Unearthing the roots of ectomycorrhizal symbioses

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Abstract | During the diversification of Fungi and the rise of conifer-dominated and angiosperm-dominated forests, mutualistic symbioses developed between certain trees and ectomycorrhizal fungi that enabled these trees to colonize boreal and temperate regions. The evolutionary success of these symbioses is evident from phylogenomic analyses that suggest that ectomycorrhizal fungi have arisen in approximately 60 independent saprotrophic lineages, which has led to the wide range of ectomycorrhizal associations that exist today. In this Review, we discuss recent genomic studies that have revealed the adaptations that seem to be fundamental to the convergent evolution of ectomycorrhizal fungi, including the loss of some metabolic functions and the acquisition of effectors that facilitate mutualistic interactions with host plants. Finally, we consider how these insights can be integrated into a model of the development of ectomycorrhizal symbioses.

One of the most fundamental requirements for forest trees to flourish is the ability to acquire limited nutrients, such as nitrogen and phosphorus, and water from soil. As the levels of bioavailable inorganic nutrients in forest soils are often too low to sustain plant growth, most trees rely on mycorrhizal fungal symbioses for their nutrition[1,2]. As such, the establishment of the mycorrhizal lifestyle was a pivotal event in the evolutionary history of land plants[3,4]. Subsequently, soil-borne mycorrhizal fungi, such as arbuscular mycorrhizal fungi and ectomycorrhizal fungi (BOX 1), helped to shape plant communities through mutualistic relationships with rhizoid-based rooting systems and roots[5–8] (BOX 2).

A remarkable number of ectomycorrhizal basidiomycetes and ascomycetes (more than 20,000 species) have established symbioses with ~6,000 tree species, including pines, beeches, oaks, eucalypts, dipterocarps and poplars, whereas arbuscular mycorrhizal glomeromycetes have established symbioses with ~200,000 plant species, including poplars, eucalypts and some gymnosperms[2,6,9]. Thus, these symbioses have a broad influence on forest ecosystems. For example, extensive forests across the temperate, boreal, subtropical and mountainous ecoregions of the Northern Hemisphere and Southern Hemisphere are composed of tree species that have been colonized by ectomycorrhizal fungi[1,2,10]. In each of these forests, trillions of plant rootlets are colonized and interconnected by the mycelium of hundreds of different species of ectomycorrhizal fungi, forming extraradicular mycorrhizal networks that have been informally termed the 'wood-wide web' (REFS 11–13).

It is important to note that ectomycorrhizal fungi occupy a dual niche; that is, the soil and the host root. Similarly to their saprotrophic ancestors, ectomycorrhizal fungi have access to mineral nutrients in the soil that are efficiently absorbed by the perennial absorbing mycelial network and partly translocated to the host root[1,9,14]. However, ectomycorrhizal fungi have lost much of the ability of saprotrophic fungi to efficiently decay the lignocellulose that accumulates in wood and soil organic matter[15]. Adaptation to the ectomycorrhizal lifestyle has not only involved loss of functions; ectomycorrhizal fungi have also gained some of the mechanisms that are used by biotrophic plant pathogens to colonize root tissues and capture host glucose[16–18] (although ectomycorrhizal fungi lack the parasitic morphological structures that are specific to pathogens, such as the haustorium). These adaptations suggest that ectomycorrhizal symbiosis provides a useful model for the study of the evolution of nutritional modes in fungi[19,20].

Although the vast majority of ectomycorrhizal fungi share a typical anatomical pattern — a hyphal network (known as the Hartig net) that forms inside root cells, a sheathing mantle around rootlets and extramatrical hyphae that explore the rhizosphere and nearby soil niches[14,21] (FIG. 1) — the phenotypic diversity of these fungi is broad, owing to variation in morphology, anatomy, physiology, host species and ecological specialization[2,6,9,21]. However, only a small number of ectomycorrhizal symbioses have been studied at a molecular level, as studies have mainly focused on the model associations between *Laccaria bicolor* and poplars, *Hebeloma cylindrosporum*

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Box 1 | **The four most common types of mycorrhizal symbiosis**

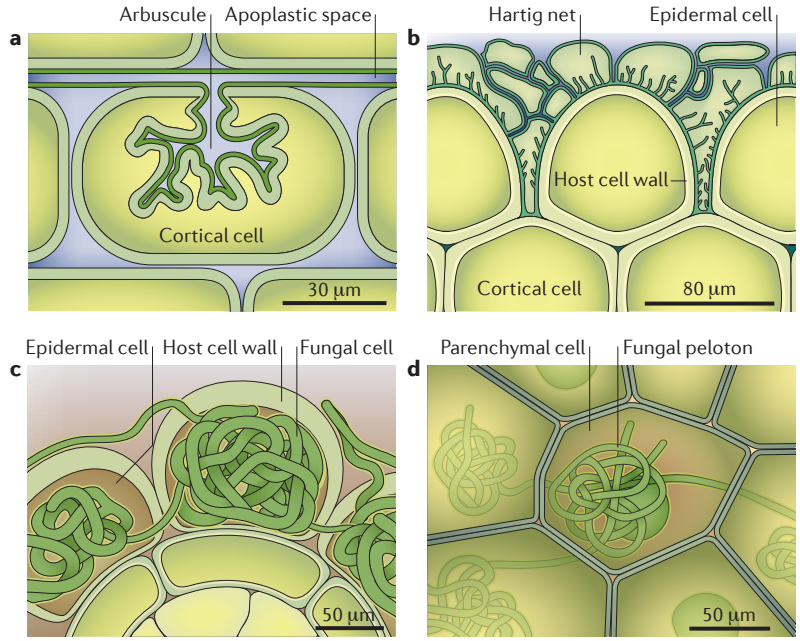
There are seven major classes of symbiotic plant–fungal interaction[1,2,6,21], among which the most common classes are ectomycorrhiza, arbuscular mycorrhiza, orchid mycorrhiza and ericoid mycorrhiza (see the figure). Each class is classified based on host plant and characteristic symbiotic structures. In arbuscular mycorrhiza (see the figure, part **a**), hyphae that emanate from germinated glomeromycete spores grow in the apoplastic space between plant root cells and then penetrate cortical cells, thereby forming tree-like arbuscules. Arbuscules are separated from the cortical cell cytoplasm by a periarbuscular membrane and an interfacial polysaccharide matrix, which are both derived from the fungus (the development of this symbiosis is reviewed in REF. 18).

In ectomycorrhiza (see the figure, part **b**), hyphae from basidiomycetes and ascomycetes from a soil propagule, or from older ectomycorrhizal rootlets, attach onto epidermal cells of emerging lateral roots at a site known as the mycorrhizal infection zone[2,21]. Attached hyphae proliferate and differentiate into a series of hyphal layers, to form a pseudoparenchymatous tissue that is known as the sheathing mantle[21,87] (FIG. 1); these structures contain air and water channels that transport nutrients into the symbiotic cells[21,87]. The network of hyphae, which is known as the Hartig net, develops around epidermal root cells in angiosperms and around both epidermal and cortical root cells in gymnosperms[2,21,87]. The colonization of the host apoplastic space mainly relies on the mechanical force that results from hyphal extension, although the intrusion is probably enhanced by the secretion of fungal polysaccharide lyases[21], such as symbiosis-upregulated GH5 endoglucanases and GH28 polygalacturonases[40,41,43,78]. The Hartig net — with its complex labyrinthine hyphal branching and large surface area — forms an efficient interface for the bi-directional transport of nutrients through the cell wall that forms the surface of host cells[14,21] (FIG. 1). Although the vast majority of ectomycorrhizal roots that develop in symbioses with the thousands of species of ectomycorrhizal fungi share these fundamental anatomical features, ectomycorrhizal associations vary widely in the importance of their different hyphal structures (that is, the mantle sheathing, Hartig net and extramatrical hyphal networks), cellular anatomy[88] and physiological properties (such as the intense bi-directional transport of metabolites)[2].

