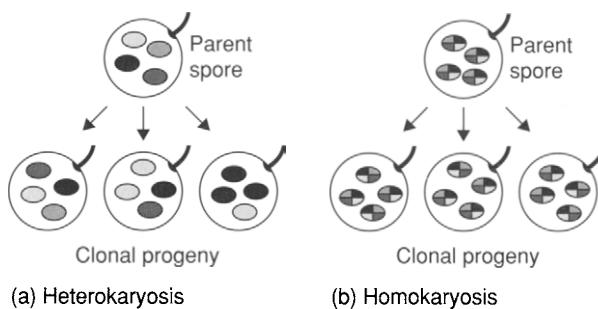


**Figure 1.3** (a) Bright field micrograph of anastomosis (arrowed) between hyphae of the external mycelium of *Glomus mosseae*. Fusion of hyphal walls has occurred and cytoplasmic continuity is evidenced by staining indicating succinate dehydrogenase activity. From Giovannetti et al. (2001), The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. *New Phytologist* **151**, 717–724, with permission. (b) Bright field micrograph illustrating an incompatible interaction (arrowhead) between hyphae of *Glomus mosseae* originating from geographically different isolates. Hyphal contents have been withdrawn and septa (arrows) formed, as evidenced by absence succinate dehydrogenase activity. From Giovannetti et al. (2003), Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical and molecular analysis. *Applied and Environmental Microbiology* **69**, 616–624, with permission of the American Society for Microbiology. (c) Epifluorescence micrograph of anastomosis between two hyphae of *Glomus mosseae* following DAPI staining. Note presence of nuclei in the middle of the fusion bridge (arrow). From Giovannetti et al. (1999), Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* **65**, 5571–5575, with permission of the American Society for Microbiology.

## Genetic organization

The genetic organization of AM fungi is not well understood, although rapid advances in unravelling long-standing problems are being made as new techniques clear the way to dealing with these 'difficult' organisms. The outline below can do no more than provide an overview of progress, acknowledging that much remains in doubt. As mentioned above, those AM fungi that have been studied are haploid; the suggestion that some may be polyploid (Pawlowska and Taylor, 2004) requires further investigation. Morphologically identifiable sexual structures have not been observed, leading to the assumption that the Glomeromycota are asexual and clonal. However, there is very high genetic diversity even in single isolates, as shown by the regular occurrence of several different ITS sequences in the nuclear rDNA of single multinucleate spores (Sanders *et al.*, 1995; Lloyd-MacGilp *et al.*, 1996; Lanfranco *et al.*, 1999; Antoniolli *et al.*, 2000; Pringle *et al.*, 2000; Rodriguez *et al.*, 2001; Jansa *et al.*, 2002b) as well as in AFLP patterns (Rosendahl and Taylor, 1997) and apparently in some genes involved in cellular function (Kuhn *et al.*, 2001; Sanders *et al.*, 2003; Pawlowska and Taylor, 2004). The way the variant sequences are arranged among the nuclei in a multinucleate spore or hypha is of considerable interest from the perspective of origin and transmission of genetic information to new generations of spores or different clonal mycelia. There are two possibilities; either the nuclei are genetically different and the organisms heterokaryotic or the nuclei are identical (homokaryotic), each containing all sequence variants (Figure 1.4).

There is evidence for both arrangements. The heterokaryotic model is supported by inheritance of spore shape in *Scutellospora pellucida* (Bever and Morton, 1999) and by the indication that nuclei in single spores of *S. castanea* contain different numbers of two divergent sequences in the ITS region of rDNA, as shown by fluorescent *in situ* hybridization (FISH) (Kuhn *et al.*, 2001). Furthermore, within-spore variation of a DNA polymerase- $\alpha$  (POL1-like sequence) in *G. etunicatum* seems unlikely to be explained by polyploidy (Hijri and Sanders, 2005). If these asexual organisms are heterokaryotic, the implication is that an assemblage of different nuclei, represent-



**Figure 1.4** Sorting of variants of a polymorphic marker in single-spore cultures of *Glomus* according to two models proposed to explain the organization of genetic variation in AM fungi. (a) Sorting under the heterokaryotic model; (b) sorting under the homokaryotic model. Variants of the polymorphic genetic marker contained in nuclei are represented by different shading. Reprinted by permission from MacMillan Publishers Ltd: Pawlowska and Taylor, *Nature* **427**, 733–737 (2004).

ing different genomes, is transmitted through the coenocytic mycelia and from one generation to the next. This would be an extremely unusual situation and hence requires critical investigation. Both Pawłowska and Taylor (2004) and Stukenbrock and Rosendahl (2005a) provide data which can only be aligned with the heterokaryotic model if frequencies of different nuclei are maintained in absolutely constant proportions during fungal development. This seems unlikely in a coenocytic and frequently anastomosing mycelium that may cover many meters (Rosendahl and Stukenbrock, 2004). Stukenbrock and Rosendahl (2005b) suggest that the conflicting evidence could be explained by differences between fungal species investigated or isolates and/or in the types of sequences (based on DNA or cDNA) used as markers to detect variation and heterokaryosis.

The genetic organization is important because the existence of multiple genomes (heterokaryosis) would be highly unusual and could pose problems for control of gene expression in the organism as a whole. The homokaryotic model requires refinement to explain the existence of marked genetic diversity in the absence of polyploidy. An understanding is relevant to determining the flow of genetic information within mycelia and between generations, the evolution of the group and also the mechanisms of control of gene expression which could be highly complex in a multigenomic organism.

If AM fungi are both ancient and asexual, then we need to ask how they avoid accumulation of large numbers of deleterious mutations (Judson and Normark, 1996). It may be that they are not asexual at all, but engage in cryptic sexual events or other processes with similar outcomes in 'cleaning up the genome', such as gene conversion or mitotic crossing over. Evidence for recombination has been sought and results indicate that it may be absent or extremely rare (Rosendahl and Taylor, 1997; Kuhn *et al.*, 2001; Stukenbrock and Rosendahl, 2005a), but more frequent recombination is indicated by the analyses of Vandenkoornhuyse *et al.* (2001) and Gandolfi *et al.* (2003). Again, differences in methodology may underlie the disparate findings. It must be stressed that identification of recombination events does not tell us how often they occur or by what mechanism, or indeed how long ago. If AM fungi are completely asexual and recombination events are rare, then it follows that mutation (and possibly heterokaryosis) provides the main bases of the variation necessary to permit adaptation to environmental change and continuing evolution. This may not be a serious problem because the pressures for change are likely to be lower in mutualistic symbioses than in parasitic interactions, for which it is argued that the host and parasite need to recombine and evolve continually to keep pace with one another (Taylor *et al.*, 1999). Indeed, Law and Lewis (1983) suggested that, in mutualistic symbioses, the endobiont (in this case the fungus) will evolve away from a sexual habit because the selection pressures will be to maintain similarity to, rather than difference from the parents. Superficially, this seems to fit with the situation in AM fungi, for which the important ecological niche for organic carbon (C) acquisition is the apoplast of the root cortex, in which homeostasis exercised by the plant will maintain reasonably constant environmental conditions. However, the idea has been criticized because it ignores the extraradical phase of the fungus that is subjected to fluctuating soil conditions; it includes the implicit assumptions that there is little diversity in the physiological outcomes of the symbiosis for either host or fungus; and it assumes that specificity in the interactions between AM fungi and their hosts is low. Any variations in these characters would lead to pressures selecting for more

advantageous partnerships and hence pressures for recombination and the evolution of sex.

Rather little attention has been paid to the relationship between the genetic diversity described above and the phenotypic diversity which has also been shown to be high both between species of AM fungi and among isolates of a single species. One investigation showed that in a group of *G. intraradices* isolates from the same site the genetic diversity was greater than phenotypic diversity in hyphal growth and spore production expressed in monoxenic root organ culture (Koch *et al.*, 2004). It will be interesting to determine whether there is real genetic redundancy in terms of function in symbiosis with whole plants growing in soil or whether the findings are a consequence of the uniform and artificial culture environment. The critical importance of phenotypic diversity in symbiotic outcomes and consequences for plant interactions in ecosystems will be discussed in later chapters. In any event, the extent of both specificity and phenotypic diversity is probably much greater than previously realized, with important consequences for pressures leading to evolution.

## Systematics and phylogeny

The difficulties of culturing AM fungi and of identifying vegetative stages of different taxa in soil or roots has meant that, until very recently, classification was based almost entirely on the development and wall structure of the spores. These can be collected from almost any soil and used to establish cultures on plant hosts ('pot cultures'), providing spores for further analysis or inoculum for experiments. Many cultures have deliberately been started from single spores to ensure that only single species were cultured. In consequence, isolates raised in this way are likely to have a much narrower genetic base than the species they are presumed to represent (Clapp *et al.*, 2002).

