CHAPTER 1
Origins of the mycorrhizal symbioses

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1.1 Introduction

Symbiosis means an intimate and often long-term association between two or more different species. Ahmadjian and Paracer (1986) commented: "It is such a universal and important phenomenon that it should be an integral component of the education of biologists". However, despite or because of its importance, this term has experienced much confusion, variation in usage, and controversy (Martin and Schwab, 2013 and references therein). De Bary coined the term in his monograph *Die Erscheinung der Symbiose* (1879) to mean "the living together of unlike organisms," using it to describe a broad range of relationships (mutualism, commensalism, parasitism).

Our usage follows the original definition, rather than the more restrictive sense (i.e. symbiosis = mutualism) proposed by some biologists about 30–50 years ago (Martin and Schwab, 2013 and references therein). Symbioses encompass a wide variety of organismal associations in diverse environments, including: bacteria and fungi that form close alliances with the roots of plants; dinoflagellates that live within the endoderm of tropical corals; bacteria that sustain giant tube worms in the deep ocean; and so on. In addition, animals harbor many different microorganisms in their gastrointestinal tracts (Paracer and Ahmadjian, 2000; Benson *et al.*, 2010). At the time De Bary developed his concept of symbiosis, Albert Bernhard Frank was working on plant-fungal relationships. He already published the word *Symbiostismus* (1877), and he was the one who introduced the term mycorrhizas to designate the type of dual organ he observed: "*the entire structure is neither tree root nor fungus alone but resembles the lichen thallus, a union of two different organisms into a single, morphological organ. It can be appropriately designated as a 'fungus-root' or 'mycorrhiza'*" (Frank, 1885; English translation, Trappe, 2005).

The ability of fungi to form mycorrhizas with plants is one of the most remarkable and enduring adaptations to life on land. The relationship is a mutualistic one, and its occurrence is now well established in many plant species (Wang and Qiu 2006; Akhmetzhanova *et al.*, 2012). By contrast, the number of fungal partners involved is less clear, and varies depending on mycorrhizal type (van der Heijden *et al.*, 2015).

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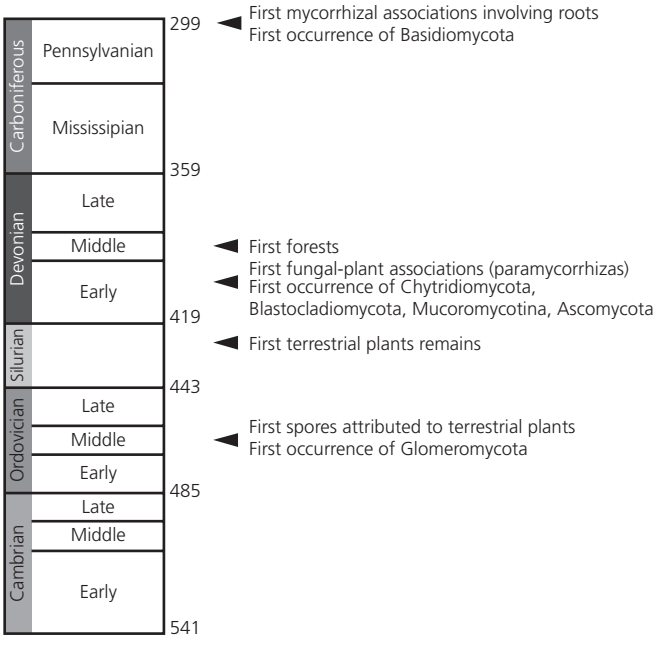


Figure 1.1 Earliest occurrences of fungi, plants and fungal-plant interactions in Palaeozoic times. Ages in millions of years are taken from the International Chronographic Chart of the International Commission on Stratigraphy, 2014. (*See insert for color representation of the figure*.)

Molecular phylogenetics is providing insights into the evolution of different types of mycorrhizal association through time, and genomic studies of both plants and fungi are shedding light on how the complex set of interactions evolved (e.g., Floudas *et al.*, 2012; Kohler *et al.*, 2015). Evidence from fossils is also providing additional perspectives (e.g., Remy *et al.*, 1994; Taylor *et al.*, 1995; Krings *et al.*, 2007a, 2007b, 2011; LePage *et al.*, 1997), and recent work shows how a carefully targeted program of research can yield highly informative results (Strullu-Derrien *et al.*, 2009, 2014a). Moreover, extinction can generate a false signal regarding the origin of evolutionary novelties in a group when only living species are taken into account (Jablonski and Shubin, 2015). As a result, the fossil record has an important role to play in establishing a chronology of when fungi and key fungal associations evolved, and in understanding their importance in ecosystems through time (Figure 1.1).

Here we present a brief review of our current knowledge of the fossil record of mycorrhizas in the context of plant evolution. In addition to providing an overview of what is known, our aim is to identify areas in which the fossil record (palaeomycology) can be of relevance to genomics, and to recommend an approach that would bridge the two disciplines.

1.2 Extant mycorrhizal diversity

Mycorrhizas are widespread, occurring in over 80% of living plant species (Strullu, 1985; Smith and Read, 2008). The fungus uses the host as a source of carbon, while

the host is supplied with mineral elements by the fungus. The two partners also protect each other against soil biotic (e.g., parasites) and abiotic (e.g., drought, toxic compounds) adversities. Some plants, such as the mosses and the angiosperm families *Brassicaceae*, *Caryophyllaceae, Proteaceae, Cyperaceae,* are generally believed to be predominantly non-mycorrhizal (Smith and Read, 2008), although mycorrhizas are rare in some other families (e.g., Nymphaeaceae – Wang and Qiu, 2006).

Today, the most common associations are the arbuscular mycorrhiza (AM) symbioses, in which fungi are all members of the phylum Glomeromycota, which form a single and ancient clade (e.g., Redecker and Raab, 2006; Blair, 2009; Berbee and Taylor, 2010). These fungi can be found in the roots of 80% of all vascular plant species, and they are obligate symbionts. With our present state of knowledge, it is impossible to grow them independently from a host plant (Fortin *et al.*, 2005).