In ericoid mycorrhiza (see the figure, part **c**), symbioses are formed between fungi and plants of the Ericaceae family (commonly known as heath plants); fungal hyphae contact the thickened epidermal cell walls of the very fine roots (known as hair roots) of the heath plant, penetrate through these walls and form intracellular hyphal complexes in epidermal cells[21]. Intracellular fungal coils are surrounded by the plant membrane.

In orchid mycorrhiza (see the figure, part **d**), fungal hyphae penetrate through plant cell walls in the parenchyma cells of protocorms and roots of orchids to form hyphal complexes called pelotons[2,21]. These complexes are encased by a plant-derived membrane and, at the plant–fungus interface, a polysaccharide matrix[21].

Box figure adapted with permission from Peterson, R. L. & Massicotte, H. B. Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. *Canadian Journal of Botany* **82**, 8, 1074–1088 (2004), © Canadian Science Publishing.



and pine, *Tuber melanosporum* and hazel, and *Pisolithus* spp. and eucalypts. These studies have shown that the development of symbioses entails the functional activity of several gene networks that are involved in a complex series of partially interrelated sequential steps[22,23]. As such, ectomycorrhizal symbioses could provide useful model systems in which to decipher how gene networks modulate the functioning of an ecosystem. However, the molecular mechanisms that underlie the morphology, anatomy and physiology of these symbioses have yet to be elucidated. Indeed, a molecular definition of the development of ectomycorrhizal symbioses remains a major challenge for the field[9] — a challenge that recent genome-sequencing projects[19,23–26] are helping to address.

In the light of the contributions of genomic data, the study of the evolution of the ectomycorrhizal lifestyle and the mechanisms of ectomycorrhizal development is now primed for rapid advances that will provide a broader understanding of forest ecosystems and the roles of ectomycorrhizal fungi therein. In this Review, we highlight the main insights that have been gleaned from these recent developments in ectomycorrhizal genomics, focusing on the loss of the lignocellulose decay apparatus and the acquisition of mycorrhiza-induced small secreted proteins (MiSSPs) that facilitate interactions with plant hosts as key evolutionary processes that drive the convergent evolution of the ectomycorrhizal lifestyle. Furthermore, we discuss molecular studies that have shed some light on how MiSSPs are able to dampen plant defences so that ectomycorrhizal fungi are able to colonize the roots of plant hosts. Finally, we identify the major questions that remain unanswered and propose new avenues of research that may help to do so.

Loss of genes for the decay of organic matter

Ectomycorrhizal hyphae are in close contact with lignocellulose during their exploration of the organic matter in soil and when colonizing the apoplastic space between the rhizodermis and host root cortex[1,2,21]. However, the ability of ectomycorrhizal fungi to decay lignocellulose has been debated, as molecular phylogenetic analyses have suggested that ectomycorrhizal fungal species, which have evolved repeatedly from diverse soil and wood saprotrophic ancestors, do not revert to free-living conditions, thereby suggesting a lack of metabolic capacity[27–29]. Therefore, whether ectomycorrhizal fungi have a crucial role in the decomposition of biopolymers, such as lignocellulose components, that accumulate in plant cell walls and soil organic matter is also debated[15,30–33]. By contrast, wood-decaying fungi and soil saprotrophs produce a diverse array of secreted enzymes that degrade organic matter, most of which belong to families of plant cell wall-degrading enzymes (PCWDEs) that depolymerize cellulose, hemicellulose and pectin, which are the main structural polysaccharide components of the plant cell walls that form wood[34] (FIG. 2). These PCWDEs all belong to carbohydrate-degrading families of carbohydrate-active enzymes (CAZymes), such as glycosyl hydrolases (for example, cellulases and endoglucanases), carbohydrate esterases and polysaccharide lyases (for example, polygalacturonases). The genomes

Box 2 | **Evolution of mycorrhizal symbioses**

Even before roots evolved, the early land plants, which arose in the Early Ordovician period (450–460 million years ago (Mya)), had fungal associations that resembled extant arbuscular mycorrhiza[3,5–8]. However, it is debated whether these paramycorrhizal interactions occurred subsequent to, or coincided with, the origin of land plants[6]. Lycopsids (order Lepidodendrales), which were the tree-like plants that formed the first extensive swamp forests during the Carboniferous period (300–359 Mya), had arbuscular mycorrhiza-like associations[89] in their below-ground organs. Later, early conifers (species in the Araucariaceae, Podocarpaceae, Cupressaceae and Taxodiaceae families) from the Triassic period (201–252 Mya) and Jurassic period (145– 201 Mya) were colonized by arbuscular mycorrhizal fungi that formed symbioses that resemble those observed in extant arbuscular mycorrhiza[6]. Later still, members of Pinaceae family, which became established in the Late Jurassic and Early Cretaceous periods (140–180 Mya), formed primitive ectomycorrhizal associations. Phylogenetic and biogeographical studies support the diversification of symbioses between ectomycorrhizal fungi and conifers until about 50–60 Mya, during the Late Palaeocene and Early Eocene[27–29]; the fossil records of ectomycorrhizal roots date back to about 50 Mya, in the Middle Eocene[90]. Ancestral ectomycorrhizal clades from the Early Cretaceous and Late Cretaceous periods were probably involved in symbiotic associations with angiosperms from Palaeotropical regions[28]. The rise and rapid diversification of the major ectomycorrhizal Basidiomycota lineages, such as species in the Cortinariaceae, Boletaceae, Amanitaceae and Russulaceae families, seem to have closely tracked the expansion of the angiosperm and conifer hosts of these fungi[27–29] and were probably driven by increasing host and habitat specificity that began in the Late Cretaceous, Oligocene or Eocene periods (30–90 Mya; partly attributed to the rise of the Fagales-dominated temperate forests[91] that occurred as a consequence of drying and cooling from the Late Eocene[1]). The switch from saprotrophic to mycorrhizal nutrition modes probably happened convergently during fungal evolution, and in many independent lineages[20,27,29,43] (FIGS 2,3).

Rhizoid-based rooting systems
Simple hair-like protuberances that extend from the epidermal cells of certain plants. Rhizoids are similar in structure and function to the root hairs of vascular land plants.

Basidiomycetes
(Formally known as Basidiomycota). A division or phylum within the kingdom Fungi that, together with the ascomycetes (formally known as Ascomycota), constitute the subkingdom Dikarya (often referred to as 'higher fungi'). Basidiomycetes reproduce sexually through the formation of specialized club-shaped end cells, known as basidia, that contain meiospores.

Ascomycetes
(Formally known as Ascomycota). A division or phylum in the kingdom Fungi that, together with the basidiomycetes, form the subkingdom Dikarya. Members of the Ascomycota are commonly known as the sac fungi. The defining feature of ascomycetes is the ascus, a microscopic sexual structure in which meiospores, known as ascospores, are formed.

Glomeromycetes
(Formally known as Glomeromycota). One of the seven currently recognized phyla in the kingdom Fungi. The 230 recognized species are all obligate symbionts of land plants that form arbuscular mycorrhizal associations.

Mycorrhizal networks
Underground networks of hyphae that are produced by mycorrhizal fungi. Mycorrhizal networks connect individual plants together and transfer water, carbon and other nutrients.

Saprotrophic fungi
Fungi that obtain their nutrition from non-living organic material.

Rhizosphere
The soil that surrounds and is influenced by the roots of a plant.

of these fungi also encode other CAZymes, such as glycosyltransferase and auxiliary activities (enzymes that have redox functions that are auxiliary to carbohydrate metabolism, such as class II peroxidases and laccases)[34]. It has been suggested that CAZymes that attack crystalline cellulose, which include glycosyl hydrolase 6 (GH6) and GH7, expanded early in the evolution of the Agaricomycetes, followed soon thereafter by the innovation of lignin oxidation, which resulted in families of lignin-oxidizing enzymes, such as class II peroxidases. These enzymes were the defining feature of the first white-rot fungi, which arose around the time of the divergence of the Auriculariales (~280 Mya)[35,36]. However, genome analyses have revealed decreases in the size of families of class II peroxidases and other decay-related enzymes that have occurred in parallel in the independent lineages of ectomycorrhizal fungi (and also in lineages of orchid mycorrhizal fungi and saprotrophic brown-rot fungi; FIG. 3).