It was assumed until recently that AM fungi were most closely related to the Zygomycota because of their aseptate hyphae and, despite absence of typical sexual stages, this view was not challenged until the use of DNA sequences forced a re-evaluation of their relationships. The first Linnaean classification of the Endogonaceae (Zygomycota) made no attempt to relate taxonomy to the phylogeny of the group (Gerdemann and Trappe, 1974, 1975). Gerdemann and Trappe regarded their classification as a 'temporary solution to a difficult taxonomic problem', but it was important because it put the study of AM fungi on a firm basis, with descriptions of four genera, *Gigaspora*, *Acaulospora*, *Glomus* and *Sclerocystis*. The number was later increased to five, with the addition of *Scutellospora*. The classification allowed researchers to refer to the species of fungi used in their investigations, rather than using descriptive terms such as 'E3' and 'yellow vacuolate' as had been the common practice. Up to 1993, about 150 species, most widely distributed globally, had been described and, although there has been a major re-evaluation of the taxonomy and phylogeny of the group, rather few new species have been added. Considering the long evolutionary history, the paucity of species is surprising as it suggests remarkably little diversification. However, as we have seen, a single species can, in fact, encompass very high genetic and functional diversity.

The structure and development of the spores, particularly their walls, remain important components of taxonomic descriptions and phylogenetic analysis. The first approach to a classification representing phylogenetic relationships was made

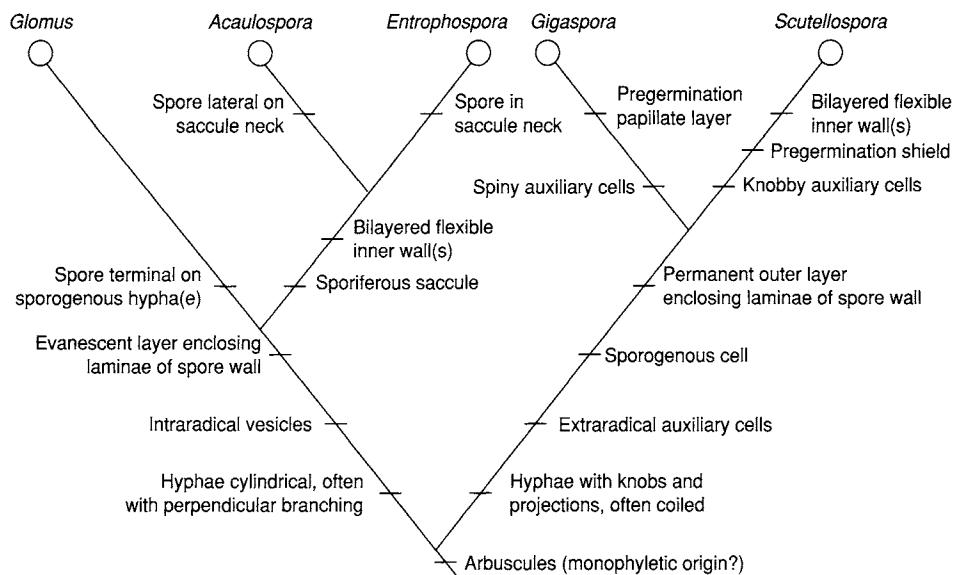
by Morton (1990a) using cladistic tools and assuming evolutionary significance for 27 characters. A new order, Glomales, was separated from the Endogonales (but still included in the Zygomycota) and defined as a monophyletic group containing only those fungi for which 'carbon is acquired obligately from their host plants via intraradical dichotomously branching arbuscules' (Morton and Benny, 1990). Although this was an attempt to define AM fungi as a separate group, it was widely recognized as a very restrictive definition because it makes a number of assumptions which have not been substantiated physiologically (Gianinazzi-Pearson *et al.*, 1991a; Smith and Smith, 1996) and it also created some practical difficulties. It was assumed that the arbuscule is a key unifying structure and that it is the sole site of C acquisition by the fungi. This is by no means certain, for there is no *a priori* reason why intercellular hyphae or intracellular coils should not be sites of organic C transfer (see Chapter 4). There are other difficulties: the definition requires that all descriptions of AM fungal species should be accompanied by evidence that the fungi can form arbuscules, which is problematic considering that, in some symbioses, hyphal coils predominate; fungi with typical spore development and vegetative morphology, but 'atypical' C transfer also exist (see Chapter 13); and the fungal partner in the unique cyanobacterial (*Nostoc*) symbiosis *Geosiphon pyriformis* is certainly related to AM fungi on the basis of DNA sequence information as well as spore morphology, but forms no structures similar to those in roots (Schüßler *et al.*, 1994; Schüßler, 2002). Setting aside these problems, the remaining characters used by Morton and Benny were based on spore characteristics that vary qualitatively and are stable and discrete. Vegetative structures (other than existence of arbuscules) were not used because of their developmental plasticity and variations within different host plants. Application of the cladistic approach to determination of evolutionary relationships yielded the phylogenetic tree shown in Figure 1.5a, which has served as a hypothesis for subsequent investigations.

The key features of the tree are the separation of the *Glomus/Sclerocystis* group from *Gigaspora/Scutellospora* and the existence of *Acaulospora/Entrophospora* as a line apparently diverging from *Glomus*. Initial DNA sequence analysis provided evidence confirming the 'glomalean' fungi as true fungi of monophyletic origin, divided into the same three families shown by the cladistic approach. Lipid analysis also supported the existence of three families (Sancholle and Dalpé, 1993), but the carbohydrates in the fungal walls suggested a less clear-cut phylogeny and, significantly, called into question the relationships of AM fungi with the Zygomycota (Gianinazzi-Pearson *et al.*, 1994b; Lemoine *et al.*, 1995).

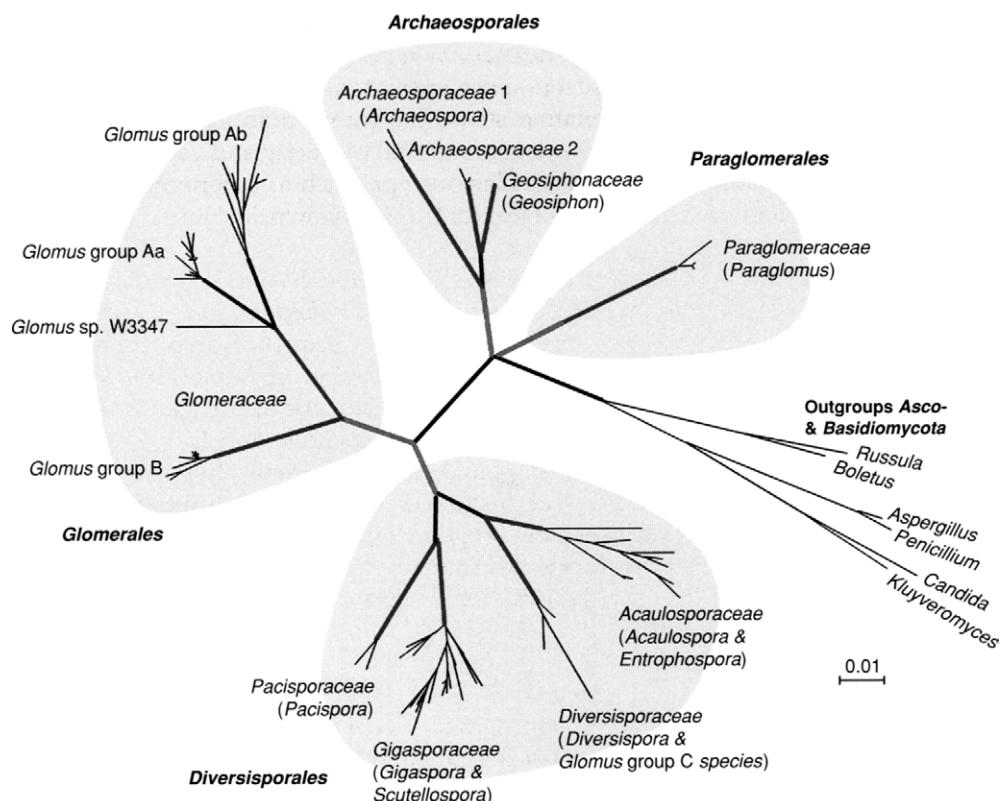
Development of molecular methods has transformed our knowledge of the evolution and phylogeny of AM fungi. Using SSU rRNA sequences, Simon *et al.* (1993)

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**Figure 1.5** Phylogenetic trees for AM fungi, derived from different types of information. (a) A cladogram showing taxonomic and phylogenetic divergence among genera of AM fungi, based on comparative developmental sequences of the spores. Courtesy Joe Morton. (b) A maximum likelihood tree based on molecular data from near full-length SSU rRNA gene sequences, which reflects the topology of different neighbour joining and parsimony bootstrap analyses, showing the phylogeny of the AM fungi (Glomeromycota). Courtesy Arthur Schüßler, modified from Schüßler (2002).