AM associations are characterized by branched, tree-like, intracellular fungal structures (i.e. arbuscules, hyphal coils) and, sometimes, storage organs termed vesicles (Strullu, 1985; Genre and Bonfante, 2016). Some complex and simple thalloids, liverworts (Marchantiopsida), hornworts (Anthocerophyta), lycophytes and fern gametophytes also form associations with Glomeromycota, which are structurally (e.g., Strullu, 1985; Read *et al.*, 2000; Selosse, 2005; Ligrone *et al.*, 2007; Pressel *et al.*, 2010) and functionally (Strullu *et al.*, 1981; Humphreys *et al.*, 2010), similar to those of vascular plants.

Recently, it has been discovered that members of several early diverging clades of land plant (liverworts, hornworts, lycopods and ferns) develop symbiotic associations with Mucoromycotina fungi, and this might also represent an ancestral land plant-fungal symbiosis (Bidartondo *et al.*, 2011; Desirò *et al.*, 2013; Rimington *et al.*, 2015, 2016). Interestingly, some of these extant plants also form partnerships, sometimes simultaneously, with Glomeromycota. This symbiosis is characterized by an intracellular phase showing fine fungal coils with terminal, thin-walled swellings, and an extracellular phase with the hyphae forming semi-parenchymatous structures and thick-walled spores (Pressel *et al.*, 2010; Rimington *et al.*, 2016). We designate this CM symbiosis (coiled mycorrhizas) to distinguish its fine coiled intracellular phase from the arbuscular intracellular phase of AM symbiosis. Because bryophytes, lycopods and fern gametophytes do not have roots, both AM and CM associations are best referred to as mycorrhizal-like (Smith and Read, 2008) or paramycorrhizas (Strullu-Derrien and Strullu, 2007).

Several Ascomycota, Basidiomycota and a few members of the Zygomycota form ectomycorrhizas (ECMs), mostly on shrubs and trees from temperate and Mediterranean regions, and in some parts of tropical forests. Ascomycota and Basidiomycota have been recruited more recently and on multiple occasions (van der Heijden *et al.*, 2015 and references therein). ECM symbiosis is clearly distinguishable from all others on the basis of the absence of intracellular penetration by the fungus (Strullu, 1985; Smith and Read, 2008). The root colonization remains intercellular, and a hyphal sheath is formed around the plant root (Balestrini and Kottke, 2016). This is the type of mycorrhiza originally observed by Frank (1885).

Compared to AM, the range of plants colonized by ECM is relatively small; only a mere 3% of seed plants are ECM (Moore *et al.*, 2011). Within the gymnosperms, ECMs are known from many Pinaceae and

from the genera *Gnetum* and *Welwitschia*. In Cupressaceae, some species in *Juniperus* and *Cupressus*, as well as the angiosperms *Poplar* and *Alnus*, can develop both AM and ECM (Smith and Read, 2008). The same fungus sometimes forms ectendomycorrhizas, where some hyphae penetrate the host cells – for example, in basal *Ericaceae* (Selosse *et al.*, 2007).

Finally, in two plant families, namely Orchidaceae and Ericaceae, mycorrhizas involve intracellular colonization by hyphal coils. A range of Basidiomycota form orchid mycorrhizas (ORMs) while both Asco- and Basidiomycota form Ericoid mycorrhizas (ERMs) (Strullu, 1985; Selosse *et al.*, 2007; Smith and Read, 2008). Fungi forming mycorrhizas with orchids (Dearnaley *et al.*, 2016) typically live as saprotrophs in the soil, and likely as endophytes, or even form ECM associations with neighboring trees (Dearnaley *et al.*, 2013; Dearnaley *et al.*, 2016). Orchid seeds are extremely small and, in natural ecosystems, the seedlings (protocorms) of most orchids are completely dependent on colonization by fungi for carbon supply. ERM is most common under acid and infertile heathland conditions. Some ERM fungi (Helotiales, Ascomycota) are soil saprotrophs; however, recent evidence suggests that others are plant endophytes (Selosse *et al.*, 2009). Some fungi can also form both ERM and ECM associations with different host plants (van der Heijden *et al.*, 2015).

1.3 Early land plants to early forests

Land plants evolved from freshwater algae originating and diversifying through the Ordovician, Silurian and Devonian Periods (Figure 1.2). The fossil record reveals that prior to the origins of forest ecosystems (mid-Devonian; ca 387 million years ago [MYA]) early plants differed in notable ways from those of later floras, and especially from modern species (Edwards and Kenrick, 2015). Plants were small and herbaceous, with simple vascular tissues and typically leafless bifurcating axes, some of which functioned as upright stems and others as rhizoid-based rooting systems (Kenrick and Strullu-Derrien, 2014). Here, the term "axis" is preferred over stem, rhizome, and root because, in the first land plants, these organ systems differed in important aspects of structure and function from their equivalents in living plants (Tomescu *et al.*, 2014). Another key difference from modern bryophytes or tracheophytes (vascular plants) is that life cycles showed a much greater degree of similarity between gametophytes (haploid sexual phase) and sporophytes (diploid phase; Kerp *et al.*, 2004; Taylor *et al.*, 2005). Similar organ and tissues systems were expressed in both phases of the life cycle.

The vascular plants, or tracheophytes, are defined by the possession of a vascular system which is composed of phloem and xylem, but it is the latter that is more commonly encountered in the fossil record, due to the resilience of its cellular components, which typically possess robust cell walls containing the polyphenolic polymer lignin (Boyce *et al.*, 2003). Vascular tissues first appear in the fossil record in the lower part of the Devonian period (410–407 MYA), when terrestrial sediments containing fossil plants first became abundant (Kenrick *et al.*, 2012). The evolution of lignified tissues led to arborescent plants by the mid- to late Devonian (Stein *et al.*, 2007).

