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Evolutionary trends in root-microbe symbioses

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SUMMARY

Some of the various symbiotic associations that occur between plant roots and microbes are found on a very wide range of plants, others on very few. By far the most widespread association is the arbuscular mycorrhizal symbiosis, which appears to be an ancestral characteristic; other mycorrhizas and all bacterial nodule symbioses have much more restricted distributions. It has recently been shown that the ability to form nitrogen-fixing symbioses is a trait that occurs in only one clade of the angiosperms. Here we analyse the phylogenetic pattern of mycorrhizal associations, and show that the ectomycorrhizal association has almost certainly arisen more than once, although other types are more concentrated phylogenetically. A detailed comparative analysis of these symbioses awaits a more secure dataset, but it has been possible to undertake such an analysis for arbuscular mycorrhizas in the British flora, which has revealed that the non-mycorrhizal state is a derived one representing habitat and other forms of specialization.

1. INTRODUCTION

In common with most other organisms, the normal state of seed plants is symbiotic. They coexist with a range of symbionts, including leaf-inhabiting fungal endophytes, but the most widespread symbionts are in roots. Possibly 90% of all seed plant species have fungal symbionts in their roots, forming the structures known as mycorrhizas. A very much smaller number form nodules containing symbiotic bacteria that can fix atmospheric dinitrogen gas. There is strong evidence (discussed below) that the original colonization of land by plants was at least assisted by the evolution of mycorrhizal symbioses, but it is also certain that other types of symbiosis, both mycorrhizal and bacterial, have evolved subsequently. In this paper, we aim to investigate the evolutionary history of these symbioses as it relates to the phylogeny of the seed plants, seeking any correlations between the development of different types of symbiosis and important ecological traits of the hosts. We shall concentrate on the mycorrhizal symbioses, because they are more varied and widespread than the other types.

Although the term mycorrhiza lacks a clear definition, it is best viewed as a sustainable, non-pathogenic, biotrophic interaction between a fungus and a root; this paper will not cover pathogenic associations or the range of poorly characterized associations that have been identified by mycologists, such as those involving 'dark septate hyphae' (Haselwandter & Read 1981; Stoyke *et al.*1992) or dematiaceous fungi resembling *Phialocephala fortinii* (O'Dell *et al.* 1993). Mycorrhizas range widely in form and in the type of fungus involved, demonstrating that they do not represent a single evolutionary class of association, but rather a type of association that has evolved repeatedly, in response to distinct selection pressures. They have been classified in various ways, for example into ectomycorrhizas (EcM), typically formed between woody species and long-lived fungi (generally Basidiomycotina and Ascomycotina), and endomycorrhizas. The latter themselves contain a number of disparate types, notably the ubiquitous arbuscular mycorrhizas (AM, sometimes referred to as vesicular-arbuscular mycorrhizas), involving a very wide range of plants and a small group of fungi in the Glomales (Zygomycotina); the ericoid mycorrhizas, formed between fungi in the Ascomycotina (notably *Hymenoscyphus* spp.) and plants in the Ericales; and the orchid mycorrhizas, formed between various fungi, often imperfect forms, and orchids. There are also numerous other types that have been wholly or partially described and are apparently confined to restricted plant taxa; they illustrate our imperfect understanding of this symbiosis. Functionally, these symbioses behave in distinct ways, but they share the transfer of fixed carbon from the plant to the fungus as a unifying physiological feature. The characteristics of the main types of mycorrhiza are summarized in table 1.

In contrast, bacterial root symbioses are more sharply defined. There are two main types: first, the rhizobial symbiosis is formed between most (but importantly not all) species of the Fabales and a single genus in the Ulmaceae (*Parasponia*), and bacteria in what was formerly the genus *Rhizobium*, now split into three or even four genera, including *Bradyrhizobium*, *Azorhizobium*, and recently *Sinorhizobium* (Young & Haukka, 1996); second, actinorhizas develop between bacteria of the genus *Frankia* and an apparently diverse group of plants in 24 genera in eight families. In each case the bacteria develop within a nodule formed on the root and fix nitrogen within it, receiving fixed carbon from the plant, as in mycorrhizas.