Organic matter decay by saprotrophic fungi. During their colonization of wood, white-rot basidiomycetes use oxidoreductases, such as class II peroxidases, to oxidize lignin and simultaneously use a large arsenal of glycosyl hydrolases (such as cellulases, hemicellulases, carbohydrate esterases, polysaccharide lyases and lytic polysaccharide monooxygenases) to degrade cellulose and hemicellulose[37–39]. Whereas white-rot fungi oxidize lignin, brown-rot fungi release sequestered carbon from lignocellulose without substantially removing the recalcitrant lignin that encases the structural polysaccharides. Instead, they use highly oxidizing hydroxyl radicals, which are produced as a result of extracellular Fenton reactions ($H_2O_2 + Fe^{2+} + H^+ \rightarrow H_2O + Fe^{3+} + OH^{\bullet}$), to modify lignocellulose through mechanisms such as hydrogen abstraction and demethylation or demethoxylation; these hydroxyl radicals also facilitate the oxidative-then-hydrolytic mechanism of the restricted set of glycosyl hydrolases that are expressed by brown-rot fungi[37–39]. The Fenton reaction depends on the reduction of Fe^{3+}, which is present in wood, to Fe^{2+}, as well as on the reduction of O_2 to H_2O_2; cellulose dehydrogenase or other members of the glucose–methanol–choline (GMC) family of oxidoreductases (CAZy subfamily AA3.2) are thought to be involved in these reductions[37–39].

Organic matter decay by ectomycorrhizal fungi. The sequencing of ectomycorrhizal genomes has enabled the assessment of the phylogenomic relationships between symbiotic fungi and their saprotrophic cousins[40–44]. Analysing the gene repertoires of symbiotic basidiomycetes and ascomycetes (10 ectomycorrhizal, two orchid mycorrhizal and one ericoid mycorrhizal species) confirmed the repeated independent evolution of ectomycorrhizal fungi from ecologically diverse ancestors (brown-rot and white-rot wood-decaying fungi and other saprotrophs), which led to a radiation of ectomycorrhizal fungi that occurred in parallel in most Agaricomycete orders (FIGS 2,3; although we note exceptions, such as Polyporales, Gloeophyllales and Corticiales)[43]. The polyphyletic evolution of the ectomycorrhizal lifestyle and the associated shift in nutritional mode is marked by convergent losses of numerous PCWDEs and lignin-oxidizing class II peroxidases from ancestral saprotrophs (FIG. 3), and genome erosion is still ongoing in some lineages. However, the ancestors of ectomycorrhizal fungi are diverse in their saprotrophic mechanisms, and gene losses in ectomycorrhizal lineages are not uniformly distributed across PCWDE and oxidoreductase gene families. Losses are most pronounced in genes that encode class II peroxidases, which are essential for lignin oxidation, and exocellulases that degrade crystalline cellulose, for which the nearly complete absence of genes that encode the GH6 and GH7 families of cellobiohydrolases is remarkable[40,41,43]. Both the GH6 and GH7 gene families are present in soil saprotrophs and white-rot fungi, as well as the orchid

mycorrhizal fungi *Serendipita vermifera* (formerly known as *Sebacina vermifera*) in the order Sebacinales and *Tulasnella calospora* in the order Cantharellales, and the beneficial endophyte *Serendipita indica* (formerly known as *Piriformospora indica*) in the order Sebacinales, but they are absent from the genomes of the Agaricomycetidae, which are ectomycorrhizal fungi that include species in the orders Agaricales and Boletales, and most brown-rot fungi[43] (BOX 3). In the Ascomycota, the Périgord black truffle (*T. melanosporum*) lacks genes in the GH6 and GH7 families[41], whereas the sole ectomycorrhizal species in the Dothideomycetes, *Cenococcum geophilum*, has retained these genes[44]. In contrast to GH6 and GH7, all ectomycorrhizal fungi have retained at least one gene that encodes a lytic polysaccharide monooxygenase (LPMO), which is a newly classified oxidoreductase that seems to be important for the decomposition of cellulose and chitin[38]. Nonetheless, phylogenetic tree reconciliation analyses suggest that the loss of LPMOs occurred in the ectomycorrhizal Agaricomycetidae and Tuberaceae lineages[41,43]. Compared with white-rot fungi, brown-rot fungi and ectomycorrhizal fungi have a much lower co-occurrence of oxidoreductases that degrade lignocellulose, including LPMO–cellobiose dehydrogenase genes[45]. Some LPMOs have been shown to carry out oxidative cleavage of chitin, which is the primary constituent of fungal cell walls. Accordingly, it is possible that the LPMO genes that are retained in ectomycorrhizal genomes have functions other than lignocellulolysis, perhaps including hyphal growth and the development of fruiting bodies.