Arborescence is known to have evolved independently in many different groups,

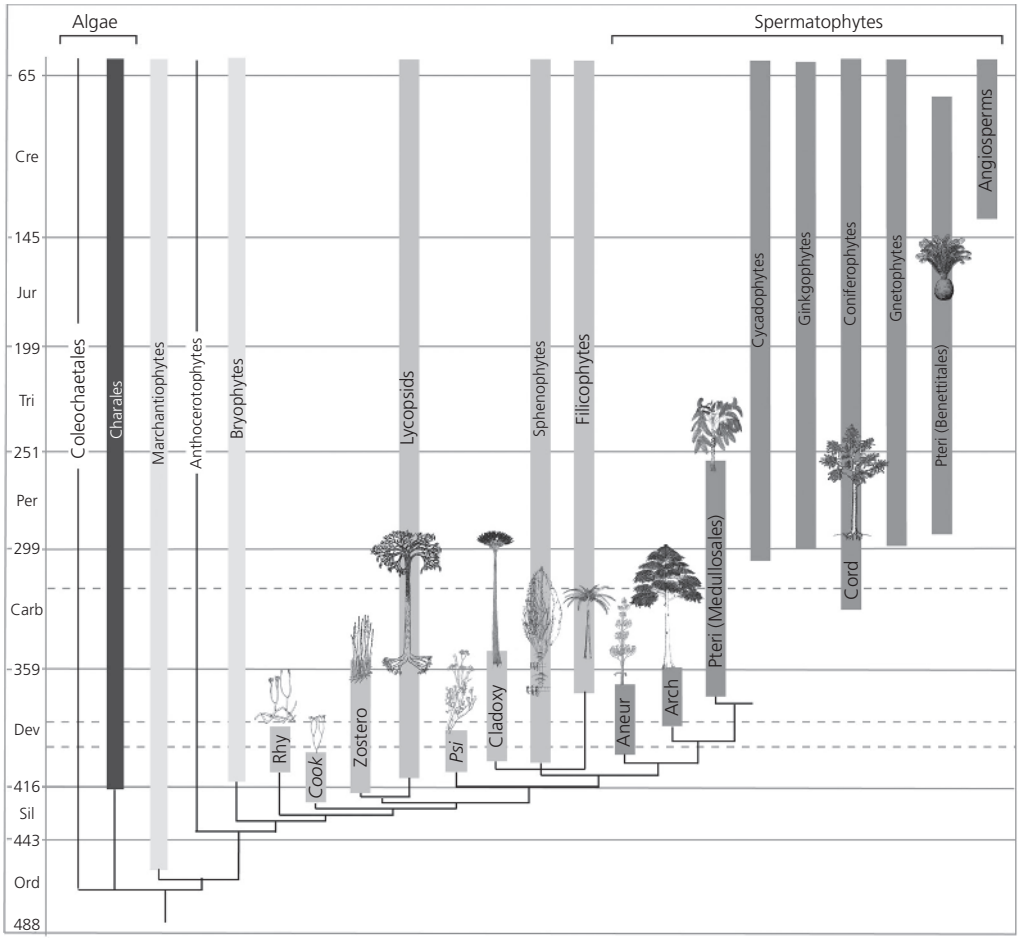


Figure 1.2 Simplified phylogenetic tree showing the minimum stratigraphic ranges of selected groups based on fossils (thick bars) and their minimum implied range extensions (thin lines). Extinct and living plant groups are shown. Adapted from Kenrick and Crane (1997) and Strullu-Derrien (2010). Ord = Ordovician, Sil = Silurian, Dev = Devonian, Carb = Carboniferous, Per = Permian, Tri = Triassic, Jur = Jurassic, Cre = Cretaceous. Rhy = Rhyniophytes, *Cook* = Cooksonia, Zostero = Zosterophyllophytes, *Psi* = Psilophyton, Cladoxy = Cladoxylopsids, Aneur = Aneurophytales, Arch = Archeopteridale, Pteri = Pteridosperms, Cord = Cordaitales. Pteridosperms or seed ferns are paraphyletic. They comprise hydrasperman Pteridosperms, Medullosales, Callistophytales Peltaspermales, Glossopteridales, Benettitales, and Caytoniales. The relationships among gymnosperms are still not well resolved. (*See insert for color representation of the figure*.)

and a variety of biomechanical strategies were employed (Spicer and Groover, 2010; Pittermann, 2010 and references therein). This dramatic increase in size was, in most groups, a consequence of the evolution of the cambium. The bifacial cambium gave rise to secondary xylem (wood) and secondary phloem, and was present in the extinct progymnosperms, which comprised two groups: the Aneurophytales and the Archaeopteridales

(Figure 1.2). However, it was recently demonstrated that wood evolved initially (407–395 MYA) in plants of small stature that were members of Euphyllophytes, a clade that includes living Sphenophytes (horsetails), Filicophytes (ferns) and Spermatophytes (seed plants) (Figure 1.2) (Strullu-Derrien, 2010; Gerrienne *et al.*, 2011; Hoffman and Tomescu, 2013; Strullu-Derrien *et al.*, 2014b).

The earliest tree-sized plants developed progressively between the early mid-Devonian and early late Devonian (393 to 380 MYA) (Figures 1.2 and 1.3). Cladoxylopsid trees (an extinct group of uncertain affinity) (Stein *et al.*, 2007, 2012) bore digitate lateral leafless branches and had long, narrow, undivided roots originating from the base of the trunk. Lycopsid trees had principally cormose bases with narrow undivided rootlets, trunks covered in microphyllous leaves, and a branched crown. Progymnopsperms had conifer-type wood but reproduced with spores only; the aneurophytales had a large woody rhizome with simple narrow roots, and aerial shoots with iterative branching patterns; the Archaeopteridales had a vertical woody trunk with extensive, woody, highly-branched rooting systems, and truly leafy branchlets (or compound leaves) (Figure 1.3).