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Table 1. *The characteristics of the main types of mycorrhiza*

	ectomycorrhiza	arbuscular mycorrhiza	ericoid mycorrhiza	orchid mycorrhiza
taxa				
fungi involved	Basiodiomycotina, Ascomycotina, one genus in Zygomycotina	Zygomycotina (Glomales)	Ascomycotina (esp. *Hymenoscyphus*)	Basidiomycotina, Mycelia Sterilia, Deuteromycotina
plants involved	taxonomically diverse, many	taxonomically diverse, few	Ericales	Orchidales
morphology				
diagnostic features	Hartig net	arbuscule	intracellular hyphal complexes in epidermis	hyphal coils (pelotons) in cortical cells
other structures	sheath	vesicles, coils		
functionality (changes in plant function)				
P acquisition	important	important	?important	exclusive[a]
N acquisition	important	marginal	important	exclusive
water acquisition	?important	marginal	unknown	unknown
protection from pathogens	?important	?important	unknown	unknown
others	?protection from ionic toxins	?micronutrients	protection from ionic toxins	carbon supply to plant

[a] 'exclusive' is used to indicate that all the plant's resource acquisition is achieved by this route

2. THE SYSTEMATIC PATTERN

The distribution of root-microbe symbioses can be viewed under two heads: abundance and phylogenetic concentration. By far the most abundant of these symbioses is the arbuscular mycorrhiza. Trappe (1987) analysed available records of mycorrhizal colonization and found that 55% of angiosperm species examined (which was only 3% of all known angiosperm species) formed AM; a further 12% were regarded as facultatively mycorrhizal. Since this figure is probably biased towards preferential inclusion of non-mycorrhizal species such as annual weeds and crops, it can be estimated that around two-thirds of land plants normally form AM symbioses. Such figures have to be interpreted with great care. A sure demonstration that a plant species is 'normally mycorrhizal' ideally requires the following:

1. identification of characteristic structures (e.g. arbuscules in AM, Hartig net in EcM symbioses) in field-collected roots, and from a range of habitats if the species typically occurs in several;
2. re-synthesis of the association under controlled conditions, with appropriate plant and fungal taxa (i.e. Koch's postulates should be applied);
3. a demonstration of functional attributes of the association.

Unsurprisingly, this has been performed only for a handful of cases, and the vast bulk of the literature on mycorrhizal associations is based upon some subset of these criteria, with the first being the most frequently achieved. In many cases, however, even that most basic test is only partially satisfied, and records of arbuscular mycorrhizal associations without evidence of arbuscules, or of ectomycorrhizal associations without visible Hartig nets (both are diagnostic characters) abound. It is common, for example, to find apparently AM-colonized roots in which only hyphae and vesicles or coils are visible; experienced workers feel confident about identifying these as AM structures, even though they are not strictly diagnostic. More seriously, many of the older records of EcM associations are based on the presence around trees of carpophores of fungi known to be ectomycorrhizal, with no examination of roots at all. Even the basic data are therefore suspect; extrapolations from them must be treated with extreme caution.

Nevertheless, it is certain that the AM symbiosis is the most abundant type. It also shows the smallest degree of phylogenetic concentration, being found in almost all groups including the Bryopsida. Some of the symbioses demonstrate extreme concentration, occurring in only a single plant taxon, notably the orchid and ericoid mycorrhizas, and (with the sole exception of *Parasponia*) the rhizobial nodule symbiosis. To some extent, this is a circular argument: there may be a tendency for investigators to describe a symbiosis found in such a group as being of the type defined by that group, but even so the statement appears robust. The other two types to be discussed here, actinorhizas and ectomycorrhizas, are often cited as cases where there is no apparent phylogenetic pattern to their distribution. Actinorhizas are found in eight families including Betulaceae, Casuarinaceae, Myricaceae and Rosaceae, while ectomycorrhizas appear in families as disparate as the Pinaceae, Fabaceae, Dipterocarpaceae and Cyperaceae.

(*a*) *The ancestral symbiosis*

Pirozynski & Malloch (1975) were the first to suggest not only that the evolution of the arbuscular mycorrhizal symbiosis was contemporaneous with the first land plants, but that symbiosis between a photosynthetic aquatic ancestor and a fungus was actually a prerequisite for the development of a land flora. The argument was largely based on the inability of essentially rootless plants to acquire sufficient phosphorus: the phosphate ion is virtually immobile in soil (phosphate ions will diffuse 0.1 to 1 mm per day in typical soils) and its acquisition requires the external hyphae of the fungal partner in the symbiosis. Other important evidence was the remarkable ubiquity of the symbiosis in the plant kingdom, and the tentative identification of structures in 400 million year old Early Devonian fossils (*Aglaophyton*) from the Rhynie Chert as being hyphae and vesicles from an AM fungus (Kidston & Lang, 1921).