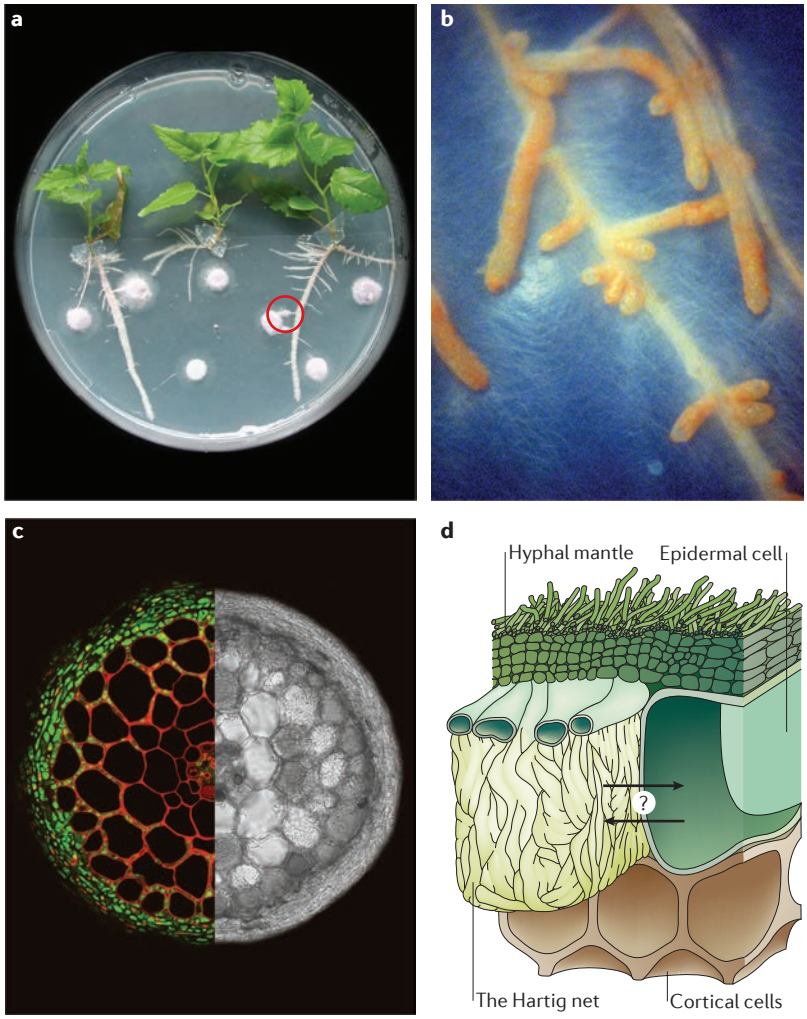


Figure 1 | **The *Populus* spp.–*Laccaria bicolor* ectomycorrhizal symbiosis: an *in vitro* model system.** *Populus* spp.–*Laccaria bicolor* is extensively used as an *in vitro* model system for the investigation of the development and function of mutualistic associations in ectomycorrhizal symbioses. **a** | The *in vitro* growth of an ectomycorrhizal symbiosis between *Populus tremula x alba* and *L. bicolor*; *P. tremula x alba* plantlets and *L. bicolor* hyphae can be seen emanating from agar plugs. An example of an ectomycorrhizal rootlet is highlighted with a red circle. **b** | The ectomycorrhizal roots of a *P. tremula x alba*–*L. bicolor* S238N symbiosis. Note the extensive clustering of ensheathed rootlets that results from substantial changes in auxin metabolism[51,56]. **c** | A transverse section through a rootlet of *Populus trichocarpa* that has been colonized by *L. bicolor* (green). A dense pseudoparenchymatous mantle of fungal mycelium ensheaths the external surface of the rootlet, and intrusions of *L. bicolor* hyphae can be seen between the cell walls (red) of epidermal and cortical cells; these intrusions collectively form a fungal network in plant root cells that is known as the Hartig net. **d** | A 3D reconstruction of the ectomycorrhizal interaction that shows the hyphal mantle, which is formed by layers of aggregated hyphae that cover the root surface, and the labyrinthine Hartig net. The proliferation of membranes favours the bi-directional movement of signals and nutrients between symbionts; however, the type of signals and metabolites that move between the two partners is unknown. Image in part **a** courtesy of A. Jambois, French National Institute for Agricultural Research (INRA). Image in part **b** courtesy of J. Felten, Swedish University of Agricultural Sciences. Image in part **c** courtesy of J. Plett, Western Sydney University, Australia. 3D reconstruction in part **d** courtesy of H. Lagrange, INRA.

In contrast to the diverse set of class II peroxidase genes that are responsible for lignin modification and degradation that are found in white-rot wood-decaying fungi, ectomycorrhizal fungi and brown-rot wood-decaying fungi have few, if any, such genes. *H. cylindrosporum* and *Cortinarius glaucopus* are the only sequenced ectomycorrhizal species to have genes that encode lignin-targeting peroxidases (these genes encode atypical manganese peroxidases (MnP) that are related to other atypical MnP genes found in wood-decaying fungi)[31,43]. The peroxidases of *L. bicolor* are not expressed in symbiotic tissues, whereas one of the *H. cylindrosporum* peroxidases is upregulated in root tips, which suggests that it may be have a role in the colonization of the host root, rather than in the degradation of lignin[43]. Parallel losses of lignin peroxidase genes have occurred in each lineage of ectomycorrhizal Agaricomycetes, but several ectomycorrhizal species are probably still capable of lignin oxidation and/or oxidation of polyphenolic residues that are found in the soil litter, which would enable access to nitrogen that is sequestered in complex polyphenolic sources[46].

All sequenced ectomycorrhizal fungi have maintained genes that encode laccases, dye-decolorizing peroxidases and haem–thiolate peroxidases[43,44], which suggests that these genes confer substantial fitness advantages, although not all of their functions may be related to plant cell wall degradation and may instead reflect a role in the degradation of humic materials by hyphae prospecting the soil litter[15,46,47]. *Paxillus involutus* and some other ectomycorrhizal fungi modify major biopolymers of the litter organic matter during the assimilation of nitrogen by releasing hydroxyl

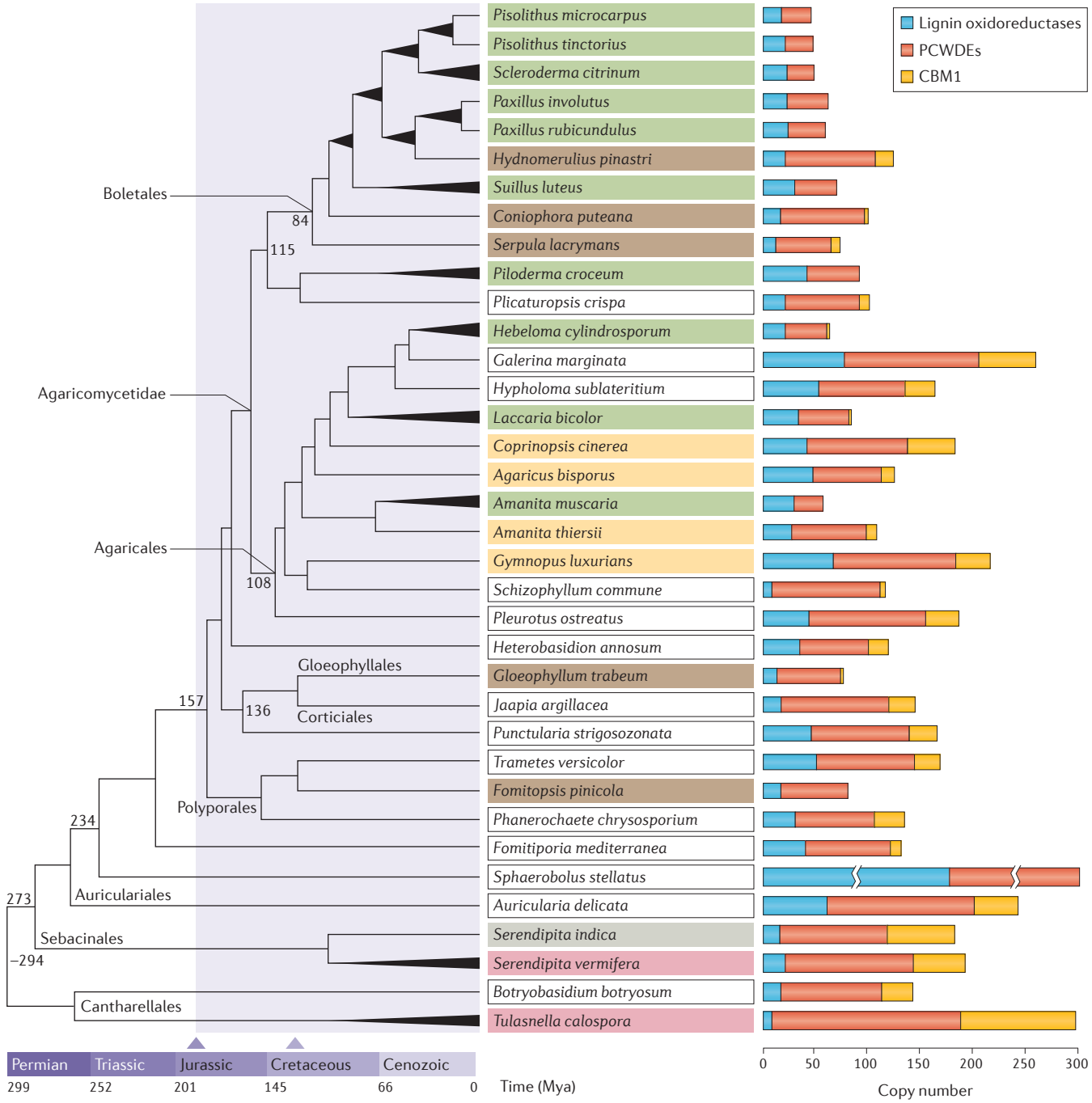
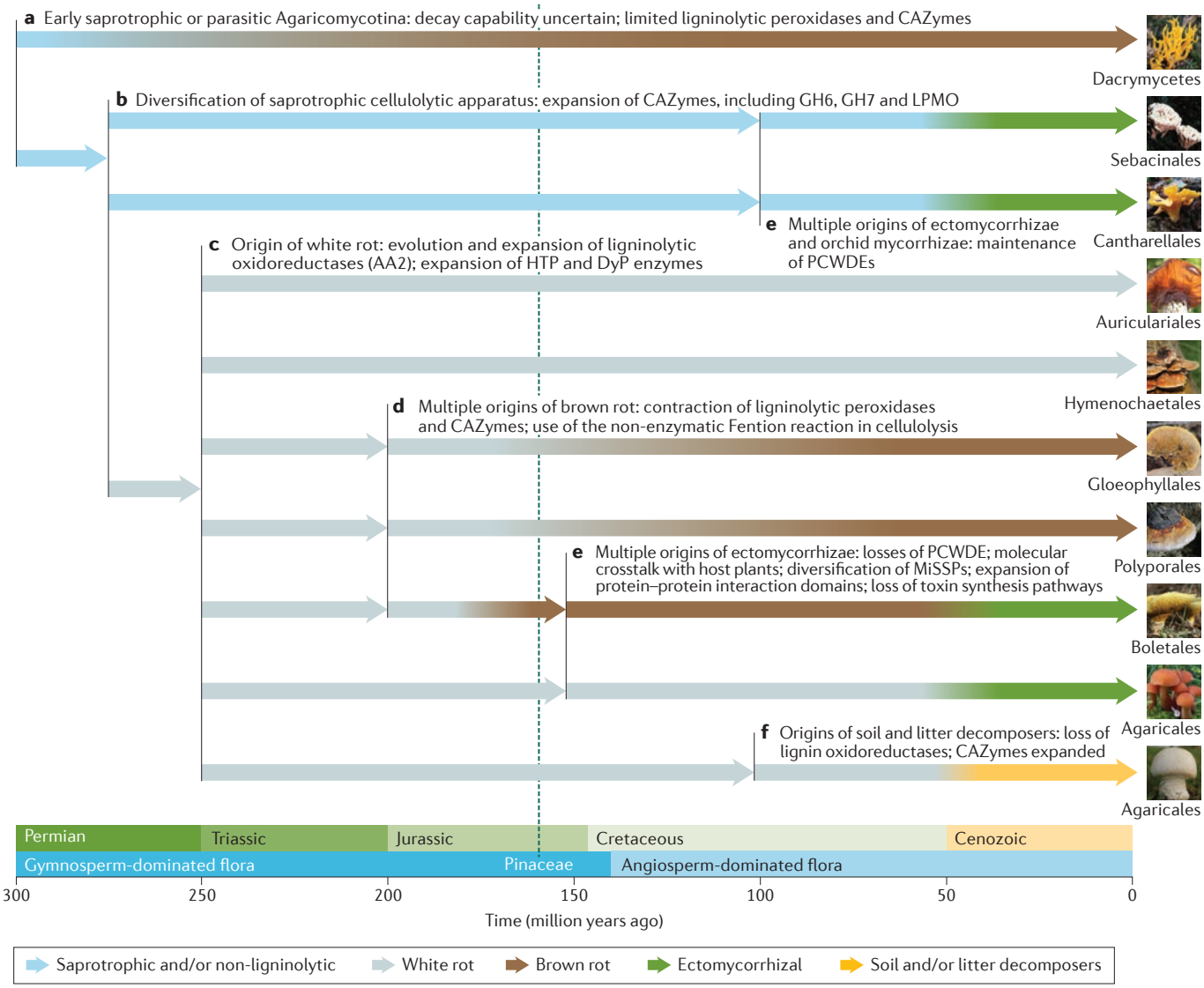