In situ fossil forests from these times are quite rare. At the fossil forest of Gilboa,

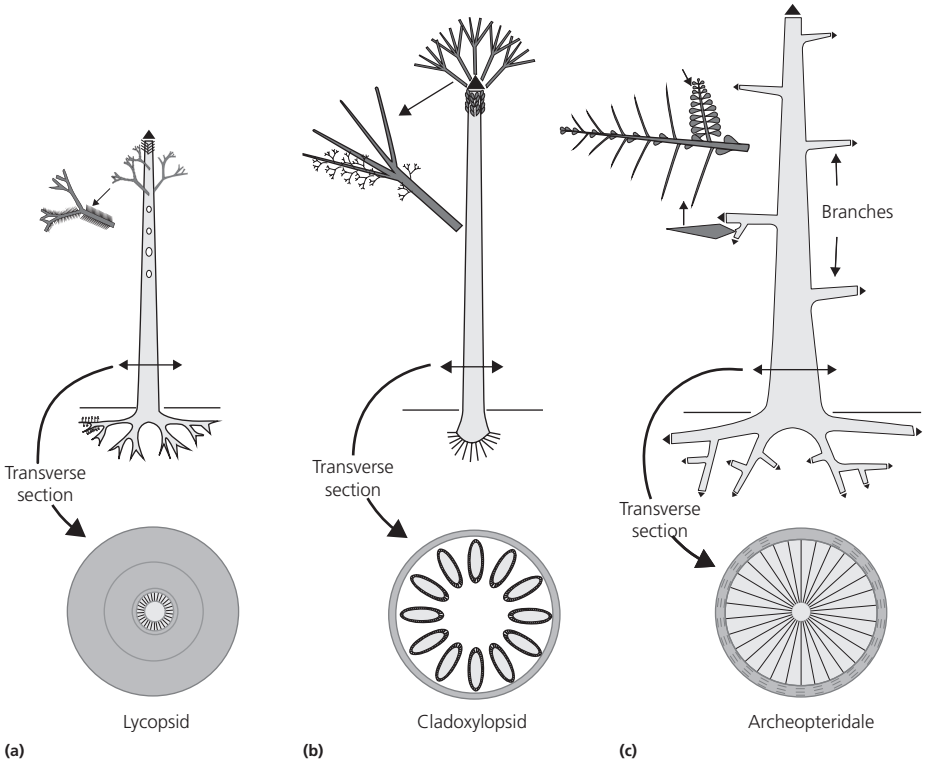


Figure 1.3 (a) to (c) Comparative architecture of three principal arborescent strategies of the middle-upper Devonian and transverse section of the corresponding trunks (Lycopsid, Cladoxylopsid and Archaeopteridale). The color scheme is as follows: yellow, cortex; grey, primary vascular tissue; striped secondary tissue. Scheme courtesy of B. Meyer-Berthaud, modified from *Géochronique* 134, June 2015). (*See insert for color representation of the figure*.)

New York, pseudosporochnaleans and aneurophytaleans dominate in a soil that undoubtedly was quite wet (Stein *et al.*, 2012). Nearby at Cairo, NY, a slightly older forest floor reveals archaeopteridalean and pseudosporochnalean rooting systems in a dry soil (Berry, pers. comm.). In Svalbard, separate stands of lycopsids and archaeopteridaleans are found in partially wet soils (Berry and Marshall, 2015). These forests demonstrate early spatial diversity.

By the Carboniferous Period (229–359 MYA), forests were well established in lowland coastal sites. The best known environments are also wetland communities (Greb *et al.*, 2006), comprising arborescent lycopods reaching a height of 30–40 meters. The trunks contained very little wood. Structural support was instead derived from a thick, bark-like periderm that enclosed soft pith. Ferns and horsetails were other important components of the plant communities, with arborescent forms that could reach heights of 20 m and 10–15 m, respectively. In addition, these forests also provided habitat for smaller pteridosperms (seed ferns), early conifers, and a wide range of smaller ferns, including epiphytes (Taylor *et al.*, 2009). The geological periods of the Devonian and the Carboniferous are significant because they witnessed the evolution of many of the fundamental organs and tissue systems, leading to the evolution of truly large plants and the first forest ecosystems.

1.4 AM symbioses in early (Palaeozoic) land plants

Microfossils in rocks of the mid-Ordovician period (ca 460–470 MYA) provide the earliest evidence of both plants and glomalean fungi (Rubinstein *et al.*, 2010; Redecker *et al.*, 2000), but no direct links between these organisms has been proven. The earliest direct evidence of mycorrhizal symbiosis is based on plants and fungi fosssilized *in situ* in the 407 million year old Rhynie Chert (Trewin, 2004). This site, discovered in 1912 near the village of Rhynie, about 50 km NW of Aberdeen (Scotland), is highly remarkable, both in terms of organismal diversity and the quality of preservation. The cherts formed from erupted hydrothermal fluids that periodically inundated vegetation on a low-energy alluvial plain formed by a braided river channel. Minor variations in topology across the floodplain gave rise to habitats that ranged from terrestrial to fully freshwater or brackish water. Plants, animals and fungi were petrified *in situ* or close to their sites of growth at low temperature, and fossilization is thought to have been relatively rapid, preserving remarkable details of cellular and subcellular structures (Trewin and Rice, 2004).

Between 1917 and 1921, in a series of five classic papers, Kidston and Lang described in detail four early land plants and, in the last paper, several fungi (Kidston and Lang, 1921). Observing the plants *Rhynia gwynne-vaughanii* and *Rhynia major* (now known as *Aglaophyton major*), they reported : "*The distribution and appearance of the layer of cells with very persistent dark contents immediately below the outer cortex suggests the possibility that this region might have contained a symbiotic organism…. Thus in the case of (the two species of) Rhynia also the only conclusion at present seems to be that proof of the existence of mycorrhizas is wanting, though there are grounds for further enquiry into the question*".

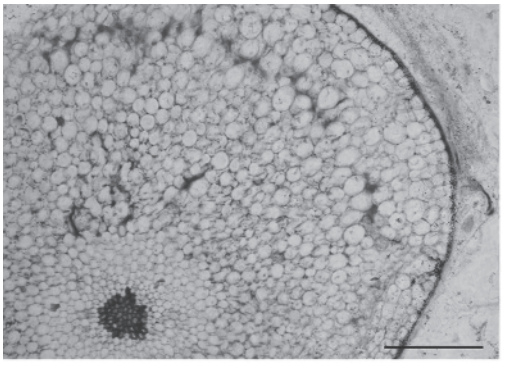
It is interesting to note that, simultaneously, Kidston and Lang discovered the plants and pioneered the concept of early symbiotic relationships. 50 years later,

Boullard and Lemoigne (1971) showed hyphae and vesicles and concluded that the same fungus was involved in a biotrophic, likely mutualistic association with both *Rhynia gwynne-vaughanii* and *Rhynia major* (= *Aglaophyton major*). However, they did not find the arbuscules characteristic of AM association. Unequivocal evidence of arbuscules was first provided by Remy *et al.* (1994) and Taylor *et al.* (1995) in the sporophyte *Aglaophyton major* (Figure 1.4a,b). This plant developed sinuous prostate axes which produced rhizoids in areas in contact with the substrate, allowing fungal colonisation to occur. Arbuscule-like structures were also recorded in *Lyonophyton rhyniensis* (the gametophyte of *A. major*) (Taylor *et al.*, 2005). Only vesicles (Karatygin *et al.*, 2006) have been described in *R. gwynne-vaughanii*, but a clear zone of fungal colonization was present in the outer cortex of the aerial axes, similar to that observed in *Aglaophyton*. Colonisation was not observed in the rhizoids. The fungus involved in the colonization of these plants has been recorded as belonging to Glomeromycota.