This hypothesis has recently received convincing confirmation from two additional pieces of evidence. First, molecular sequence data have been used (Simon *et al.* 1993) to estimate the origin of the order Glomales, which includes all known AM fungi, at between 353 and 462 million years ago, consistent with the believed origin of a land flora. Second, Taylor *et al.* (1995) have re-examined the *Aglaophyton* material and shown that the endophytic fungus produced arbuscules, confirming its AM nature. It seems certain, therefore, that the AM symbiosis developed shortly after or even simultaneously with the appearance of a land flora, and Pirozynski & Malloch's (1975) perceptive theory appears well-founded. This means that ancestors of all land plants had the potential to form arbuscular mycorrhizas, and those that do not do so nowadays have either lost or suppressed the genes involved. Evidence that the genes are not lost in many cases comes from the widespread occurrence of dual symbioses (typically AM and EcM in the same root) and from the recent discovery that many species in the Pinaceae are naturally colonized by AM fungi in the wild (Czares & Trappe 1993; Czares & Smith 1996).

From the standpoint of comparative analysis, the questions that need to be asked, therefore, are not about the distribution of the AM symbiosis and its ecological correlates, but about the pattern of the other symbioses and, importantly, of the 10% or so of plant species that appear to have abandoned this symbiosis altogether.

3. PHYLOGENETICALLY CONCENTRATED SYMBIOSES

(*a*) *Orchid and ericoid mycorrhizas*

These two symbioses both occur within a single family (Orchidaceae) or order (Ericales), as far as is known, and it is reasonable to postulate that the evolutionary event that led to their development occurred early in the establishment of the respective taxa. However, not all species within these taxa necessarily form the eponymous symbiosis: there are records of AM associations in orchids (Hall 1976) and certainly in other families of the Orchidales, such as Burmanniaceae (Leake 1994). The Ericales includes families such as the Pyrolaceae and Monotropaceae which have their own distinctive mycorrhizal types. Nor is it the case that the fungus partner in these symbioses shows a high degree of faithfulness: the orchid fungi are typically abundant species that may even be pathogens of trees, and Duckett & Read (1995) have shown that the ericoid endophyte *Hymenoscyphus ericae* can colonize the rhizoids of a number of leafy liverworts; whether the liverwort association is truly mycorrhizal awaits confirmation.

The orchid association appears to be unique in that all identified material fluxes in the symbiosis are from fungus to host: in other words the orchid appears to parasitize the fungus. This is transparently true of the achlorophyllous orchid genera such as *Neottia* and *Epipogium* which have neither chlorophyll nor roots and are therefore incapable of autonomous resource acquisition. But even in green orchids such as *Goodyera repens*, no carbon movement from plant to fungus was seen by Alexander & Hadley (1984). This extreme mycotrophy, associated with achlorophylly, has also evolved in two families of the Ericales, the Monotropaceae and the Pyrolaceae (reduced by some taxonomists to subfamilies of the Ericaceae). A phylogeny of the Ericales based on 28S rRNA sequences (Cullings 1994) shows that the mycorrhizal types are congruent with the host phylogeny, to the extent that the subfamily Monotropoideae (syn. Family Monotropaceae) is both polyphyletic on morphological grounds, and contains more than one mycorrhizal type (figure 1). *Monotropsis ordata* appears in the phylogeny in subfamily Vaccinioideae (third from top in figure 1) and is the only member originally classified as Monotropoideae not to have a typical monotropoid mycorrhiza. Cullings *et al.* (1996) have shown that some monotropoid mycotrophs, including *Monotropa hypopithys* and, especially, *Pterospora andromeda*, are specialist parasites of a small number of EcM associations, and that such specialization has developed within in a single clade of the Monotropoideae.

The complexity of mycorrhizal types in the Ericales is greater than in any other order: at least four distinct types have been recognized, including ectomycorrhiza, ericoid, arbutoid and monotropoid mycorrhiza (see Harley & Smith 1983 for details of these associations). Each of these is defined morphologically but named for the particular plant taxon involved. Molecular analyses confirm that the mycorrhizal status of a plant species is an important taxonomic character within this group: in other words, the nature of the mycorrhiza is a determinant of ecological and evolutionary behaviour.

(*b*) *Nitrogen-fixing nodules*

The rhizobial symbiosis has a curious distribution, with most members of two subfamilies of the Fabaceae (Papilionoideae and Mimosoideae) normally forming the symbiosis, but only about a third of the species in the third subfamily, Caesalpinioideae. In addition, the single genus *Parasponia* (Ulmaceae) has also been shown to be rhizobial. This curious pattern has provoked speculations of unusual evolutionary phenomena such as horizontal transfer.