(a)

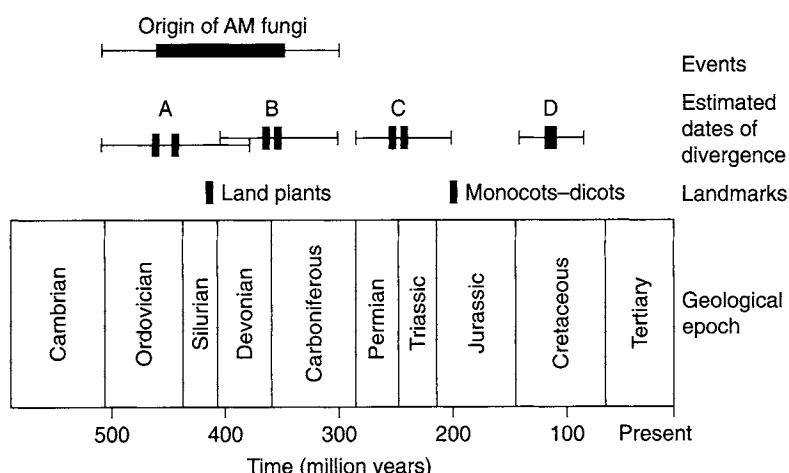


(b)

**Figure 1.5** (Caption opposite)

dated the probable origin of the group to between 460 and 350 million years ago, which appeared to accord well with the fossil record of AM colonization in vascular land plants (Figure 1.6 and see below). Reappraisal now puts the timing much earlier, at between 1400 and 1200 million years ago, and the origin of the first land plants at around 900 million years ago, but there is still some uncertainty because of the absence of fossils with which to calibrate the molecular data (Heckman *et al.*, 2001; Schüßler, 2002). In any event, the discovery that the fungus *Geosiphon pyriformis* appears to be ancestral to other Glomeromycota suggests a role for these fungi in very early plant colonization of land, via symbiosis with cyanobacteria (Gehrig *et al.*, 1996).

A major advance in phylogeny was enabled by the availability of large numbers of sequences of SSU rRNA genes of a wide range of fungi, including many members of the Zygomycota and AM fungi, as well as the very well studied basidiomycetes and ascomycetes. The new data, taken with molecular, morphological and ecological characteristics, unequivocally show that AM fungi are quite different from other fungal groups and should be separated from them in a new monophyletic clade, given the status of phylum. According to SSU sequence phylogeny, this phylum, designated as Glomeromycota, probably diverged from the same common ancestor as the Ascomycota and Basidiomycota, but is not related to the Zygomycota (Schüßler *et al.*, 2001) (see Figure 1.5b). The monophyletic status of the Glomeromycota based on SSU sequences is supported by studies of  $\beta$ -tubulin, actin and elongation factor alpha gene phylogenies, although these suggest that the sister groups may not be ascomycetes and basidiomycetes, but chytridiomycetes or zygomycetes (*Mortierella*) (Helgason *et al.*, 2003; Corradi *et al.*, 2004). The confirmation of ascomycetes and basidiomycetes as the closest living relatives of the Glomeromycota therefore remains a subject for future research.



**Figure 1.6** Estimated dates of origin and divergence of arbuscular mycorrhizal fungi. From Simon *et al.* (1993). Reprinted with permission from *Nature*, 363, 67–69. Copyright McMillan Magazines Ltd.

At a finer scale, SSU sequence phylogeny supports the existence of four groups, given the status of order by Schüßler *et al.* (2001) (see Figure 1.5b). The Glomerales (formerly Glomales) as understood by Morton and Benny (1990) is now represented by two orders, the Glomerales containing two families Glomus group A and Glomus group B, and the Diversisporales containing Acaulosporaceae, Diversisporaceae, Gigasporaceae and Gerdemanniaceae (Schüßler *et al.*, 2001; Walker *et al.*, 2004). Two more ancestral lineages are also recognized, Archaeosporales, containing Archeosporaceae and Geosiphonaceae, and Paraglomerales, currently containing a single family, the Paraglomeraceae. These taxa are well supported by the SSU sequence analysis. Of the very well known groups, the Gigasporaceae is monophyletic and contains *Gigaspora* and *Scutellospora*, but the genus *Glomus* is not monophyletic, with members distributed in at least four families, Glomus group A, Glomus group B, Diversisporaceae (Schwarzott *et al.*, 2001) and Gerdemanniaceae (Walker *et al.*, 2004).

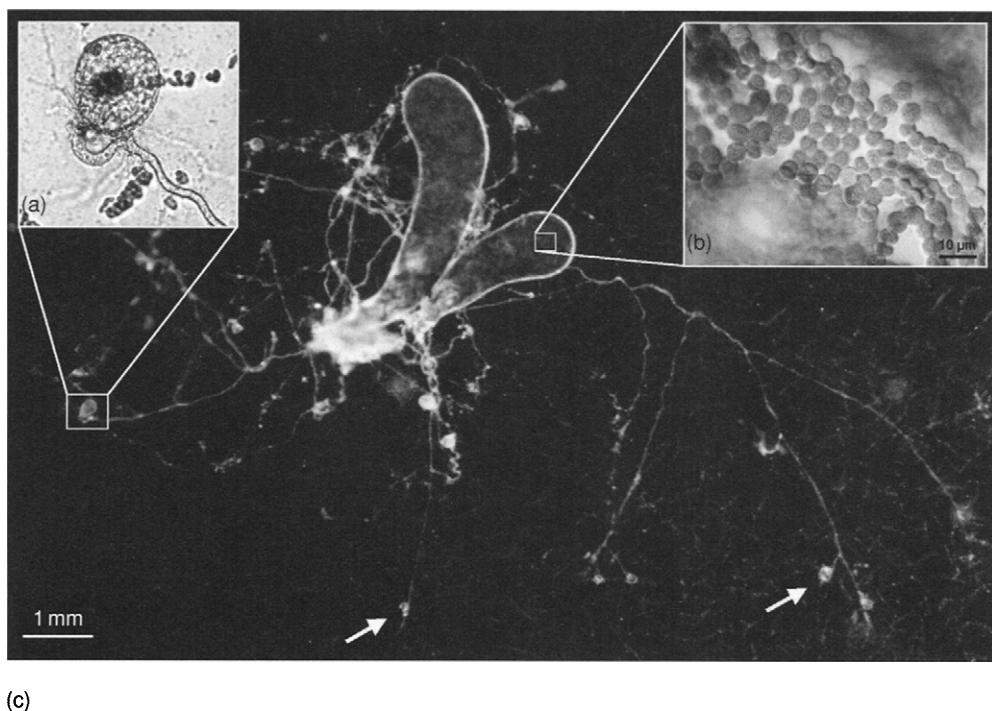
## A species concept for AM fungi

The asexual and clonal nature of members of the Glomeromycota means that the Biological Species Concept is inappropriate and they should be regarded as phenetic or form species (Morton, 1990b; Walker, 1992). The increasing availability of SSU rDNA sequences means, as we have seen, that the classification and phylogeny based on spore characteristics (Morton *et al.*, 1992) can be supplemented by sequence data, providing a species with a range of 'sequence variants' that represent its molecular diversity and 'bar codes' by which it or its isolates could be identified. This information has already proved useful in ecological investigations of the distribution of AM fungi among root systems of different species and in different environments. The low number of species (~150) in the Glomeromycota based on spore characters superficially might suggest very low diversity in the group. However, it is becoming increasingly apparent that the high genetic diversity among different isolates of one phenetic species also extends to phenotypic diversity at the levels of development, function and symbiotic performance (Hart and Reader, 2002a; Koch *et al.*, 2004; Munkvold *et al.*, 2004). The genetic control of developmental variation is highlighted by different abilities of isolates of *Glomus intraradices* (separated on the basis of rDNA sequences as well as origin) to enter roots of a reduced mycorrhizal colonization (*rmc*) mutant of tomato. Most isolates are blocked at the root epidermis, but *G. intraradices* WFVAM23 is able to develop normally in the root cortex and forms a functional symbiosis (Gao *et al.*, 2001; Poulsen *et al.*, 2005; Jansa *et al.*, unpublished). If phenotypic diversity is high within a phenetic species, then we need to question the value of the species name in terms of understanding its evolution, characterizing it phenotypically and functionally and comparing different investigations purporting to use the same fungus. A major challenge for the future is to provide a workable species concept for the Glomeromycota and the tools to permit rapid identification and description of species or subspecific variants so that names have developmental and functional relevance and predictive capacity in relation to symbiosis. In this context, the variations in ability of AM fungi to anastomose within and between isolates and species, if linked with information on sequence similarities and symbiotic function, may be an avenue worth exploring.

## The range of plants forming arbuscular mycorrhizas

The range of potential plant partners for AM fungi is extremely wide and has been responsible for the oft-quoted statement (Gerdemann, 1968) that 'the symbiosis is so ubiquitous that it is easier to list the plant families in which it is not known to occur than to compile a list of families in which it has been found'. This continues to hold good. Some members of most families of angiosperms and gymnosperms, together with sporophytes of ferns and lycopods develop arbuscular mycorrhizas. Additionally, the free-living gametophytes of pteridophytes, as well as those of some hepatic (liverworts, see Chapter 14), are often colonized by fungi now identified as members of the Glomeromycota, regardless of structural features of the colonization and photosynthetic capacity of the plant. One fungus from the Glomeromycota engulfs the cyanobacterium *Nostoc* to form the rare bladder-like symbiosis known as *Geosiphon pyriformis* (Schüßler *et al.*, 1994) (Figure 1.7; see Colour Plate 1.2).