Among the three endophytes observed in *Nothia aphylla* (Krings *et al.*, 2007a, 2007b) only one closely resembles *Glomites rhyniensis* (Glomeromycota), the endomycorrhizal fungus of *Aglaophyton major*. However, a different mode of colonization was reported for *Nothia*. Intracellular fungal colonization was observed in the rhizoids and the tissues of the rhizoidal ridge, and intercellular vesicles and spores were produced in the cortex of both prostate and aerial axes, but arbuscules were not observed (Krings *et al.*, 2007a, 2007b).

Recently, two new endophytes were described colonizing the Rhynie Chert plant *Horneophyton lignieri* (Strullu-Derrien *et al.*, 2014a; Figure 1.4c,d). The rooting system of *Horneophyton* is easily distinguished from all other Rhynie plants. It comprises a corm at the base of the aerial axis, with numerous unicellular rhizoids emerging from the

Figure 1.4 Fungal partnerships in Devonian and Carboniferous plants. (a) and (b) Fungal endophyte of the glomeromycotan type in *Aglaophyton major* from the Devonian Rhynie Chert. (a) Transverse section of an aerial axis, showing the well-defined colonized zone in the outer cortex (slide PB V15637 from the Natural History Museum, London). (b) Arbuscule-like structures in an aerial axis (slide from the University of Munster; photograph courtesy of H. Kerp). (c) and (d) Colonization of the mucoromycotean type in *Horneophyton lignieri* from the Devonian Rhynie Chert. (c) Transverse section of a corm; a zonation of fungal colonization is visible within the corm. (d) Intercellular branched thin-walled and intercellular thick-walled hyphae are present. (e) Arborescent clubmoss rootlet from the Upper Carboniferous of Great Britain (slide PB V11472 from the Natural History Museum, London). (f) AM-like fungi in stigmarian appendage. Trunk hyphae, intercalary vesicle (left), and putative arbuscule-like structures (right) are visible (slide BSPG 1964X from the Bavarian State Collection for Paleontology and Geology; photograph courtesy of M. Krings). (g) *Cordaites* rootlet from the Upper Carboniferous of Grand'Croix, France, colonized by AM fungus. The cortex comprises a reticulum of phi thickenings that are prominent in cells located close to the vascular cylinder (slide: Lignier Collection no. 194 from the University of Caen). (h) Detail of an arbuscule-like structure. The hyphal trunk of the arbuscule-like structure branches repeatedly forming a bush-like tuft within the cell (slide: Lignier Collection no. 194 from the University of Caen). Bars = 0.55 mm in A, 30 mm in B, 1.1 mm in C, 120 mm in D, 1.5 mm in E, 70 mm in F, 1.25 mm in G, and 18 mm in H. Copyright American Society of Plant Biologists (from Kenrick and Strullu-Derrien, 2014). (*See insert for color representation of the figure.*)



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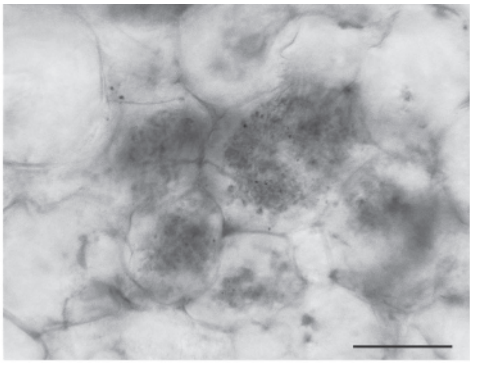

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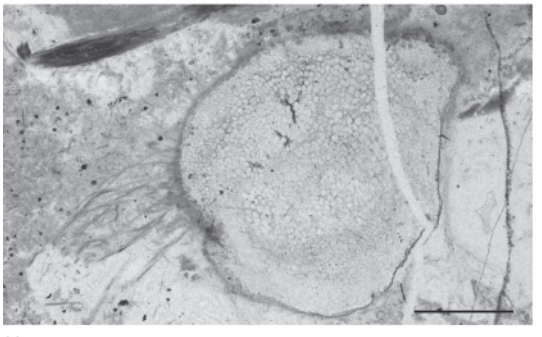

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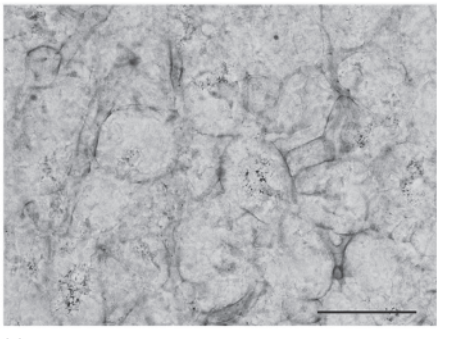

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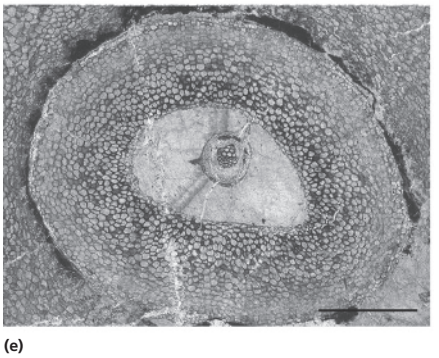

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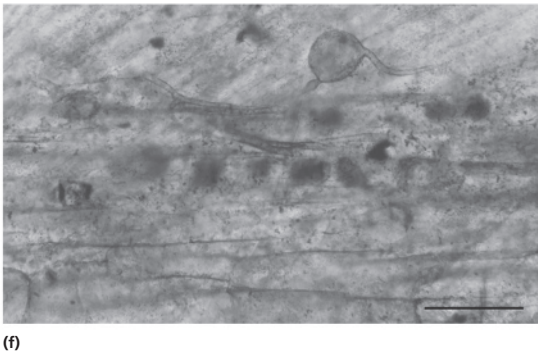