In contrast, the actinorhizal symbiosis has been viewed as phylogenetically diffuse. Associations have been found in 24 genera in eight families. In traditional angiosperm classifications, notably that of Cronquist

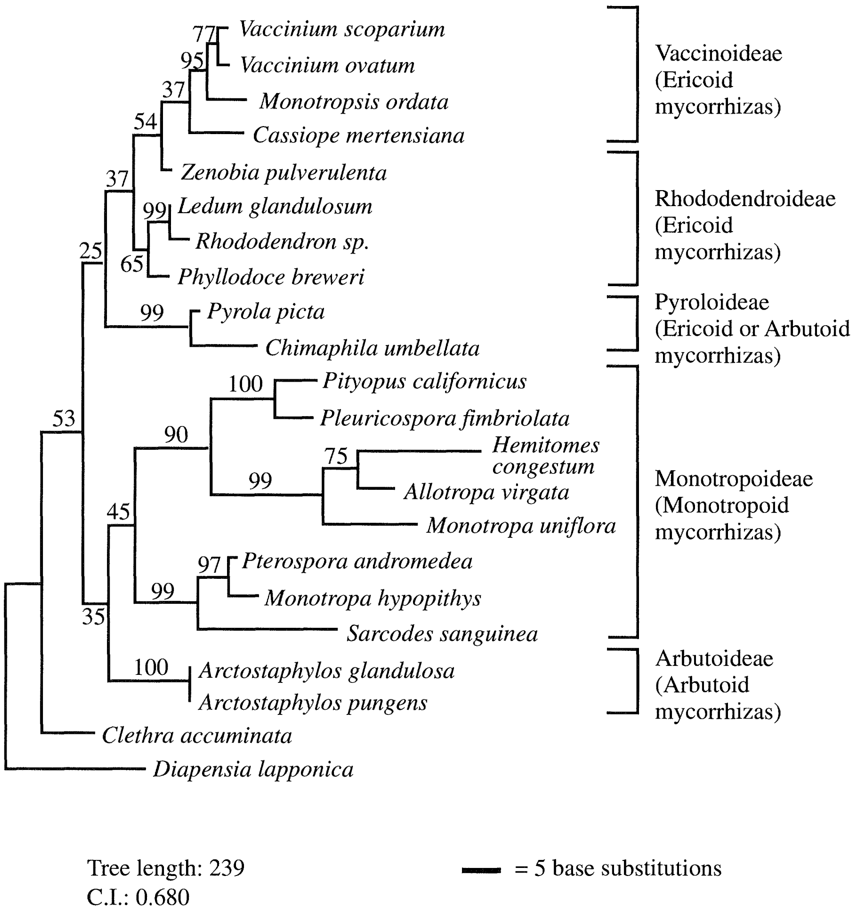

Figure 1. Phylogenetic tree of the Ericales based upon 28S rRNA gene sequences, showing the conformity of phylogeny and mycorrhizal type (Cullings 1994)

(1981), this distribution showed no obvious pattern. Seven orders were involved: Rhamnales, Proteales and Rosales in the Rosanae; Casuarinales, Myricales and Fagales in the Hammamalidanae; and Ranunculales in the Magnolianae. A quite different picture emerges using a different phylogeny, based on DNA sequences of the chloroplast *rbcL* gene (Chase *et al.* 1993). This suggests that all N-fixing root symbioses, both rhizobial and actinorhizal, occur in a single clade, Rosid I (Soltis *et al.* 1995), implying a single origin of the potential to form the symbioses, followed either by the development of distinct types of symbiosis in some subsequent lineages, or possibly the loss of the symbiosis in others. Whichever is true, it opens the possibility of asking comparative questions about the species in the Rosid I clade.

The traditional adaptationist view of the N-fixing symbioses has been that they permit species to grow in N-deficient habitats. This has always been hard to maintain. Some N-fixers do indeed occur in, for example, early successional habitats: the well-documented case of *Alnus crispa*, an actinorhizal species, in the succession at Glacier Bay, Alaska, is a good illustration. But many legumes, in particular, are not obviously associated with extreme N-deficiency. An alternative view, put forward by McKey (1994), is that the legumes (Fabaceae) were a group with inherently high nitrogen demand, characterized by high leaf tissue N concentrations and consequent high photosynthetic rates (Field & Mooney 1986). As a result, a symbiosis that increased N acquisition ability would be strongly favoured. If this view is accepted, the interesting question becomes why some legumes are non-rhizobial. In Caesalpinoideae (Fabaceae), the nodulating tribes Caesalpiniae and Detariae are predominantly AM, whereas ectomycorrhizal associations are only frequent in the tribe Amherstiae, members of which do not normally nodulate (Alexander 1989). Possibly the carbon cost of maintaining both symbioses is too great, though certainly some species (e.g. the actinorhizal *Alnus* and the rhizobial *Acacia*) can achieve such multiple symbiosis. In contrast, most Papilionoideae are both nodulated and arbuscular mycorrhizal: the carbon cost of AM symbiosis is almost certainly less than that of EcM. An alternative explanation for the paucity of EcM nodulators is that the same genes are required, as is true of *nod*$^-$ peas, in which both nodulation and AM formation are suppressed (Ginaninazzi-Pearson *et al.* 1996).