On the other hand, some plants characteristically do not form mycorrhizas of any type and it is possible to generalize that colonization is unlikely to occur in



**Figure 1.7** *Geosiphon pyriformis*, a glomeromycotan soil fungus forming endosymbiosis with a cyanobacterium and representing an *in-vitro* culture of a mycorrhiza-like association. (a) Spore and subtending hypha of *G. pyriformis*; (b) cyanobacterial partner; (c) bladders of *G. pyriformis*, with emanating external mycelium. From Schüßler and Wolf (2005), *Geosiphon pyriformis – a glomeromycotan soil fungus forming endosymbiosis with Cyanobacteria*. In *In Vitro Culture of Mycorrhizas*. Eds S Declerck, DG Strullu, JA Fortin pp. 272–289, with kind permission of Springer Science and Business Media. See also Colour Plate 1.2.

several major families, including Chenopodiaceae, Brassicaceae, Caryophyllaceae, Polygonaceae, Juncaceae and Proteaceae. However, even in these, colonization of the roots varying from both arbuscules and vesicles to scattered hyphae and perhaps vesicles, is sometimes observed (see below). The physiology of these interactions has rarely been explored and, in consequence, it is unwise to be too categorical about the functional characteristics of the symbioses. These may well vary from parasitic utilization of organic C, through neutral interactions to typical mutualistic symbioses (see Chapters 4 and 5).

Trappe (1987) produced a most valuable compilation of the incidence of all types of mycorrhizas within the angiosperms, taken from published material. Records of arbuscular mycorrhizas are to be found in all the orders from which plants have been examined and are about equally frequent in Dicotyledonae and Monocotyledonae (Table 1.1). He stressed that only about 3% of species had actually been examined at that time and our knowledge of the mycorrhizal status of some taxa is still very poor, despite an increasing number of surveys. Nevertheless, the more we look the greater number of species turn out to be mycorrhizal, even in supposedly non-mycorrhizal families and in habitats such as wetlands, salt marshes and arid or disturbed environments, where the incidence of mycorrhizas has long been viewed as relatively unlikely (Bagyaraj *et al.*, 1979; Clayton and Bagyaraj, 1984; McGee, 1986; Khan and Belik, 1995; O'Connor *et al.*, 2001; Carvalho *et al.*, 2004). Harley and Harley (1987a, b) surveyed the literature on the incidence of

**Table 1.1** Numbers and percentages of species of subclasses and classes of Angiospermae examined for mycorrhiza formation and percentage of examined species by type of mycorrhiza.

Taxon	Per cent with mycorrhizal types						
	Total species	Species examined	Per cent examined	AM only	Other	AM+ other	NM
Division Angiospermae	223 400	6507	3	50	15	5	18
Class Dicotyledonae	173 500	5020	3	50	14	6	17
Subclass							
Magnoliidae	12 000	270	2	66	3	4	17
Hamamelidae	3400	265	8	27	44	11	6
Caryophyllidae	11 000	317	3	14	4	2	59
Dilleniidae	25 000	792	3	33	29	7	20
Rosidae	62 100	1838	3	56	16	5	12
Asteridae	60 000	1538	3	63	2	5	15
Class Monocotyledonae	49 900	1487	3	49	18	2	21
Subclass							
Alismatidae	500	26	5	4	0	0	88
Arecidae	5600	61	1	56	3	3	30
Commelinidae	15 000	826	6	55	1	2	28
Zingiberidae	3800	28	1	71	4	0	11
Liliidae	25 000	546	2	37	48	2	7

Based on Trappe, 1987. Plant classification according to Cronquist (1981). AM: arbuscular mycorrhiza; Other: mycorrhizas formed by ascomycetes and basidiomycetes; NM: non-mycorrhizal (non-host).

mycorrhizas at the species level in the very-well-studied British flora. In many families, over 40% and sometimes as high as 80% of the species had been investigated, often more than once. All families listed contained mycorrhizal species and, frequently, these constituted a very high proportion of the total. Similar trends are repeated in most surveys carried out more recently (Fitter and Moyersoen, 1996; Wang and Qiu, 2006). In many cases, species have been recorded as occurring in both mycorrhizal and non-mycorrhizal states and members of some plant families typically form mycorrhizas of types other than AM or, indeed, more than one type of mycorrhiza (Newman and Reddell, 1987; Wang and Qiu, 2006). The reasons for failure of a potentially mycorrhizal species to become colonized are many and include lack of inoculum of an appropriate fungus at the site, environmental conditions such as high nutrients, cold or waterlogging and seasonal variation in the development of the fungi in roots. Species which are sometimes, but not always, colonized are often referred to as ' facultatively mycorrhizal', to distinguish them from those ' obligately mycorrhizal'<sup>1</sup> species that are consistently colonized. It is best to avoid using these terms in relation to the extent to which plants respond to colonization, because responsiveness of a plant (whether positive or negative) is markedly influenced by the identity of the AM fungus colonizing the roots and by the environmental conditions (Chapter 4).

Arbuscular mycorrhizas are found in most herbaceous species that have been studied (see above for exceptions), but are by no means restricted to herbs. As long ago as 1897, Janse examined 46 species of tree in Java and found them all to have arbuscular mycorrhizas. More recent work, reviewed by Smits (1992), Janos (1987) and Wang and Qiu (2006), confirms the widespread occurrence of AM in trees. Even the Dipterocarpaceae, long thought to be exclusively ectomycorrhizal (Smith and Read, 1997), has recently been suggested to include as many as 75% of species that may form arbuscular mycorrhiza (Tawaraya *et al.*, 2003; Wang and Qiu, 2006), confirming the prevalence of AM symbioses in taxonomically diverse tropical forests, as well as in some temperate forest systems. Thus, Baylis (1961, 1962) stated that arbuscular mycorrhizas are ecologically the most important type of mycorrhiza in New Zealand forests and this can sometimes be true also of northern temperate broadleaf forests, even when the canopy dominants form ectomycorrhizas.

In general terms, arbuscular mycorrhizas are characteristically found in species-rich ecosystems, in contrast to ectomycorrhizas which predominate in forest ecosystems where only a few host species are present. Whereas the Pinaceae are predominantly ectomycorrhizal (ECM), all other conifer families are mainly AM, as are most other gymnosperms, all of which are woody. Although arbuscular mycorrhizas have often been ignored by foresters, they are characteristic of such valuable trees as *Acer*, *Araucaria*, *Podocarpus* and *Agathis*, as well as all the Cupressaceae, Taxodiaceae, Taxaceae, Cephalotaxaceae and the majority of tropical hardwoods. The importance of considering the appropriate mycorrhizal associates for trees used in reafforestation programmes in temperate and tropical ecosystems is now widely recognized

<sup>1</sup> We avoid the use of 'mycotrophic' which is both vague and potentially confusing. The term is used in at least two different ways: to indicate whether or not a plant becomes colonized by mycorrhizal fungi, which can be determined by microscopic investigation or molecular ecological techniques; and/or to indicate whether a plant is 'responsive' to colonization, which depends greatly on identity of the fungus involved.

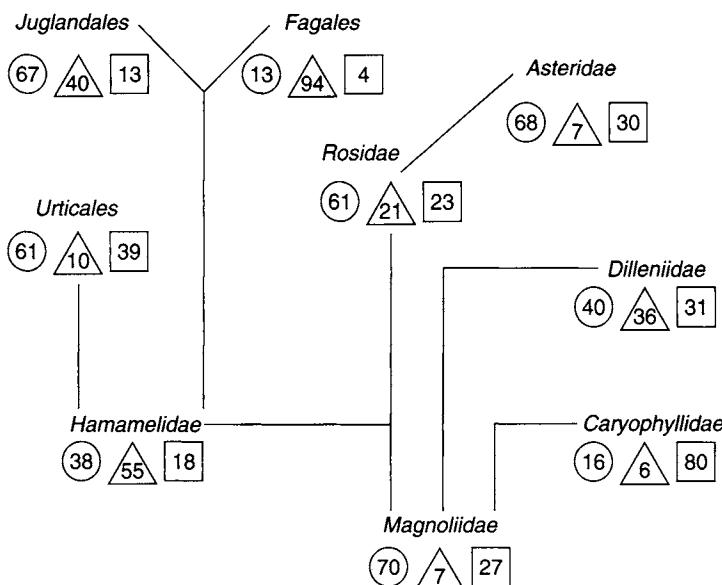
(see Chapter 17). While most of the experimental work on arbuscular mycorrhizas has been done with herbs, some trees have also been used and include *Malus* (apple), *Citrus*, *Salix*, *Populus*, *Persea* (avocado), *Coffea*, *Araucaria*, *Khaya*, *Anacardium* (cashew), *Liquidambar*, *Acacia* and many others. It is certainly important to realize that arbuscular mycorrhizas may be significant in nutrient absorption and in nutrient cycling of woody species. Therefore, work with trees and other perennials, as well as in associated understorey plants, is very important both from an ecological point of view and from a need to consider forest and crop production. Indeed, although work with herbs allows greater control of conditions in growth rooms etc., the propagation of some woody species from cuttings may have great advantages in providing genetically uniform experimental material which may partly offset the lengthy growth periods necessary for the study of long-lived plants. The extensive work on *Citrus* mycorrhiza, by groups in California and Florida, exemplifies the experimental use of an economically important tree species with a view both to increase production and to understand the development and physiology of the AM symbiosis (Menge *et al.*, 1982; Graham and Syvertsen, 1985; Graham, 1986; Eissenstat *et al.*, 1993; Graham *et al.*, 1997). It is to be expected that the genome mapping project in *Populus*, which forms both ecto- and arbuscular mycorrhizas (Martin *et al.*, 2004; Strauss and Martin, 2004), will provide a rich avenue for future exploration of AM symbioses with woody plants.