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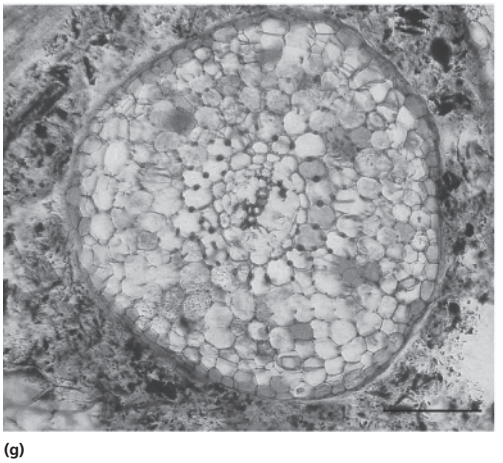

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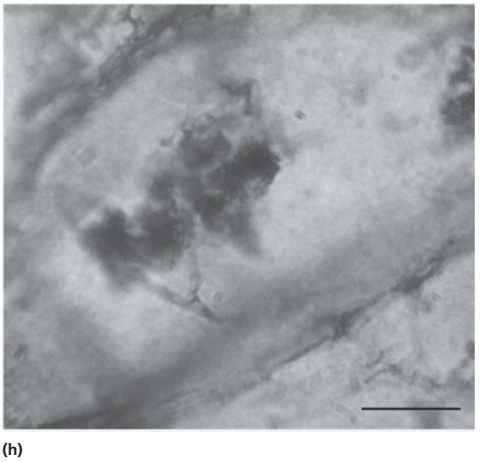

(h)

epidermis. A glomeromycotean fungus (*Palaeoglomus boullardii*) was observed in the outer cortex of the aerial axes, forming arbuscules, vesicles and spores. A fungus of the Mucoromycotina type (*Palaeoendogone gwynne-vaughaniae*) was observed in the corm of the plant, where it was present in intercellular spaces and as intracellular coils but absent from the rhizoids (Strullu-Derrien *et al.*, 2014a; Figure 1.4c,d). Krings *et al.* (2007a, 2007b) speculated that the intra- and intercellular phases of the colonization in *Nothia* might belong to different fungi. Strullu-Derrien *et al* (2014a) suggested that, as in the corm of *Horneophyton*, the intercellular hyphae in *Nothia* were most likely mucoromycotean in nature.

Colonization of the upright axes (Glomeromycota) in *Horneophyton lignieri* probably occurred through the epidermis. The mode of colonization in the corm is unclear, but fungal entry was probably not via the rhizoids. Several modes of fungal entry have been described in Rhynie Chert plants, but caution must be exercised in drawing firm conclusions, because this feature is very difficult to observe in fossils. Critical comparisons between the newly discovered *Horneophyton* endophytes, fungi previously described from the Rhynie Chert, and fungal colonization in extant lower land plants reveal several features characteristic of both Mucoromycotina and Glomeromycota. This finding indicates that early fungal symbioses were more diverse than assumed hitherto, overturning the long-held paradigm that the early endophytes were exclusively Glomeromycota (Strullu-Derrien *et al.*, 2014a). Because Devonian fossil plants are evolutionarily and structurally closer to extant bryophytes and lycophytes, comparisons with these groups, rather than the more derived vascular plants, is appropriate (Field *et al.*, 2015). These geologically early fungal-plant associations are considered to be mycorrhizal-like or paramycorrhizas (Strullu-Derrien and Strullu, 2007).

1.5 Evolution of the mycorrhizal symbioses

During the early phases of land colonization by plants, rooting systems evolved into a broad range of complex multicellular organs specializing in anchorage and nutrient acquisition (see paragraph above). However, the relationships between fungi and early trees are still not documented. Unfortunately, neither the type nor the quality of preservation allows us to observe fungal associations. The bases of the trees when found *in situ* are mostly preserved as casts, with very little anatomy remaining. To develop an understanding of mycorrhizal associations in the earliest forests, new information is needed from permineralized rooting systems or soils in the middle to latter part of the Devonian period (393–359 million years ago). Newly discovered fossils from Eurasia, on which we are currently working, may begin to provide this crucial information.

The following Carboniferous period (359–299 MYA) is famous for its extensive wetland forest communities, which gave rise to extensive coal fields in Eurasia and North America. Krings *et al.* (2011) reported an AM-like fungus in the underground organs of arborescent lycopsids from the Upper Carboniferous (ca 315 MYA). These plants had unique rooting organs (called *Stigmaria*) that developed into large, shallow bifurcating trunks that bore numerous narrow "rootlets"

(Rothwell *et al.,* 2014). The stigmarian base apparently formed by dichotomy of the shoot during embryogeny, and the "rootlets" are considered to be leaf homologues. The fungus developed near the tip of the appendages, and occupied the inner portion of the middle cortex. Hyphal threads grew along the long axis of the rootlet. Extending from these trunk hyphae were narrower hyphae that may have produced large vesicles or spores. Other branches penetrated individual cells of the cortex to form multibranched structures, interpreted as arbuscules (Krings *et al*., 2011) (Figure 1.4e,f).

The earliest fungal colonization of seed plant roots (eumycorrhizas) to date was observed in *Cordaites* (basal Coniferophytes) from the Upper Carboniferous (ca 315 MYA) (Strullu-Derrien *et al.,* 2009). AM associations developed on young rootlets exhibiting only primary growth (0.5 to 0.65 mm diameter). The fungus colonized a discontinuous zone in the central layers of the cortex. Colonization was characterized by the absence of an intercellular phase, and by the development of intraradical hyphae. While vesicles were not observed, small arbuscules did develop in some of the cortical cells (Figure 1.4g,h). Additional details of the association are difficult to resolve, owing primarily to the prominence of cortical thickenings in the rootlets. A similar masking of fine details of the mycorrhiza by cortical cell thickenings has been recorded for extant plants (cf. *Thuya occidentalis*).