A wider view of the evolution of nodulation symbioses might allow a range of selection pressures to have acted within a clade that possessed the requisite genetic mechanisms. Doyle (1994) suggests that not only is the subfamily Caesalpinoideae polyphyletic, but that nodulation has arisen several times within the family. In some cases the driving force may have been a suite of characters that demanded high rates of N acquisition; in others, both legumes and actinorhizal non-legumes, nodulation may have been favoured in taxa that occupied N-deficient habitats.

Actinorhizal symbioses share some striking phylogenetic patterns with some mycorrhizas, notably a match between the phylogeny of the plant and microbial partners. This matching has been described in ericoid mycorrhizas (see above) and also in actinorhizas (Cournoyer *et al.* 1993). It seems likely that these symbioses have a powerful effect on the ecology and consequently constrain the evolution of both partners.

4. PHYLOGENETICALLY DIFFUSE SYMBIOSES

Where symbioses have evolved within well-defined clades and are characteristic of most members of that clade (e.g. orchid mycorrhizas), there is little scope for comparative analysis. In some cases, such as N-fixing symbioses, the patterns are much more amenable to analysis, but current uncertainties about phylogenetic relationships or lack of basic biological and ecological information about the species makes progress difficult. There are, however, two groups of associations that offer more scope, even with current data, namely ectomycorrhizal and (stretching a point) non-mycorrhizal species.

(*a*) *Ectomycorrhizas*

On the basis of the Cronquist (1981) classification, EcM species of plant are found in all the major groups of dicots and in one group of monocots, the Cyperales. Trappe (1987) has already presented a comprehensive analysis of the phylogenetic trends that can be detected in this way. He suggested that the most pronounced evolutionary trend was towards a reduced dependence on mycorrhizal associations, and also noted the absence of mycorrhizal types other than AM in annual plants. There is a parallel association with woodiness: virtually all EcM plant species are woody (trees, shrubs, lianes), as shown by the results of a principal components analysis of angiosperm orders, based upon the presence and absence of various types of symbiosis (table 2). The first component in this PCA has large loadings for EcM, dual (EcM plus AM) mycorrhiza and N-fixation. An analysis of variance of the first axis scores of each order using the presence or absence of woody species in the order as the factor, reveals that non-woody orders have negative scores (-0.867 ± 0.177; mean and 95% confidence limit) whereas orders with at least some woody species have positive scores (0.458 ± 0.432). The difference is highly significant: $F_{1,79} = 18.81$, $P < 0.001$. The association between woodiness and the occurrence of both EcM and N-fixation is therefore strong at the level of order.

Table 2. *Loadings on the first two axes of a principal components analysis of orders of angiosperms*

(Each variable refers to the presence of at least one record of that characteristic in the order. The first two axes accounted for 49% of the variation.)

variable	axis 1	axis 2
eigenvalue	2.091	1.319
ectomycorrhiza	0.593	−0.089
dual mycorrhiza (AM/EcM)	0.611	−0.007
non-mycorrhizality	−0.064	0.584
other mycorrhizal types	−0.178	−0.310
nitrogen fixation	0.436	−0.188
parasitism (plant-plant)	0.025	0.528
insectivory	0.220	0.491

There are a number of records of EcM in non-woody species, and these illustrate the severe difficulties of relying on published records for this type of analysis. Warcup (1980) reported that a number of Australian Asteraceae, including some annual herbs, could form ectomycorrhizas. Subsequently, Warcup & McGee (1983) tested numerous species of Asteraceae in culture with one or two ectomycorrhizal fungi and found that 20 annual species could apparently form EcM structures. The illustration they show of *Podolepis rugata* displays typical external morphology and also the Hartig net in a TS of a mycorrhizal root, but the generality of these findings remains unclear. Very few other workers have discovered EcM structures in non-woody (or at least in short-lived) species; whether the Australian species studied by Warcup would have formed fully functional EcM under natural conditions is unknown. However, Fontana (1963), Haselwandter & Read (1981) and several workers since have identified sedges in the genus *Kobresia* (Cyperaceae) as being EcM. This appears to be the only reliable record of an EcM monocot, if that for *Pandanus* is discounted (B. Moyersoen, unpublished data), and poses interesting problems for a phylogenetic analysis. Numerous reports of EcM plants, especially from field surveys, meet few of the criteria suggested on p. 1368, and must be treated with caution. Consequently in the analysis that follows, we have restricted our analysis to the level of the family.