Strong emphasis has been placed on angiosperms and gymnosperms largely because of their significance in ecosystems and in primary production, but AM symbioses are also characteristic of fern sporophytes, as was well recognized by Boullard (1951, 1958) and later by many others who have examined the group or included them in more general surveys (Cooper, 1976). Sporophytes of Lycopodiaceae and Psilotaceae are now known to be AM and, although information on gametophytes is more scanty, those of most of these families, plus many thalloid liverworts are also characteristically AM (Read *et al.*, 2000; Winther and Friedman, 2007). The gametophyte stages provide a fascinating area for study because some are achlorophyllous and hence require an external source of organic C, possibly supplied by their AM fungal symbionts. Accordingly, these symbioses may have carbohydrate physiology in which the fungus supplies the plant with organic C (see Chapters 13 and 14).

Recent surveys worldwide have greatly increased the number of species known to form more than one kind of mycorrhiza (Wang and Qiu, 2006). Indeed, it is possible that, due to their ancient origin and significance during terrestrial plant evolution, AM fungi may have the ability to invade the underground organs of almost all land plants. It follows that failure of a plant to form arbuscular mycorrhizas or to form another type is likely to be a derived or secondary character and all plant genomes may carry evolutionary footprints of present or former AM status. Evidence from the phylogeny of host plants and the fossil record supports this conclusion (see below).

## Phylogenetic relations of arbuscular mycorrhizal plants

The first significant attempt to examine the mycorrhizal status of plants in relation to their phylogeny was made by Trappe (1987) who, using only those angiosperm taxa for which the mycorrhizal status was known in at least 10% of the species and



**Figure 1.8** Phylogenetic dendrogram for the subclasses of Dicotyledonae, with percentage of species with arbuscular mycorrhizas (circles), asco- and basidiomycetous (ericoid, and ecto) mycorrhizas (squares), or no mycorrhizas (triangles). Many species have mycorrhizas in more than one category, so that percentages total more than 100. From Trappe (1987) in *Ecophysiology of VA Mycorrhizal Plants*, ed. GR Safir. Copyright CRC Press, Boca Raton, Florida.

the phylogenetic classifications of Cronquist (1981), prepared dendograms which allow some preliminary evolutionary conclusions to be drawn (Figure 1.8).

More recent studies (Fitter and Moyersoen, 1996; Brundrett, 2002; Wang and Qiu, 2006), using the phylogenetic classification system of angiosperms based on molecular sequence data (Soltis *et al.*, 1992; Stevens, 2004) have come to very similar conclusions. Using reports from the literature, Wang and Qui surveyed 3617 species from 263 families for mycorrhizal status of any type. They show that, among land plants as a whole, 80% of species and 92% of families potentially form at least one type of mycorrhiza. For angiosperms, the figures are 84 and 94%, closely similar to numbers reported by Trappe (1987) (Tables 1.1 and 1.2). Arbuscular mycorrhizas appear as the ancestral type, occurring in the vast majority of land plant species and, very frequently, in all the early diverging lineages of the major clades. Mycorrhizal types other than AM and non-mycorrhizal species appear in lineages of more recent origin. Orchid mycorrhizas appear to have evolved only once, but ecto- and ericoid mycorrhizas probably had independent origins in many unrelated plant lineages. The same is true of both mycoheterotrophs (achlorophyllous plants in symbiosis with various fungal associates including fungi in the Glomeromycota, see Chapter 13) and non-mycorrhizal plants.

## Non-mycorrhizal plants

Supposedly completely non-mycorrhizal plant families appear among mosses, ferns and many families of angiosperms distantly related to each other. Additionally,

**Table I.2** Mycorrhizal status of four major groups of land plants, indicating numbers and percentages of the families and species that have always (obligate), sometimes (facultative) and never (non-mycorrhizal) been observed to form mycorrhizas.

Group	Number of families/species surveyed	Number (percentage) of mycorrhizal families	Number (percentage) of obligate mycorrhizal species	Number (percentage) of facultative mycorrhizal species	Number (percentage) of non-mycorrhizal families	Number (percentage) of non-mycorrhizal species
Bryophyta	28/143	20 (71)	60 (42)	6 (4)	8 (29)	77 (54)
Pteridophyta	28/426	26 (93)	185 (43)	39 (9)	2 (7)	202 (47)
Gymnospermae	12/84	12 (100)	83 (99)	1 (1)	0 (0)	0 (0)
Angiospermae	195/2964	184 (94)	2141 (72)	396 (3)	11 (60)	427 (14)
Total	263/3617	242 (92)	2469 (68)	442 (12)	21 (8)	706 (20)

Modified from Wang and Qiu (2006). For more detailed information see the original publication. Angiosperm classification according to Soltis *et al.* (1992).

some families have both mycorrhizal and non-mycorrhizal members and, even in predominantly non-mycorrhizal groups, some mycorrhizal species do occur. As these groups include the Brassicaceae, Caryophyllaceae, Proteaceae and Cyperaceae, all traditionally thought of as wholly non-mycorrhizal, it is clear once again that we must not be too dogmatic about the mycorrhizal status of a species or group that has not actually been examined.

The independent (polyphyletic) origin of the non-mycorrhizal condition in different plant lineages suggests that the condition is secondary and that the cellular and physiological bases for it may be quite diverse. Although families containing large numbers of species in which mycorrhizal colonization is characteristically absent are relatively rare, they are worth studying in their own right, with respect to environmental conditions that may have led to loss of the symbiosis, mechanisms by which the fungi are excluded, as well as the means by which the plants acquire nutrients from soil and compete effectively with mycorrhizal plants in ecological situations (see Chapters 3, 15 and 16) (Lamont, 1981, 1982; Pate, 1994; Marschner, 1995; Lambers *et al.*, 1998). It is thought that several factors may have led to loss of mycorrhizal status, including adaptation to aquatic habitats and growth in nutrient rich, extremely nutrient poor or disturbed environments. These plants often appear to have evolved compensatory mechanisms of effective nutrient uptake, such as fine roots with well developed root hairs, proteoid and dauciform roots and exudates that increase the solubility of P in the soil. However, as noted above, complete absence of mycorrhizas from either particular taxonomic or ecological groups of plants is not observed.

## Fossil history of arbuscular mycorrhizas

Phylogenetic arguments about mycorrhizal origins are supported by the fossil record. The long fossil history of both spores of AM fungi and colonized absorbing structures is well recognized. Fossils resembling the spores of AM fungi date from as early as the Silurian and Ordovician (440–410 million years ago) (Redecker *et al.*, 2000) and *Glomus*-like spores are also common within plant axes or decaying plant material from the famous and beautifully preserved Rynie chert flora (Kidston and Lang, 1921), which is approximately 400 million years old. This flora includes some of the earliest terrestrial tracheophyte fossils. Recent re-examination has revealed arbuscules, vesicles and intercellular hyphae within the protosteles of sporophytes of several fossil species including *Aglaeophytton major*, as well as a distinct tissue layer containing arbuscules in the gametophytes of the same plant (*Leonophytton rhynensis*), leaving no doubt that arbuscular mycorrhizas had evolved by that time (Remy *et al.*, 1994; Taylor *et al.*, 2004, 2005) (Figure 1.9a). The presence of AM structures in these early tracheophytes agrees with the supposition that arbuscular mycorrhizas were the first of all mycorrhizal types to evolve and that the AM condition is ancestral for land plants. Even earlier associations of Glomeromycota with plants are suggested by the cyanobacterial symbiosis *Geosiphon* and by the AM status of many present-day liverworts. These supposed links with early colonizers of land have led to the assumption that AM symbioses have continued in an unbroken line from the earliest, pre-tracheophyte land flora and that ascomycete or basidiomycete symbioses with modern liverworts came later. Some doubt has been cast on this