Figure 2 | **Evolution of ectomycorrhizal and orchid mycorrhizal symbioses.** Phylogenomic analysis of the genes that encode enzymes that function in lignocellulose oxidation, the degradation of cellulose and hemicellulose, and other aspects of plant cell wall degradation, can reveal insights into the evolutionary history of ectomycorrhizal symbioses and orchid mycorrhizal symbioses in the Agaricomycetes fungal clade. The tree shown here is a chronogram that was constructed on the basis of a maximum-likelihood phylogeny inferred with the RaxML software[43]. The mean estimation of the age of the common ancestor, in millions of years, is indicated adjacent to the selected nodes, and the estimated ages of the ectomycorrhizal host taxa Pinaceae (dark purple triangle) and Rosid (light purple triangle) are indicated on the timescale. As can be seen in the phylogeny, adaptation to different nutritional modes is polyphyletic, as taxa of ectomycorrhizal fungi (green), orchid mycorrhizal fungi (pink), endophyte (grey), brown-rot fungi (brown), white-rot fungi (white) and soil or litter saprotrophs (yellow) do not always cluster, and are estimated to have arisen independently (black triangles). The identity of the lineage in which the ectomycorrhizal lifestyle first arose is not yet known, but the time window (light-purple shading) in which the first ectomycorrhizal fungus might have plausibly existed dates back to the origin of the Pinaceae. For each species in the tree, stacked bars indicate the copy numbers of genes that encode three groups of carbohydrate-active enzymes (CAZymes): enzymes that function in lignin oxidation (such as class II peroxidases), plant cell wall degrading enzymes (PCWDEs) and enzymes that contain the cellulose binding motif carbohydrate-binding module 1 (CBM1). Note that the copy numbers of CAZymes in *Sphaerobolus stellatus*[35] are truncated to enable visualization alongside the copy numbers of other species. Mya, million years ago. Figure adapted from REF 43, Nature Publishing Group.



radicals through the Fenton reaction that are similar to mechanisms of decomposition used by brown-rot fungi, as has been shown by using Fourier transform infrared spectroscopy and transcriptome profiling[48]. The alteration of litter material probably results from the secretion of enzymes such as LPMOs (which oxidize polysaccharides), aromatic peroxygenases, haem peroxidases, laccases and tyrosinases (which oxidize polyphenols), and peptidases. As a consequence of the loss of their ability to degrade lignocellulose, ectomycorrhizal fungi obtain carbon from host plants rather than soil. It has thus been proposed that the main role of litter decomposition by these fungi is to scavenge nitrogen that is trapped in organic matter[48,49]. In this scenario, ectomycorrhizal fungi would act as 'coincidental decomposers' that release carbon into the soil as a by-product of obtaining nitrogen by decomposing organic matter[49]. The released carbon compounds would be used by commensal saprotrophic bacteria and fungi that live in the vicinity of the ectomycorrhizal hyphae. Therefore, it should be emphasized that, although several ectomycorrhizal fungi are predicted to be decomposers of organic matter, they are not predicted to be saprotrophs[49], as they do not use the released carbon. Furthermore, the abilities to decompose lignocellulose and degrade cellulose are several orders of magnitude lower in ectomycorrhizal fungi than in wood-decaying fungi and soil and/or litter saprotrophs[1,15].

The genome analyses discussed here have shown how, through contraction and the loss of major gene families (such as PCWDEs, invertases and genes families that are involved in toxin synthesis), ectomycorrhizal fungi have become highly reliant on the availability of a continuous flux of photoassimilates from their host plant, while preserving plant cell integrity by avoiding the release of PCWDEs (FIG. 3). We suggest that the loss of gene families is a general attribute of the symbiotic mode of nutrition that no longer requires lignocellulose decaying capabilities, as the host roots provide a ready source of simple carbohydrates, and is thus a symptom rather than a cause of the obligate nature of ectomycorrhizal interactions[24,43].

Protocorms
Tuber-shaped bodies with trichomes that are produced by the young seedlings of various orchids and other plants that have associated mycorrhizal fungi.

Apoplastic space
In plants, the apoplastic space, or apoplast, is formed by the continuum of cell walls of adjacent cells as well as the extracellular space. It is the space outside of the plasma membrane.

Rhizodermis
The epidermis that is formed by the outermost layer of primary cells in the plant root.