Use of the *rbc*L phylogeny by Soltis *et al.* (1995) offered a novel insight into the evolution of N-fixing symbioses. We have therefore used the same phylogeny to investigate the distribution of ectomycorrhizas, using data obtained from a wide literature, although with a considerable contribution from the dataset compiled by Newman & Reddell (1987), and kindly lent by Dr E. I. Newman. On this basis it appears that the EcM symbiosis (or the propensity to form it) has probably evolved at least twice (figure 2), once or more in the branches leading to the Pinaceae and Gnetales, and once in the root of the clade that includes all the Rosid and Asterid lineages of Chase *et al.* (1993), and possibly the Hamamelids as well. A complication of this analysis is the small number of records of EcM in the Pteropsida. Although some of the records may be discounted, a few seem secure, even though ferns and their allies are largely AM. Did the ability to form EcM arise, therefore, at a very early stage in land plant evolution, becoming lost subsequently in many clades? It seems more plausible that odd EcM ferns are secondary developments, just as we assume the EcM *Kobresia* to be.

Mapping the distribution of EcM symbioses on to the trees of Doyle & Donoghue (1993) allows a comparison of the evolution of woodiness and the EcM habit. They propose two trees of the base of the

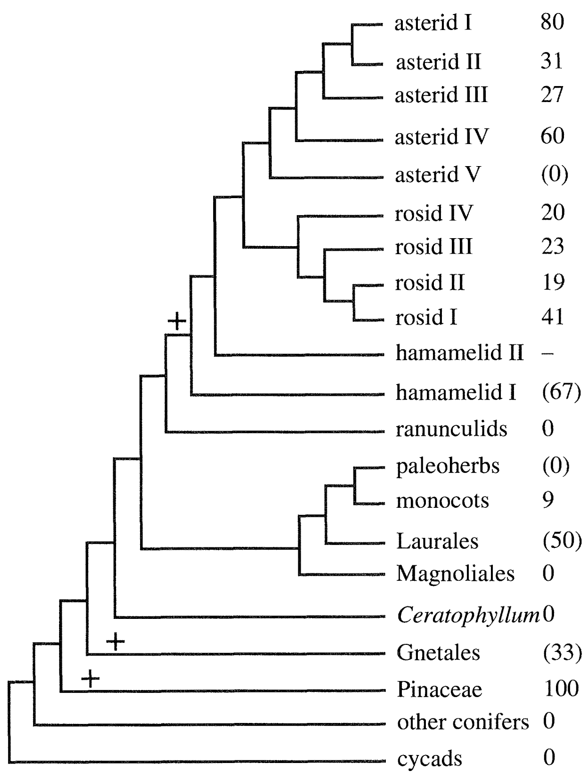

Figure 2. Seed-plant phylogeny based on *rbcL* (Chase *et al.* 1993), showing the distribution of ectomycorrhizas. Numbers for EcM represent the percentage of families within each group that contain EcM species; figures in parenthesis are where there are fewer than five families for which data are available and a dash represents 'no data'.

angiosperm phylogeny, alternatively rooted in the palaeoherb or magnolioid clade. If the magnolioid root is correct, which is the traditional view, then woodiness evolved long before the EcM habit, and many early branches of the tree were woody but not EcM (figure 3*a*). If palaeoherb-rooting is preferred, as is indicated by RNA data (Doyle & Donoghue 1993), then early angiosperms were non-woody (possibly aquatic), but again it is likely that woodiness evolved independently of the ability to form EcM (figure 3*b*).

Among modern plants, the great majority of EcM species are woody, but many woody species are not EcM. Read (1991) has put forward a persuasive hypothesis that the type of mycorrhizal association found is determined by habitat type and the distinct function of the various symbioses. Cool, wet habitats, where nutrient cycling is very slow, favour the ericoid association, in which the fungus can actively mineralize both N and P from organic matter as well as protect the plant from toxic ions. In more productive boreal forests, where nutrient cycling is still potentially limiting, ectomycorrhizal symbioses occur, with mineralization capacities greater than those of AM associations but less effective than ericoid fungi. In the most productive habitats, where N limitation is least and possibly P limitation greater, the ubiquitous AM association is found. The phylogenetic analysis above demonstrates that within the main eudicot lineage, both EcM and AM potentialities are present, and it is safe to make such comparisons as those implied by the hypothesis of Read (1991).