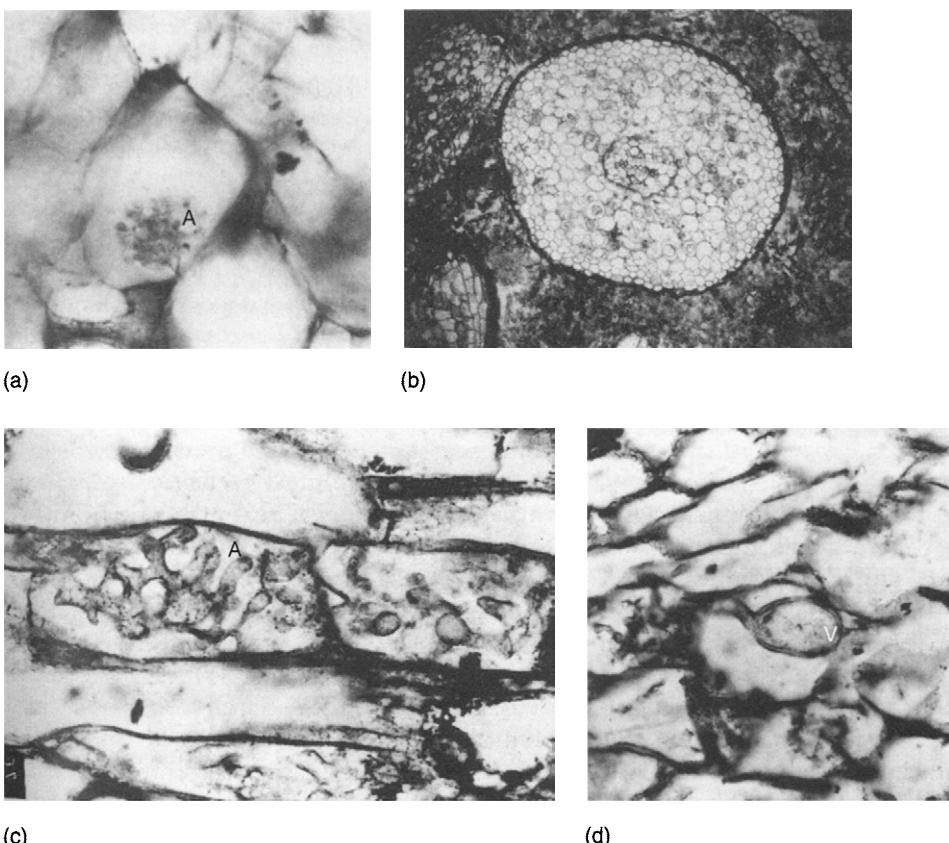
simple view, based on structural features of colonization and the association of some modern liverworts with members of the relatively recently evolved Glomus group A (Selosse, 2005; see Chapter 14).

The significance of the fungal associates of the sporophyte fossils was noted by Kidston and Lang themselves (1921) and by Nicolson (1975). Subsequently Pirozynski and Dalpé (1989) provided a critical review of the geological history of the fungi. This shows continuous occurrence of *Glomus*-like structures into the Quaternary period and the occurrence of *Glomus*-like spores from silicified peat from the Triassic deposits in the Antarctic (Stubblefield *et al.*, 1987a, 1987b). Unfortunately, no reliable fossils of other genera in the Glomeromycota have been found which would shed more light on phylogenetic relationships within the group.

The fossil record of symbiotic organs in later Carboniferous deposits has revealed many gymnosperm fossils with arbuscular mycorrhizas. The best known and preserved is *Amyelon radicans*, which again resembled the arbuscular mycorrhizas of living gymnosperms (Nicolson, 1975). The Triassic flora from Antarctica (250–210 million years ago) has also yielded important evidence for the development of intraradical vegetative structures, including intercellular hyphae and arbuscules and well-developed intracellular coils (Stubblefield *et al.*, 1987a, 1987b; Phipps and Taylor, 1996). Beautifully preserved roots of *Antarcticycas*, containing both septate and aseptate hyphae and structures strongly resembling mycorrhizal arbuscules, vesicles and spores have been described by Stubblefield *et al.* (1987a). Sections and peels of these fossils are virtually indistinguishable from present-day mycorrhizal cycad roots (Figure 1.9b, c, d).

The emphasis has been on identifying the presence of arbuscules as easily recognized and supposedly defining features of arbuscular mycorrhizas and some variation in arbuscular structure has been noted. Those of *Aglaeophyton* are delicate structures with secondary branches of 1–2 µm diameter, similar to present-day arbuscules in *Arum*-type mycorrhizas (see Chapter 2), but different from the better preserved arbuscules found in *Antarcticycas* from the Triassic (compare Figures 1.1a and 1.9a and c). With the exception of the work of Phipps and Taylor (1996) on cycads, little attention has been given to identifying the diversity of structures, including coils, which are increasingly recognized as providing important intracellular interfaces between AM fungi and their hosts (*Paris*-type mycorrhizas) in modern plants, including the mycotrophic gametophytes of some hepaticas and pteridophytes (see Chapters 2 and 14). Coils are of course harder to diagnose as 'AM', but would provide a significant avenue of research into structural diversity in the early land flora and might provide some evolutionary insights into development of different morphological types.

We cannot, of course, be sure about the physiology of these fossil arbuscular mycorrhizas but, if they functioned in a manner similar to present-day forms, their role in colonization of the land and in subsequent plant evolution is likely to have been considerable. The early land plant sporophytes did not have roots and their underground protosteles were poorly developed with respect to accessing nutrients from soil. The gametophytes had slightly swollen basal protocorms bearing rhizoids which, again, would have had limited access to the substratum. The soils colonized by early land plants were likely to have been poorly developed and deficient in available mineral nutrients. In consequence, it is likely that mutualistic symbioses with fungi in the Glomeromycota were important to the success of the autotrophic plants invading the terrestrial environment (Baylis, 1972; Nicolson, 1975; Pirozynski and Malloch, 1975).



**Figure 1.9** Fossil arbuscular mycorrhizas. (a) Arbuscule (A) in a cell of *Aglaeophyton* from the Devonian flora of the Rhynie Chert. From Remy et al. (1994). Copyright, National Academy of Sciences. (b) Transverse section of a mycorrhizal root of *Antarcticycas*, from the Triassic deposits of Antarctica. Note the colonized central cortex of the root. (c) and (d) Details of colonization in *Antarcticycas*. (c) Dichotomously branched arbuscule (A), with relatively robust branches; (d) vesicle (V). (b), (c), (d) from Stubblefield et al. (1987a; 1987b), with permission.

## Fungus–plant specificity

So far, we have confined the discussion to the potential of different taxa of plants to form arbuscular mycorrhizas in field or experimental conditions without concerning ourselves greatly with questions about whether a species is always AM, how extensively the roots are colonized, or how far the plant may be dependent on the AM fungal symbionts for growth or reproductive success. These are complex issues which are important in discussions of cellular interactions and plant–fungus specificity and compatibility, as well as of ecology. Here we consider the range of potential partners that are available for plant and fungal symbionts. This aspect of specificity needs to be considered at both taxonomic and ecological levels. Taxonomic specificity indicates whether a given species of fungus can form an AM relationship with more than one species of plant or whether a given species of plant associates with more than one species of fungus. This can be extended to lower taxonomic levels, where subspecific genetic strains or isolates of a fungus may form AM attuned in

some way to the species or subspecific genetic strains of the plant. Molecular tools will enable this issue to be explored.

The early investigators Magrou (1936), Stahl (1949) and Gerdemann (1955) reached the conclusion that there is no absolute specificity between taxa of AM fungi and taxa of potential host plants and until recently almost all research continued to support this view. Specificity appeared to be very low or absent at the level of species, but to be exerted at higher taxonomic levels, with generalizations possible about lack of mycorrhiza formation or the type of mycorrhiza formed in particular families, as discussed above. There is still agreement that much specificity is not qualitatively absolute, that it may be generally low at the level of currently described species and that it encompasses a continuum of variations in extent of colonization or effects on host plants.

Many observations support the existence of low or very low specificity and hence wide choice of partners in many plant-fungus combinations. The low number of AM fungal species (~150, based on current species concept) compared with the very large number of potential host species (perhaps 200 000 or 80–90% of terrestrial plants), means that each fungal species theoretically must have many hosts. Field observations (using both conventional and molecular detection methods) indicate that a single plant root system can contain many AM fungi and that different plant species at the same site often contain the same fungi or fungal sequence variants.  $^{14}\text{C}$  can be transferred between roots of different plant species, indicating that the same fungus colonizes both. Finally, a very large number of experiments with a wide range of plant and fungal species have shown that an AM fungus isolated from one species of host plant can be expected, with reasonable confidence, to colonize any other species which has been shown to be capable of forming arbuscular mycorrhizas. Furthermore, it has been argued that AM fungi (like many soil-inhabiting fungi) have relatively ineffective dispersal mechanisms and will benefit from low specificity, enabling them to access organic C from a wide range of plant species. Additionally, mutualistic symbioses, like arbuscular mycorrhizas, are not expected to show the same specificity as pathogenic symbioses (Vanderplank, 1978; Law and Lewis, 1983; Smith and Douglas, 1987; Douglas, 1998). As Vanderplank said: 'Mutations to resistance in mycorrhizal plants are eliminated by selection because they are disadvantageous; and the elimination also eliminates a major source of specificity'.