◀ Figure 3 | **The evolution of saprotrophic and ectomycorrhizal lifestyles in the subphylum Agaricomycotina.** A simplified schematic representation of the adaptation of species in the subphylum Agaricomycotina to ectomycorrhizal and saprotrophic lifestyles, with images of the fruiting bodies of select species of extant taxa for each lifestyle. **a** | Early Agaricomycotina were saprotrophic or parasitic; extant descendants that have retained the ancestral lifestyle include osmotolerant moulds (class Wallemiomycetes), parasites (class Tremellomycetes) and brown-rot wood-decaying fungi (class Dacrymycetes, such as *Calocera viscosa* (shown in the figure)), which all have limited repertoires of lignocellulolytic enzymes. **b** | The expansion of gene families that encode enzymes that degrade crystalline cellulose (glycosyl hydrolase 6 (GH6), GH7 and lytic polysaccharide monooxygenases (LPMOs)) occurred early in the evolution of species in the class Agaricomycetes, and in the orders Sebacinales and Cantharellales; extant descendants of Sebacinales (such as *Sebacina pallida* (shown in the figure)) and Cantharellales (such as *Cantharellus cibarius* (shown in the figure)) seem to have retained the ancestral cellulolytic apparatus and include taxa that are variously saprotrophs, ectomycorrhizal fungi or orchid mycorrhizal fungi. **c** | The diversification of class II peroxidases and other ligninolytic peroxidases gave rise to extant descendants that include diverse white-rot saprotrophs (in the orders Auriculariales, such as *Auricularia auricula-judae* (shown in the figure)), Hymenochaetales (such as *Inonotus hispidus* (shown in the figure)), Polyporales and the subclass Phallomycetidae). **d** | Brown-rot fungi have several independent origins, each of which is associated with the loss of ligninolytic (ligninolytic peroxidase (POD), dye-decolorizing peroxidase (DyP) and heam–thiolate peroxidase (HTP)) and cellulolytic (GH6, GH7, and LPMO) enzymes and carbohydrate-binding module 1 (CBM1), and an enhanced role of Fenton chemistry in lignocellulose depolymerization. Extant examples of brown-rot fungi that are derived from white-rot lineages include wood-decaying fungi (in the orders Gloeophyllales, such as *Gloeophyllum sepiarium*, and Polyporales, such as *Fomitopsis pinicola* (both shown in the figure)) and rare saprotrophic species in the order Boletales. Dacrymycetes are also brown-rot fungi, but do not seem to be derived from white-rot fungal ancestors. **e** | Ectomycorrhizal fungi and orchid mycorrhizal fungi arose in several independent lineages, and, as such, can be derived from diverse ancestors that include white-rot fungi, soil-decaying or litter-decaying fungi (such as species in the order Agaricales) and brown-rot saprotrophs (in the order Boletales, such as *Suillus luteus* (shown in the figure)). Erosion of degradative enzymes that are associated with a saprotrophic lifestyle has occurred in parallel in each ectomycorrhizal lineage that is derived from these ancestors. Ectomycorrhizal fungi have also arisen in an early-diverging Agaricomycetes lineage (in the order Sebacinales), and these ectomycorrhizal taxa have retained enzymes that degrade crystalline cellulose. Studies of model ectomycorrhizal fungi from the Agaricales order, such as *Laccaria bicolor*[40] (shown in the figure) and *Hebeloma cylindrosporum*[61], have revealed a diversification of effector proteins that are associated with ectomycorrhizal symbioses (mycorrhiza-induced small secreted proteins (MiSSPs)) and the loss of genes in toxin synthesis pathways. In all lineages, the ectomycorrhizal lifestyle is thought to have first arisen subsequently to the establishment of Pinaceae hosts (dashed blue line). **f** | The evolution of soil and litter decomposers is associated with a variable decrease in the number of ligninolytic enzymes, but a retention or expansion of cellulolytic enzymes and oxidoreductases; these changes are possibly associated with the degradation of humic materials by these fungi; extant examples include *Agaricus campestris* (shown in the figure), *Agaricus bisporus* and *Coprinopsis cinerea* (in the order Agaricales). CAZymes, carbohydrate-active enzymes; PCWDE, plant cell wall-degrading enzyme; Image of *Sebacina pallida* in part **b**, courtesy of P. Kaminski. All other images courtesy of F. Martin, French National Institute for Agricultural Research (INRA).

The loss of genes that encode PCWDEs was therefore a major — and perhaps irreversible — step in the evolution of ectomycorrhizal fungi from their saprotrophic ancestors[43,50]. Nonetheless, the diversity of degradative enzymes that are retained by different ectomycorrhizal lineages reflects their polyphyletic origins and perhaps their variation in capabilities for decay.

Root cortex
The outermost layer of the plant root, which is bound on the outside by the epidermis (or rhizodermis) and on the inside by the endodermis. The root cortex is usually composed of large thin-walled parenchyma cells.

Modulating plant signalling

The depletion of nitrogen, simple carbohydrates and other nutrient resources in decomposing organic matter in forest soils may have exerted a strong selective pressure on ancestral saprotrophic fungi to develop symbioses with plant roots prospecting organic layers of soil, as this would enable the acquisition and use of photoassimilates. Such symbioses would require communication with the root cells. Indeed, signalling molecules and pathways that orchestrate the recognition of symbiotic partners and the differentiation of ectomycorrhizal roots have undoubtedly had a crucial role in the evolution of mutualistic interactions between ectomycorrhizal fungi and their plant hosts, although the molecules and pathways that are involved have, until recently, been elusive[9,16]. Importantly, ectomycorrhizal fungi are able to promote extensive rhizogenesis through the modulation of auxin homeostasis[51] and then massively colonize root tissues without succumbing to plant defences. Furthermore, several studies have suggested the defence-like responses in host roots that have been colonized by ectomycorrhizal fungi are impaired[52–54], which indicates that the colonizing symbionts have acquired the ability to actively suppress plant defences[16,55]. Recent studies suggest that this is achieved through the secretion of protein effectors.

Diffusible signalling molecules. Ectomycorrhizal fungi make use of a large set of diffusible signals to manipulate the morphology and metabolism of host roots, whether by secreting signals into the plant or through the manipulation of signalling by host molecules[16,18]. By studying changes in lateral root formation and gene expression in poplar during the establishment of ectomycorrhizal symbiosis, it was found that *L. bicolor* induces an accumulation of auxin at the root apex that enhances lateral root formation and leads to an arrest of stem cell activity in the meristem. *L. bicolor* is able to manipulate auxin signalling, and thus alter developmental programmes in plant roots, by interfering with root auxin metabolism, signalling and responses[51,56]. Similarly, *Tricholoma vaccinum* also uses auxin signalling to regulate the morphology of spruce ectomycorrhiza, although, in this case, the fungus modulates signalling by secreting auxin; in this symbiosis, *T. vaccinum* was shown to use auxin to modify root colonization and increase the Hartig net[57]. By contrast, in the context of the host, integrated jasmonate and ethylene signalling limit the development of the Hartig net through differential gene expression[58]. Aside from auxin, sesquiterpenes are also involved in the stimulation of lateral roots by ectomycorrhizal fungi[59], which indicates that a complex cocktail of diffusible and volatile compounds are used by symbiotic partners to trigger and coordinate the pathways that lead to the development of mature functioning ectomycorrhizal roots. However, the transcriptional regulators and the signalling pathways that are targeted by these compounds are unknown. The manipulation of root development programmes by auxins and other hormones is not a specific feature of ectomycorrhizal symbionts and is shared by several rhizospheric and endophytic fungi[60]. The developmental pathways that are triggered *in planta* by ectomycorrhizal symbionts undoubtedly require more specific signalling molecules and pathways, such as those that involve the manipulation of plant defences by MiSSPs (see below).