Nevertheless, within tropical forests, which are predominantly AM communities, EcM trees do occur, notably the dipterocarps and some tropical legumes. There is little evidence that these EcM species grow on significantly less fertile soils and, although pure EcM stands are sometimes found, EcM species typically occur in mixed communities with AM species (Alexander 1989; Moyersoen 1993). It is possible that these

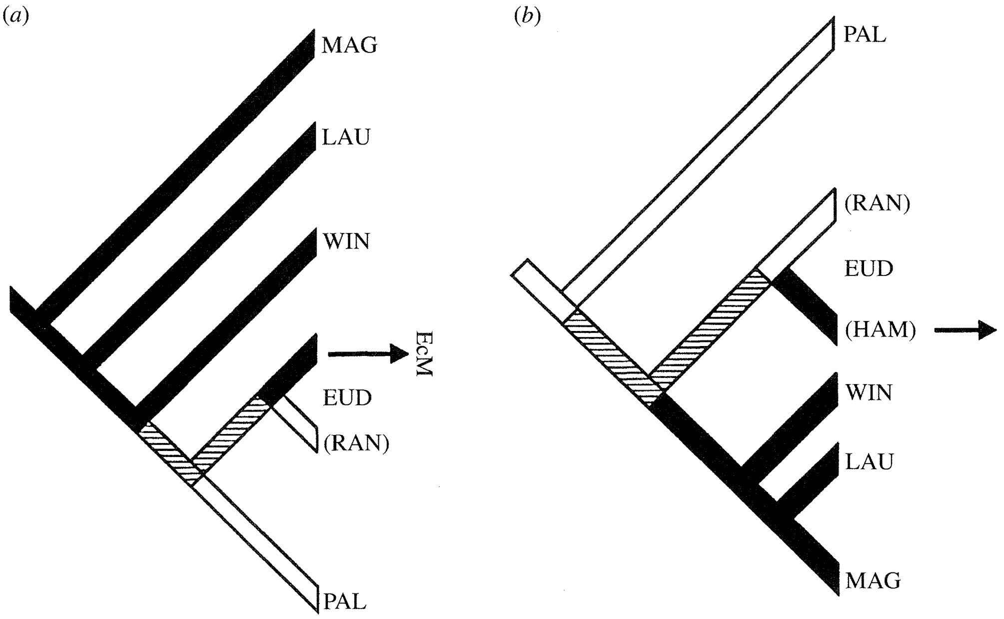

Figure 3. Simplified phylogenetic trees of the angiosperms (from Donoghue & Doyle 1993), showing (*a*) magnolioid-rooting, suggesting that woodiness is ancestral, and (*b*) paleoherb rooting, suggesting that woodiness is derived. In each case the arrow represents the origin of the clade in which virtually all EcM angiosperms (except possibly *Sassafras*, Laurales) occur. Ectomycorrhizality evolved within a woody clade, but not in all woody clades. MAG Magnoliales; WIN Winterales; LAU Laurales; EUD Eudicots; RAN Ranunculids; PAL Palaeoherbs. Solid lines represent woody clades.

forests are comparable (in terms of nutrient supply and consequent mycorrhizal relationships) with temperate broadleaf forests, where similar AM/EcM mixtures occur.

(*b*) ***Non-mycorrhizal species***

In many ways, the most interesting group are those that have lost the ability to form mycorrhizas at all (or at least do not express it). It has been suggested that about 10% of all plant species are normally non-mycorrhizal, including most members of the families Caryophyllaceae, Brassicaceae, Chenopodiaceae, Cyperaceae and Juncaceae. This set of families does not have many obvious ecological characteristics in common. A severe problem exists, however, in defining non-mycorrhizality. A single record of a plant as being non-mycorrhizal is obviously insufficient, let alone a record of some roots of a plant as being so, which is the more likely nature of such a record. Ideally, the criteria on p. 1368 need to be applied, at least to the extent of attempting to synthesize mycorrhizas with a range of appropriate fungal isolates under a range of conditions. There are few datasets that can be relied upon to provide this level of detail, but Peat & Fitter (1993) used that of Harley & Harley (1987) and the interpretation of it and other data held in the Ecological Flora Database (Fitter & Peat 1994) for the British flora. They also used an explicitly phylogenetic approach, based on a hierarchical analysis of variance using the taxonomic hierarchy as a surrogate for a true phylogeny.