Recently, mycorrhizal symbiosis was reported in the extinct gymnosperm order Glossopteridales, based on structurally preserved fossils from the Upper Permian of Antarctica (ca 260–252 MYA) (Harper *et al.,* 2013). The fungus was characterized by septate hyphae, and it was attributed to the genus *Glomites* (Taylor *et al.,* 1995), which now includes forms with aseptate to (sparsely) septate hyphae (Harper *et al.,* 2013). The fungus colonized the cortical cells of *Vertebraria* (rootlets of the seed fern *Glossopteris*) in a serpentine or helical pattern that resembles modern Paris-type mycorrhizas. Intracellular vesicles were also reported, but their occurrence was not well corroborated by the images.

Taylor *et al.* (1995) interpreted the colonization in *Aglaophyton* as symptomatic of the Arum-type, one of the two major anatomical types of colonization by AM fungi recognized in higher plants, and often associated with the fast-growing root systems of crop plants (Smith and Read, 2008). Harper *et al.* (2013) reported that the Glossopteridales specimen was the only fossil that did not have the Arum-type arbuscule morphology. However, and as also recognized by several authors (Taylor *et al*., 1995; Selosse, 2005; Strullu-Derrien *et al*., 2014a), extreme caution should be exercised when comparing fungal structures in early fossil land plants with those in modern species, especially late divergent analogues.

Root nodules (i.e. short lateral roots harboring fungal symbionts) (Russell *et al*., 2002; Dickie and Holdaway, 2011) have rarely been described in the fossil record, but recently discovered evidence suggests a lengthy geological history in gymnosperms. Schwendemann *et al.,* (2011) described root nodules in the early conifer *Notophytum* (Middle Triassic, 245–230 MYA, Antarctica) reporting probable fungal arbuscules in the cortex. This is by far the oldest known record. Cantrill and Douglas (1988) described fossil roots with nodular and abbreviated lateral roots from the Lower Cretaceous (113–100 MYA) of the Otway Basin, Victoria (Australia). A mycorrhizal

association was suggested on the basis of the general morphology of the roots, but the anatomy was not preserved and arbuscules were not observed. The roots were likely coniferous, belonging either to Taxodiaceae or Podocarpaceae.

Following a huge gap in the fossil record of mycorrhizas, material from the Middle Eocene (ca 50 MYA) has shown that both AM and ECM co-existed at that time, and that ECM occurred contemporaneously within both Gymnosperms (Pinaceae) and Angiosperms (Dipterocarpaceae). AM were described from anatomically preserved roots of the taxodiaceous conifer *Metasequoia milleri* (Stockey *et al*., 2001). Mycorrhizal structures developed in the root cortex. Coiled hyphae were most common within cells of the inner cortical region, and these produced numerous, highly branched arbuscules.

The earliest direct fossil evidence of ECM comes from roots attributable to *Pinus* in the 50 million year old Princeton Chert. The fossils show a Hartig net that extended to the endodermis, a pseudoparenchymatous mantle, and contiguous extramatrical hyphae. The mycorrhizal rootlets lacked root hairs, and they dichotomized repeatedly, to form large, coralloid clusters (LePage *et al.,* 1997). Reproductive structures were absent. The authors suggested comparison with the extant Basidiomycota genera *Rhizopogon* and *Suillus*. Recently, ECM preserved in amber were reported from an Eocene angiosperm forest (Beimforde *et al.,* 2011). Unramified, cruciform and monopodial-pinnate ectomycorrhizas were fossilized adjacent to plant rootlets, and different developmental stages of the mycorrhizas were preserved. The mycobiont *Eomelanomyces cenococcoides* is considered to be an ascomycete, and the host was most likely a species of Dipterocarpaceae.

Currently, there is no direct fossil evidence of ectendomycorrhizas or endomycorrhizas in the orchids (ORM) and Ericaceae (ERM). A first estimate of the time of origin of these mycorrhizal forms can be derived from estimates of the age of origin of their host plant clade, derived either from fossil evidence or from calibrated molecular phylogenies of angiosperms. Direct fossil evidence of Orchidaceae is extremely rare, so one must rely on calibrated molecular phylogenies. Ramirez *et al.* (2007) suggested an origin of Orchidaceae during the late Cretaceous (76–84 MYA), coupled with a Cenozoic radiation of the most diverse epiphytic clades (Figure 1.1). In contrast, Ericaceae has an extensive fossil record (Friis *et al*., 2011), and there are fossils assignable to the modern ERM genus *Leucothoe* from the Late Cretaceous (66–72 million years) of Central Europe (Knobloch and Mai, 1986), providing an indicative minimum age for the origin of ERM. In molecular phylogenies of Ericaceae, if one excludes the basal *Enkianthus* (AM) and the Arbutoideae and Monotropideae (further specializations in arbutoid and monotropoid mycorrhizas), the remainder of the species are basically ERM. The most recent calibrated molecular phylogenetic trees indicate a mid-Cretaceous origin for ERM (Schwery *et al*., 2014). Despite the absence of direct fossil evidence for ORM and ERM, indirect fossil evidence of host plants, together with calibrated molecular phylogenies, imply that they evolved much later than AM and ECM, probably during the Cretaceous period.

A current hypothesis is that at the rise of ORM and ERM, fungal taxa that usually colonize the roots of other plants as endophytes were recruited as specific symbionts (see below; Selosse *et al.,* 2009; van der Heijden

et al., 2015). Thus, the ancestral AM mycorrhizas underwent replacement by other types of mycorrhizas and fungal partners in diverse plant lineages. While an adaptation to specific soil conditions (e.g., Selosse and Le Tacon, 1998; Smith and Read, 2008) is postulated to have driven this process, its timing and causes still deserves study, especially based on a closer inspection of the fossil record.

1.6 Perspectives for bridging paleomycology and genomics

Berbee and Taylor (2010) questioned how close we are to dating the phylogenetic tree of fungi. They concluded that molecular clocks calibrated by fossils are the only available tools to estimate timing of evolutionary events in fossil-poor groups. Fungi are not simply ancient and unchanging, but have evolved just as dynamically as any other group of eukaryotes, even if limited morphological criteria are available to mark this. Our brief review of the fossil record of mycorrhizal associations shows how sparse is the evidence and yet, where encountered, how informative it can be.