Box 3 | **Saprotrophic ability in orchid mycorrhizal fungi and ericoid mycorrhizal fungi**

Symbioses in species in the orders Cantharellales and Sebacinales (phylum Basidiomycota), which form mycorrhiza with orchids, are not associated with a substantial decrease in the number of genes that encode plant cell wall degrading enzymes (PCWDEs), in contrast to ectomycorrhizal fungal genomes, for which a decrease in the number of these enzymes is one of the defining features of genome evolution[43,44]. The orchid mycorrhizal fungi *Serendipita vermifera* in the order Sebacinales and *Tulasnella calospora* in the order Cantharellales have a rich repertoire of PCWDEs, including secreted endocellulases and exocellulases from the glycosyl hydrolase 6 (GH6) and GH7 families, lytic polysaccharide monooxygenases (LPMOs; a class of enzymes that attack polysaccharides, such as cellulose and chitin) and gene families that are related to hemicellulose degradation, such as CE1 and CE16. PCWDEs that are expressed by the hyphal network that these fungi form to colonize the substratum are exploited indirectly by the orchid host as a mechanism to supply nutrients to seeds that have insufficient intrinsic carbohydrate storage for development. The orchid mycorrhizal fungus *S. vermifera* and the endophytic fungus *Serendipita indica* share expansions for gene families that are involved in plant cell wall degradation and carbohydrate binding[70,72]. Similarly, the ericoid mycorrhizal fungus *Oidiodendron maius* (Myxotrichaceae family, class Leotiomycetes) maintains several copies of genes that encode secreted GH6 and GH7 cellulases and LPMOs[43], which provide the fungus with an arsenal of PCWDEs for the decay of peat moss[92]. Notably, most of these PCWDEs are not expressed during the biotrophic phase of fungal growth[43], but instead are induced in culture that contains organic matter[92] or, for the endophyte *S. indica*, during the necrotropic phase of growth that is established on certain hosts following plant cell death[70,71]. Reconciliation analyses of phylogenetic trees of lignocellulose decay genes suggested that the divergence of the orders Cantharellales and Sebacinales occurred before the duplication of class II peroxidases in the class Agaricomycetes, which was an event that marked the origin of white-rot fungi[43] (FIG. 3). The substantial ability to degrade crystalline cellulose in these lineages and in ericoid mycorrhizal fungi may reflect a primitive mode of symbiotic lifestyle that largely relies on saprotrophic ability to decompose non-woody substrates that have low lignin contents.

Laccases
Enzymes that carry out a one-electron oxidation on phenols and similar molecules. Laccases are part of a larger group of enzymes that are termed the multicopper enzymes and that occur widely in fungi (but are also found in many plants and bacteria).

White-rot fungi
Fungi that decay wood by breaking down lignin and cellulose.

Auriculariales
An order of the kingdom Fungi in the class Agaricomycetes. Species in the Auriculariales often differentiate gelatinous fruit bodies and are thus commonly named 'jelly fungi'.

Brown-rot fungi
Fungi that decay wood by breaking down hemicellulose and cellulose, leaving the lignin behind.

Endophyte
A bacterium or fungus that lives inside a plant host without causing apparent disease.

Agaricomycetidae
A subclass of fungi in the phylum Basidiomycota.

Sesquiterpenes
A class of volatile hydrocarbons that consist of three isoprene units.

Although genes from overlapping functional gene categories (such as information storage and processing, transport and metabolism) are upregulated in all ectomycorrhizal interactions for which gene expression has been studied[43], each ectomycorrhizal fungal species expresses a distinct set of symbiosis-associated genes that are thought to be involved in redox reactions, nutrient transport and metabolism. A large proportion of these genes have orthologues in brown-rot and white-rot fungi, which suggests that they are not unique to ectomycorrhizal fungi but instead tend to be associated with essential core metabolic pathways. Owing to these alterations in gene expression, the interactions between ectomycorrhizal fungi and their hosts are characterized by changes in the levels of fungal and host plant metabolites; these changes include marked shifts in the metabolism and transport of carbohydrates, amino acids, aromatic acids, organic acids, fatty acids and water[18,22–24,43,44,53,54,61,62]. This suggests that the metabolic responsiveness of plant roots to ectomycorrhizal fungi is an additional factor in symbiotic interactions. However, although upregulation and fine-tuning of the expression of genes that are involved in nutrient and water transport and assimilation occur in all of the ectomycorrhizal interactions studied so far, symbiosis-specific genes that encode membrane transporters have not been identified in these interactions[43,63].

An insertional mutagenesis screen was used to identify *H. cylindrosporum* genes that are essential for the efficient formation of ectomycorrhizal symbioses, such as *HcMycE1* (REF. 64). *HcMycE1* mutants were unable to differentiate into a true sheathing mantle and the Hartig net, and the gene was upregulated during ectomycorrhizal development; however, although *HcMycE1* has orthologues in several fungi, it encodes a protein that has no known function and thus its role in the establishment of ectomycorrhizal symbiosis remains elusive[64].

Hundreds of mycorrhiza-induced small secreted proteins. Transcript profiling of several ectomycorrhizal interactions, orchid mycorrhizal interactions and ericoid mycorrhizal interactions has shown that 7–38% of genes that are upregulated during symbiosis are taxon-specific genes that are restricted to a single mycorrhizal species[43]. Among all symbiosis-upregulated genes, 8–28% encode candidate-secreted effector proteins (less than 300 amino acids with a predicted signal peptide) that were named mycorrhiza-induced small secreted proteins (MiSSPs)[40]. MiSSPs have also been detected in pine ectomycorrhizal roots, collected *in situ*, that were colonized by *Piloderma croceum*[65], which indicates that they are not restricted to *in vitro* systems that are used for studying mycorrhizal development. The expression of fungal MiSSPs is regulated by the identity of the plant host that is recognized by the fungus and by various environmental signals[61,62]. We hypothesize that MiSSP effectors target growth hormone signalling pathways to restructure the host root and cells (both anatomically and transcriptionally), thereby promoting the symbiotic interaction and mutualism[55]. However, functional analyses, including loss-of-function approaches, will be required to determine whether the hundreds of MiSSPs that have been inferred from symbiotic transcriptomes contribute to symbiosis by targeting host regulatory proteins and/or participating in the construction of the novel symbiotic apoplastic interface.

MiSSP7 sets the controls at the heart of the plant. During the past five years, research has focused on MiSSP7, based on molecular studies of the ectomycorrhizal symbiosis that forms between *L. bicolor* and poplar roots. *MiSSP7* is one of the MiSSP-encoding genes in *L. bicolor* that is most highly upregulated in ectomycorrhizal root tips[40], and it was the first gene to be shown to be required for ectomycorrhizal symbioses.

MiSSP7 is a 7 kDa protein that accumulates in the hyphae and is secreted into the extracellular environment after the sensing of diffusible plant signals. Secreted MiSSP7 is imported into root cells through phosphatidylinositol 3-phosphate-mediated endocytosis and is translocated to the root cell nucleus within a few minutes, where its accumulation rapidly alters gene expression[66]. Furthermore, depletion of MiSSP7 in *L. bicolor* by RNAi prevents the formation of the Hartig net; consequently, in the absence of MiSSP7, *L. bicolor* does not enter into symbiosis with poplar roots[66], which highlights the fundamental role of MiSSP7 in ectomycorrhizal symbioses.

In the host nucleus, MiSSP7 interacts with the transcriptional repressor JASMONATE ZIM DOMAIN protein 6 (JAZ6)[67], which is a master regulator of the jasmonate signalling pathway[68,69]. In resting cells, when jasmonate levels are low or absent, JAZ6 physically inhibits transcriptional activators of jasmonate-responsive genes[68,69]. However, following a wounding stimulus, such as hyphal or bacterial cell penetration, jasmonate is released in its isoleucine-conjugated form, which is sensed by the F-box protein CORONATINE INSENSITIVE 1 (COI1). COI1 forms part of the E3 ubiquitin ligase complex SCF^{COI1} that ubiquitylates JAZ6, thereby targeting JAZ6 for proteasomal degradation and relieving transcriptional repression. Similarly to transcriptional activators and COI1, MiSSP7 that is produced by *L. bicolor* also physically binds to JAZ6 in host cell nuclei (FIG. 4). The interaction between MiSSP7 and JAZ6 prevents the proteasomal degradation of JAZ6 that would otherwise be activated by the accumulation of jasmonate triggered by the fungal infection. This stabilization of JAZ6 maintains repression of jasmonate-induced genes and thus limits the jasmonate-related defence mechanisms that would otherwise preclude the colonization of the root apoplast by *L. bicolor* that is required for the establishment of symbiosis[67] (FIG. 4). Indeed, the inhibition of jasmonate signalling pathways seems to be a major role of MiSSP7, as increasing JAZ6 transcription with a transgene is able to complement RNAi-mediated depletion of MiSSP7, as is the inhibition of jasmonate-induced gene regulation[67]. Confirmation of such a role *in planta* comes from observations that the accumulation of MiSSP7 leads to the repression of several jasmonate-inducible genes in poplar[66,67]. Interestingly, among the jasmonate-induced genes that are repressed by the MiSSP7–JAZ6 interaction, several function in plant cell wall modification (such as those that encode chitinase, extensin and pectin esterase), which suggests that, in addition to inhibiting jasmonate-induced defence mechanisms, MiSSP7 is also able to modify the composition of the cell wall (probably ahead of the hyphal progression in the middle lamella)[67].