Peat & Fitter (1993) showed that the majority (80%) of the variance in the proportion of species per genus that are arbuscular mycorrhizal occurs at the level of genus and family. They undertook analyses to compare the ecological characteristics of species that were normally, occasionally, rarely or never arbuscular mycorrhizal, using either species or genera as the units of analysis as appropriate, depending on the results of hierarchical analyses of variance for each characteristic. Non-mycorrhizal species emerged as having thinner roots and smaller seeds than mycorrhizal species, and also as occurring in habitats of less extreme pH. They did not, however, have any special relationship with soil fertility or moisture status. The most striking result was that non-mycorrhizal perennial species occur in fewer habitat types on average than do mycorrhizal perennials; in other words, they are habitat specialists, and this specialization is not simply due to the fact that some non-mycorrhizal species favour particular habitat types, such as disturbed ground or wetlands, as the relationship persists when wetland species and halophytes are omitted, and is not stronger when only annuals are considered.

Nevertheless, it appears that non-mycorrhizality has evolved most frequently in two distinct groups of species: first, in species characteristic of disturbed habitats where a mycorrhizal network is unlikely to develop, retarding the development of colonization by the fungus of newly extending root systems, and so forcing plants to become dependent on their own resource acquisition abilities for at least part of their life cycle. In these habitats, competition for nutrients is unlikely to be very important, because low root length densities in soil lead to a low probability that depletion zones around roots will overlap. Second, non-mycorrhizality has evolved in species of wet habitats where diffusion of poorly mobile ions is faster than in dry soils and where the benefits of mycorrhizality, at least in terms of phosphate uptake, are smaller. Non-mycorrhizality is therefore a specialist trait, occurring in relatively few species of restricted ecological amplitude.

Consequently, one can regard AM species as generalists, with all other types (non-mycorrhizal, EcM, ericoid, etc.) as specialists in one way or another; this view is consistent with that of Read (1991). The curiosity of the AM symbiosis is that it appears to have evolved as a direct response to the inability of early, rootless land plants to acquire phosphate (Pyrozinski & Malloch, 1975). However, complex root systems have been a feature of land plants for a long time, although the fossil record is surprisingly unclear as to exactly how long. Many modern plants that have intensely branched root systems, such as the Poaceae, are normally AM when growing under natural conditions. Fitter & Merryweather (1992) demonstrated that there is no apparent benefit to the plant from the association in these circumstances, if benefit is to be defined in terms of increased P acquisition. This is even more true of plants that possess 'hairy root clusters' (Lamont 1993), dense groupings of lateral roots that are highly effective at absorbing nutrients from infertile soils, and that develop frequently in non-mycorrhizal taxa such as the Proteaceae.

The question therefore arises as to whether mycorrhizal associations in plants with well-developed root systems are non-mutualistic, perhaps even parasitic, or whether there are other benefits to the plant. Newsham *et al.* (1995*a*) have conclusively demonstrated that in the grass *Vulpia ciliata*, which is normally an AM species, there is no enhancement in P uptake as a consequence of colonization, but that the mycorrhizal fungus does protect the plant against other fungi (notably *Fusarium oxysporum*) that are root pathogens. They postulated (Newsham *et al.* 1995*b*) that as root systems evolved more complex branching patterns, and so became self-sufficient in terms of P acquisition in most soils, they also became more vulnerable to pathogens, as many such fungi attack roots where lateral roots penetrate the cortex. The ability to protect plants against pathogens therefore maintained the AM association as a mutualism.

5. CONCLUSIONS

Although root-microbe symbioses are ubiquitous among land plants and play a key role in their ecology, only one dataset has proved amenable to a full comparative analysis: that of Peat & Fitter (1993). This showed that non-mycorrhizal species do have consistent characteristics, of which the most notable is that they are habitat specialists. It is likely that similar insights could be obtained from a comparative analysis of ectomycorrhizal species were an appropriate dataset available.

Our ability to undertake systematic comparative analyses of root-microbe symbioses of plants is limited both by the rudimentary state of our knowledge of plant phylogeny and by the inadequacy of the data about the symbioses themselves, which are only really well known within a few tightly defined groups (such as the rhizobium nodule symbiosis) that do not lend themselves to comparative analysis. The new phylogenies that are appearing have already been used to demonstrate that N-fixing root symbioses all occur in a single clade (Soltis *et al.* 1995), and in this paper we demonstrate that the same phylogeny suggests that ectomycorrhizal association appeared at least twice, in the Pinaceae and in the eudicots. Future comparisons of ectomycorrhizal and other plant species must take this fundamental distinction into account.

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