However, these arguments for limited or low specificity can be questioned (Fitter, 2001). First, as we have seen, the species concept for AM fungi is inadequate and described species show remarkable intraspecific variation. A change in species concept to take this functional diversity into account could well increase the number of species and hence increase possibilities for specificity, although it is unlikely that the number of fungi would reach that of potential plant partners. Second, most fungi used in experimental tests are raised in pot cultures and, in consequence, have been selected for tolerance to disturbance and rapid colonization of many species of experimental plant; they are likely to be generalists, pre-selected for lack of specificity. Many fungi known and described from spores isolated from soil or detected as sequences in roots have never been successfully raised in pot culture, possibly because the appropriate host plant species or environmental requirements have not been met. Examples include *Entrophospora infrequens* from many sites (Rodriguez *et al.*, 2001) and *Scutellospora dipurpureascens* from Pretty Wood (a deciduous woodland in the UK) (Helgason *et al.*, 2002), although isolates of the latter fungus have been obtained in culture from other sites. Third, the arguments of Law and Lewis and of Vanderplank, fail to take into account diversity in symbiotic response both of fungi

and plants, which could exert selection pressures leading to a narrowed choice of effective symbiotic partner. Douglas (1998) argues, however, that variation in benefit as a result of environmental heterogeneity, as well as unpredictable availability of the 'best' symbiont would counter selection pressures for high specificity. Recently, Kiers and van der Heijden (2006) have outlined four hypotheses put forward to explain the long term stability and evolutionary cooperation between AM fungi and their plant symbionts. They conclude, not surprisingly, that exchange of 'surplus resources' is of key importance. They consider that such exchange is 'enforced through sanctions by one or both parties. Importantly, such sanctions and the mechanisms by which they operate are as yet speculative.

Setting these arguments aside, we need to ask if there is direct evidence for any specific interactions between particular plant species and particular AM fungal species. Genotype-dependent variations in the extent of colonization have been observed in a number of species (Smith *et al.*, 1992; Peterson and Bradbury, 1995), but caution needs to be exercised in relating this to specificity, because quantitative variations in percent of the root length colonized are a function not only of the ability of a fungus to enter and spread in a root, but also of the rate of root growth which may itself be cultivar dependent. Furthermore, both fungal spread and root growth may be strongly influenced by environmental factors such as nutrient availability, temperature and light (see Chapter 2). In any event, per cent root length colonized is not a good predictor of 'benefit' likely to accrue to the plants.

Tighter specificity has been observed in a number of investigations. The ability of three legume species, *Medicago sativa*, *Hedysarum coronarium* and *Onobrychis viciaefolia*, to be colonized by four *Glomus* species was tested (Giovannetti and Hepper, 1985). Using two soils of different phosphorus (P) availability, the results showed that whereas *M. sativa* was extensively (though variably) colonized by all four fungi, there were considerable differences in colonization of the other two plant species. *H. coronatum* showed the most striking differences, being colonized to the same extent as *M. sativa* by *G. mosseae*, but scarcely or not at all by *G. caledonium* or by one of the isolates of *G. fasciculatum*. *H. coronarium* is certainly not a 'non-mycorrhizal' plant, but there is clearly some degree of selectivity in its receptiveness to different fungi, which has not been further investigated. Similarly, Douds *et al.* (1998) showed that, in soil-grown plants, *M. sativa* was a poor symbiont with *Gigaspora margarita* but formed developmentally and functionally normal mycorrhizas with *G. intraradices*. In monoxenic cultures, there was evidence of a hypersensitive reaction of *M. sativa* roots to *Gi. margarita* and a complete failure of the fungus to colonize, whereas colonization was again normal with *G. intraradices*. More recently, Helgason *et al.* (2002) studied the colonization of several woodland species, using a combination of conventional staining and molecular detection methods. The canopy dominant *Acer pseudoplatanus* was only colonized by *G. hoi* (to which it responded positively in a pot experiment in terms of growth and P uptake), but not by other fungi from the site, including *Glomus* sp. UY1225, *Acaulospora trappei* and *Scutellospora dipurpureescens*. *Acer* therefore appeared to show selectivity in choice of fungal partner, but all the fungi appeared to have wide host ranges among the herbaceous understorey plants. Similarly, Vandenkoornhuyse *et al.* (2003) studied the occurrence of 24 groups of related fungal sequences (called by them phylotypes) in the roots of co-occurring grassland species *Trifolium repens* and *Agrostis capillaris* and found that many of them did not occur in common, although others did.

Some of the most extreme examples of specificity are provided by plant mutants or genotypes of otherwise highly AM species. These have been identified in a number of plants, particularly among non-nodulating genotypes of legumes as well as in tomato (Peterson and Guinel, 2000; Marsh and Schultze, 2001). In the majority, colonization is blocked at the root epidermis or in the hypodermal (exodermal) cell layer, but in a few cases it proceeds further, resulting in formation of intercellular hyphae or even abnormal arbuscules in cortical cells. In each case, a recessive mutation in a single gene is enough to inhibit symbiotic development (see Chapter 3). In most instances, the mutations induce the same modified developmental pattern with all fungal species tested. However, the *rmc* mutation in tomato (Barker *et al.*, 1998a), is perceived differently by different AM fungal species. Some fungi are blocked at the epidermis, others are able to penetrate as far as the exodermis and one (a variant of *Glomus intraradices*) is capable of functional colonization of the cortex (Gao *et al.*, 2001; Poulsen *et al.*, 2005). Thus, the recessive *rmc* mutation allows AM fungi to express specificity, whereas the dominant *Rmc* allele does not. These findings show that the fungal genome is important in 'cross-talk' leading to colonization and also how specificity could evolve and plants become apparently 'selective' towards their fungal partners. As long as the specific symbioses provide benefits to both partners they would be subject to positive selection and hence persist in evolutionary terms. It will be of interest to extend the analysis of genes identified by mutation to other species where host specificity has been found, to gain a better understanding of the signalling and cellular processes that underlie these interactions.

Additional examples of extreme plant–fungus specificity have been identified recently in those mycoheterotrophic symbioses that involve fungi from the Glomeromycota. *Arachnites*, a monocotyledonous species in the Corsiaceae and *Voyria* and *Voyriella*, both dicotyledons in the Gentianaceae, all associate with limited but somewhat different ranges of fungal symbionts (Bidartondo *et al.*, 2002). This fits the emerging pattern of narrower specificity in mycoheterotrophic plants forming different types of mycorrhiza, compared with autotrophic relatives (Bidartondo, 2005). It may be that only a few fungal species or sequence variants have proved amenable to involvement in the necessary tripartite symbiosis between a mycoheterotroph and an autotrophic host, which would necessitate alterations in the flow of organic C (see Chapters 4 and 13).

There are relatively few known examples of AM fungi with very narrow ranges of potential symbionts among autotrophic plants, indicating that there may well be considerable selective advantages for them to keep their options open with respect to plants which provide suitable sources of organic C. Fungal host range may, nevertheless, occasionally be restricted for, in a survey of 19 species of host plant, *Glomus gerdemanni* formed arbuscular mycorrhizas only with *Eupatorium odoratum* (Graw *et al.*, 1979). Additionally, there is much indirect evidence from use of different plant species to trap AM fungi from field soil that plant identity has a strong influence on the sporulation of different fungi (Bever *et al.*, 1996; Jansa *et al.*, 2002a) and hence on one attribute related to fungal fitness.

These examples highlight the fact that specificity or selectivity in AM symbioses may well be somewhat higher than previously believed. It will be important for investigators to remain open to this possibility, focusing on subspecific sequence variants of fungal species in relation to their abilities to form symbioses with different plant species. It seems likely that a wide range of strategies will be revealed,

ranging from the very tight specificity as exemplified by the mycoheterotrophs, to complete generalists among both plants and fungi. Generalists most certainly do exist, as shown by the fact that numerous research groups use one or a few species of plant on which to maintain pot cultures of a large number of fungal species. For example *Plantago lanceolata*, *Trifolium subterraneum* and *Sorghum sudanense* are used widely for the maintenance of pot cultures and become extensively colonized by a wide variety of fungi in the Glomeromycota, as shown in Table 1.3. The selective advantages of the different strategies and their consequences for plant and fungal interactions in ecosystems will be a fascinating area for future research.