One problem is that discoveries of fossil mycorrhizal associations have been largely serendipitous. A second is that mycorrhizas are only preserved in a very particular and restricted set of environments of fossilization (Taylor *et al.*, 2015). Essentially, what is required is soils that are petrified, preferably in silicates, and in which original plant root cells and fungal hyphae are preserved. Such systems do occur throughout the geological record (e.g., Rhynie Chert, 407 MYA: Trewin and Rice, 2004; Central Transantarctic Mountains, Antarctica, 260–252 MYA: Harper *et al.*, 2013; Hopen, Svalbard Archipelago, 220–220 MYA: Strullu-Derrien *et al.*, 2012; Princeton chert, Columbia, 50 MYA: LePage *et al.*, 1997; Stockey *et al.*, 2001). We therefore advocate an approach that targets particular environments of preservation with specific evolutionary questions in mind.

There are two main areas in which the fossil record of mycorrhizal associations and modern genomic approaches can potentially interface and benefit from reciprocal illumination. First, fossils can help to establish the sequence in which evolutionary events occurred, and they can set minimum geological ages to the origins of taxonomic groups or organismal associations. Second, fossils fill in the gaps by extending our knowledge of the diversity of mycorrhizal associations across the plant tree of life, and by broadening our understanding of the interactions of plant and fungus at the cellular level. Furthermore, the application of high-resolution imaging techniques (e.g., Confocal Laser Scanning Microscopy) now affords a new and enhanced level of precision in documenting the details of fungal plant interactions at the cellular and subcellular levels (Strullu-Derrien *et al.*, 2015). Fossils are essential to the calibration of the tree of life of fungi and of plants, and they can provide tests of evolutionary hypotheses arising from our current understanding of the evolution of mycorrhizas, and newly formed questions emerging from the fungal tree of life and from genomic studies (Selosse *et al.*, 2015).

Ectomycorrhizal symbioses evolved from ecologically diverse decayer precursors and radiated in parallel, following the origins of their host-plant lineages (Floudas *et al.*, 2012; Kohler *et al.*, 2015). The highly polyphyletic evolution of the ECM lifestyle (Hibbett and Matheny, 2009; Tedersoo and Smith, 2013)

is marked not only by convergent losses of different components of the ancestral saprotrophic apparatus, but also by rapid genetic turnover in symbiosis-induced genes (Martin and Selosse, 2008; Eastwood *et al.*, 2011; Plett and Martin, 2011; Floudas *et al.*, 2012; Wolfe *et al.*, 2012; Kolher *et al.*, 2015). In contrast, ericoid and orchid mycorrhizal fungi retained an extensive decay apparatus that is probably exploited indirectly by the plant for carbohydrate supply, thus explaining their known saprotrophic ability (Kolher *et al.*, 2015).

Recent studies (Selosse *et al.*, 2009) provided evidence that Sebacinales (basal Hymenomycetes, Basidiomycota, with diverse mycorrhizal abilities, ranging from ECM to ERM and ORM) are endophytic in many roots systems *in natura* (Selosse *et al.*, 2009) leading to the hypothesis that many mycorrhizal lineages evolved from former root endophytes, because endophytism could act as a symbiotic "waiting room", predisposing the fungus to evolution towards a tighter mutualism with some hosts (Selosse *et al.*, 2009; van der Heijden *et al.*, 2015). There is much interest in understanding how genomes evolved in both plants and fungi to make this possible. Knowledge of the chronology of these events is also important to investigating potential environmental drivers (Selosse *et al.*, 2015).

Gymnosperms were hugely diverse during the Mesozoic era, and many important groups are now extinct. A targeted study of permineralized fossil soils would provide information on the extent to which ECM were present in gymnosperms of this time, and how they might have developed in ancient Pinaceae and in the extinct relatives of the Gnetales, such as Bennettitales. Knowledge of the early evolution of mycorrhizal associations in gymnosperms and angiosperms would also benefit from a better understanding of mycorrhizas in living species across the plant tree of life. Although ECM relations are widely reported in angiosperms, they have been documented in detail for only about 3% of living species. In particular, knowledge of their occurrence and development in basal lineages of angiosperms (e.g., *Amborella*, Austrobaileyales, Chloranthaceae, magnoliids) is lacking (Wang and Qiu 2006). The genome sequences of mycorrhizal fungi which are now available, together with those already planned and in progress, will represent foundational information for understanding the development and functioning of the mycorrhizal symbiosis (Martin and Bonito, 2013).

To understand how genomic level changes within land plants impacted on the evolution of AM it is necessary to establish the original mode of infection and host response in the earliest land plants. The early development of AM symbioses is currently best documented in the plants and fungi of the 407 million year old Rhynie Chert. Although the presence of AM has been recorded in several species, very little is understood about the details of the infection pathways and the reactions of the plants to infection. Furthermore, at least two major clades of fungi (Glomeroycota and Mucoromycotina) are now implicated in mycorrhizal symbioses in both living bryophytes and early fossils (Bidartondo *et al.*, 2011; Desirò *et al.*, 2013; Rimington *et al.*, 2015, 2016). Given that Glomeromycota and Mucoromycotina are two sister lineages (Tisserant *et al.*, 2012; Lin *et al.*, 2014), it might also be possible that their common ancestor interacted with the earliest plants. This emerging possibility deserves further analyses in both fossil and living species. A focused comparative study is needed that incorporates information

from Rhynie Chert fossils with a detailed analysis of mycorrhizal development in living groups, including liverworts, hornworts, lycopsids and ferns, to infer the original modes of infection of land plants and the basic repertoire of plant responses.

Research on the origin of the genes acting in the fungal symbiotic pathway now focuses on algal lineages related to land plants, such as charophytes. A stepwise evolution of the plant symbiotic "toolkit" in algal ancestors, with several components predating the first land plants, has been proposed recently (Delaux *et al.*, 2013). Elements of this "toolkit" may, therefore, first have facilitated the interactions between aquatic charophytes and diverse symbiotic microorganisms, later being recruited and further developed for AM evolution on land. A broader survey of the distribution and function of these genes within living green algae, especially these close to land plants, is now desirable, and the investigation of living and fossil Charophyta-fungus interactions may offer further insights.

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