## Ecological considerations

Harley and Smith (1983) suggested that specificity might be closer under natural conditions than in artificial one-to-one tests of colonizing ability in pot experiments and they termed this 'ecological specificity'. There certainly are plant species that in the field may sometimes form arbuscular mycorrhizas and at other times not; the facultatively AM species referred to above. In many cases, the reasons for sporadic colonization have not been examined, but may be related to availability of inoculum, particularly in disturbed environments, as well as environmental conditions. The complexities of field environments, with simultaneous challenge of roots by many different potential AM colonizers, may also lead to quantitative variations in the extent of colonization by different fungi that would not be observed in simple one-to-one tests. Using only the broad distinction between colonization caused by the 'fine endophyte' (*Glomus tenuis*) and those caused by AM fungi with wider diameter hyphae, McGonigle and Fitter (1990) demonstrated that *Holcus lanatus* was apparently highly receptive to *G. tenuis*, which contributed over 60% of the colonized length, regardless of season. In contrast, the same fungus contributed 10% or less of the colonized length in *Ranunculus acris* and *Plantago lanceolata*, although the total percentages of the root length colonized were the same or higher than *Holcus*. In the same pasture, *Phleum pratense* was only slightly colonized by any AM fungi. Seasonal and site-related differences in the fungi colonizing woodland plants have been observed using molecular detection methods (Clapp *et al.*, 2002). Whether these cases represent variations in absolute specificity of the symbiosis is doubtful. They may equally result from variations among the fungi with respect to relative rates of colonization and competition with each other (Wilson, 1984; Vierheilig, 2004a; Jansa *et al.*, 2007), as well as variations in response to environmental variables (Chapter 2). What is important to discover is which fungi, or combinations of fungi, colonize the roots of which plants in the field and whether the symbioses thus formed are functionally similar with respect to bidirectional transfer of commodities (for example P for C) and fitness of the partners. The first question is beginning to be addressed as molecular methods of detection are developed. The second question is still very hard to answer in field-based investigations, but it is abundantly clear from pot experiments that different plant-fungus combinations exhibit enormous diversity with respect to symbiotic outcomes, not only between species but also among genotypes of plants and isolates or sequence variants of the fungi (Streitwolf-Engel *et al.*, 1997; van der Heijden *et al.*, 1998a, 1998b; Munkvold *et al.*, 2004; Smith SE *et al.*, 2004a).

**Table I.3** Species of fungi from the Glomeromycota confirmed to form arbuscular mycorrhizas with *Plantago lanceolata*, *Zea mays* and *Sorghum sudanense*. The lists are restricted to species of fungi that have been unequivocally identified; numerous other fungal species for which either the nomenclature or identification are uncertain are also associated with these plants. Gaps in the table do not imply that any species of fungus will not form an association with any particular species of plant.

Plant species	Fungal species				
	Scutellospora	Gigaspora	Acaulospora	Entrophospora	Glomus
<i>P. lanceolata</i>	<i>S. calospora</i>	<i>Gi. candida</i>	<i>A. delicata</i>		<i>G. clarum</i>
	<i>S. castanea</i>	<i>Gi. margarita</i>	<i>A. laevis</i>		<i>G. coronatum</i>
		<i>Gi. rosea</i>	<i>A. longula</i>		<i>G. dimorphicum</i>
			<i>A. scrobiculata</i>		<i>G. fasciculatum</i>
			<i>A. spinosa</i>		<i>G. fistulosum</i>
<i>Z. mays</i>			<i>A. trappei</i>		<i>G. flavisporum</i>
	<i>S. aurigloba</i>	<i>Gi. candida</i>			<i>G. geosporum</i>
	<i>S. scutata</i>	<i>Gi. margarita</i>			<i>G. intraradices</i>
					<i>G. mosseae</i>
					<i>G. occultum</i>
<i>S. sudanense</i>	<i>S. calospora</i>	<i>Gi. albida</i>	<i>A. delicata</i>	<i>E. colombiana</i>	<i>G. aggregatum</i>
	<i>S. coralloidea</i>	<i>Gi. descipiens</i>	<i>A. dilatata</i>	<i>E. infrequens</i>	<i>G. caledonium</i>
	<i>S. dipurpureascens</i>	<i>Gi. gigantea</i>	<i>A. gerdemannii</i>		<i>G. claroideum</i>
	<i>S. erythropa</i>	<i>Gi. margarita</i>	<i>A. lacunosa</i>		<i>G. clarum</i>
	<i>S. fulgida</i>	<i>Gi. rosea</i>	<i>A. laevis</i>		<i>G. clavispora</i>
	<i>S. gregaria</i>		<i>A. longula</i>		<i>G. constrictum</i>
	<i>S. heterogama</i>		<i>A. mellea</i>		<i>G. deserticola</i>
	<i>S. pellucida</i>		<i>A. morrowiae</i>		<i>G. diaphanum</i>
	<i>S. persica</i>		<i>A. scrobiculata</i>		<i>G. etunicatum</i>
	<i>S. reticulata</i>		<i>A. spinosa</i>		<i>G. fasciculatum</i>
	<i>S. scutata</i>		<i>A. trappei</i>		<i>G. fistulosum</i>
	<i>S. verrucosa</i>				<i>G. fragilistratum</i>
					<i>G. geosporum</i>
					<i>G. intraradices</i>
					<i>G. invermaium</i>
					<i>G. lamellosum</i>
					<i>G. leptotichum</i>
					<i>G. manihotis</i>
					<i>G. mosseae</i>
					<i>G. occultum</i>

Data of C.Walker and J.B. Morton.

Why are these questions important? If there is no absolute specificity, then the roots of all plants at a site may be linked together not simply in one common mycelial network (CMN) but by several separate mycelia, one for each fungal species. Links between different species of plant have been proved by direct observation (Newman *et al.*, 1994) and by transfer of  $^{14}\text{C}$  between their roots (Grime *et al.*, 1987; Robinson and Fitter, 1999). They are also implied (but not proven to exist) by the presence of the same sequence variants within the roots of adjacent species. If some combinations of plants and fungi are absolutely specific, they essentially 'opt out' of networks that involve other species. As we have seen, there is little evidence for such a completely restrictive pattern. However, there is evidence that some plant species limit their access to only one network. This would be the case of *Acer* in Pretty Wood (see above), which apparently only associates with *Glomus hoi* and would only be connected to other plants at the site via the *G. hoi* network. What selective advantages this might have remains to be investigated, but might include restricting support in terms of C to the network formed by the fungal species that provides the greatest benefit (Helgason *et al.*, 2002).

The complexities are clearly enormous and the difficulties of experimentation, particularly in the field, extremely challenging. Up to ~30 AM fungal species have been found at one undisturbed field site, together with 50 plant species (Bever *et al.*, 2001). Although disturbed agricultural sites may have a single host species and much smaller AM fungal populations (Helgason *et al.*, 1998; Boddington and Dodd, 2000), this is not invariably the case (Johnson, 1993; Jansa *et al.*, 2002a). In consequence, multiple symbioses are likely, with the potential both for functional redundancy and/or complementarity.

Despite the complexity, it is encouraging that several different groups are beginning to address the issues both at theoretical and experimental levels in both temperate and tropical environments (Bever, 1999, 2002a, 2002b; Fitter, 2001; Bever *et al.*, 2002; Sanders, 2002; Kiers and van der Heijden, 2006).

Plants need to be adaptable, responding to local environments including the local availability of symbionts. A plant may be better off associating with an 'inferior' mutualist rather than none at all. In any event, a fungus that is inferior under one set of conditions may perform well under others, so that symbionts that always 'cheat' their partners may well not exist (see Johnson *et al.*, 1997). The advantages of maintaining flexible options in highly diverse ecosystems may well outweigh the apparent advantages of evolving specific and hence restrictive 'best friend' partnerships.

## Conclusions

Arbuscular mycorrhizas are formed by members of all phyla of land plants and this mycorrhizal type is characteristic of highly diverse ecosystems, containing many potential hosts. As currently described, the fungal symbionts appear to fall into only about 150 species in relatively few genera in the order Glomeromycota. The group is monophyletic and of very ancient origin, as indicated by the fossil record and by DNA sequences of living members of the Glomeromycota. The genetic organization of the fungi is the subject of considerable interest and experimentation which should soon lead to clarification of major uncertainties, such as the existence of recombination and heterokaryosis. Fungal isolates assigned to the same species show considerable

functional as well as genetic diversity, so that current taxonomy does not necessarily have predictive value with respect to symbiotic performance. The limitations of current taxonomy also pose problems with respect to determining whether symbioses are specific or not. The symbioses are certainly ancient, probably evolved only once and most likely played an important role in colonization of the land by plants.

The number of species of present-day plants forming arbuscular mycorrhizas is very large and their diversity is considerable, not only in taxonomic position but also in life form and geographical distribution. Nearly all herbaceous plants, shrubs and trees of temperate and tropical habitats can form arbuscular mycorrhizas. Whereas most fungi are generalists, associating with a wide range of plants, there is increasing evidence for specificity or selectivity of some plant species for particular fungal symbionts. This is an important area which, together with increasing appreciation of functional diversity among plant-fungus combinations, has significant implications for roles of AM fungi in plant communities. Non-mycorrhizal plants and plants which form more than one type of mycorrhiza are found in a number of families, supporting the idea that loss of AM status or gain of another type of mycorrhiza has evolved many times, probably as a result of different selection pressures and based on different mechanisms.