Such findings have a direct effect on how we understand the evolution and development of mutualistic and pathogenic symbioses. Similarly to many pathogenic microorganisms, *L. bicolor* uses a protein effector, in this case MiSSP7, to manipulate a key hormone receptor and related signalling pathways of its host to facilitate fungal colonization. However, infection by biotrophic bacterial, oomycetous and fungal plant pathogens triggers the activation of salicylate-mediated defence pathways and the suppression of jasmonate-mediated responses through the degradation of jasmonate receptors[69]. By contrast, the MiSSP7 study shows that *L. bicolor* establishes its mutualistic association with host roots by stabilizing JAZ6, thereby inhibiting jasmonate-mediated responses. Interestingly, the manipulation of jasmonate and salicylate levels, and thus signalling, is involved in the colonization of plants by beneficial endophytic fungi, such as *S. indica* and *S. vermifera*[70–72]. It remains to be seen whether the formation of symbioses by other mycorrhizal fungi with their respective host plants similarly involves the targeting of host jasmonate signalling by MiSSPs.

During its interaction with *Medicago truncatula*, the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (formerly *Glomus intraradices*) secretes SP7, which is a protein that contains a nuclear-localization signal and a series of hydrophilic tandem repeats. SP7 is targeted to the host nucleus, where its interaction with the pathogenesis-related transcription factor ETHYLENE-RESPONSIVE FACTOR 19 (ERF19) suppresses the defence responses of the host, thereby enabling the biotrophic development of *R. irregularis* in *M. truncatula* roots[73]. Together with the studies of MiSSP7 expressed by *L. bicolor*, these findings suggest that MiSSPs have a key role in the rewiring of host plant signalling that is required to enable mycorrhizal symbioses to develop and grow, without the fungal symbiont succumbing to host defences. We speculate that only through the appropriate regulation of plant defences and root developmental pathways by fungal effectors can the state of mutual benefit that is integral to mycorrhizal symbioses be maintained. Further studies of the ability of mutualistic symbionts to interfere with host plant signalling will provide novel insights into the mechanistic basis for fungal control of the development and immunity of host plants.

Conclusions and future prospects

In this Review, we have highlighted the findings of recent genomic studies of model ectomycorrhizal fungi, and we have argued that these findings advance the concept that the development of a mutualistic relationship in ectomycorrhizal symbioses is determined by an interplay of genetic programmes that are controlled by symbiotic effectors (FIG. 5). Furthermore, we have described how comparative genomics has shown that ectomycorrhizal fungi have arisen repeatedly in several independent lineages, as a result of convergent evolution, which suggests that such adaptations have a tendency to occur in saprotrophic fungal ancestors and can be favoured by selection. Given this convergent evolution, and as detailed molecular studies have been limited to only a small number of ectomycorrhizal associations (such as the symbioses between poplars and *L. bicolor*, pine and *H. cylindrosporum*, and eucalypts and *Pisolithus* spp.), it is not yet known whether the mechanisms that are described for the adaptation to a symbiotic lifestyle in these associations are shared by all independent lineages

Middle lamella
In plants, the middle lamella is formed by a pectin layer that cements the cell walls of adjoining cells together.

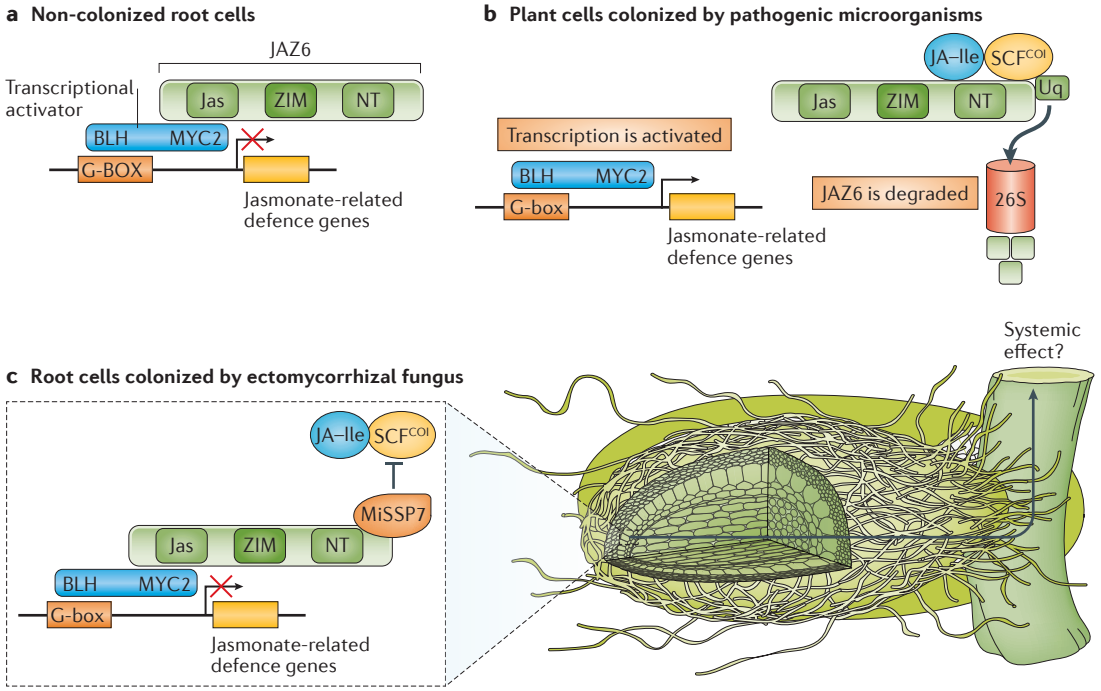


Figure 4 | **Proposed model for the regulation of jasmonate signalling in poplar by MiSSP7.** The *Laccaria bicolor* effector mycorrhiza-induced small secreted protein 7 (MiSSP7) is able to modulate defence signalling in *Populus* spp. host root tips, which helps to establish ectomycorrhizal symbiosis. **a** | Jasmonate conjugated to isoleucine (JA–Ile) is a defence hormone that is sensed by a signalling pathway that induces the expression of a set of defensive genes in the host plant. However, the JASMONATE ZIM DOMAIN-CONTAINING 6 (JAZ6) protein is a host plant protein that suppresses these genes in the absence of JA–Ile, which enables defence pathways to remain silent in non-colonized root cells. Based on the interactions that have been described for the jasmonate signalling pathway in *Arabidopsis thaliana*[68,69,93], it is thought that, in the absence of jasmonate signalling, JAZ6 interacts with basic helix–loop–helix transcription factors, such as MYC2. Binding by JAZ6 prevents these transcription factors, which bind to G-box recognition sites that are upstream of jasmonate-responsive genes, from activating gene expression. **b** | Following a wounding stimulus, such as penetration by pathogenic fungal hyphae, JA–Ile is released and sensed by the F-box protein CORONATINE INSENSITIVE 1 (COI1), which results in COI1 binding to JAZ6. COI1 is a component of the SCF^{COI1} ubiquitin ligase complex, and JAZ6 is thus targeted to the 26S proteasome for degradation. Consequently, MYC2 is no longer inhibited by JAZ6 and is able to activate transcription of jasmonate-responsive genes. **c** | Establishment of ectomycorrhizal symbiosis requires the repression of plant defences that would otherwise prevent fungal growth inside the root; therefore, the ectomycorrhizal fungus *L. bicolor* uses the effector MiSSP7 to ensure the suppression of jasmonate-responsive genes. After wounding caused by the colonization of the apoplastic space by its hyphae, *L. bicolor* secretes MiSSP7 as an effector protein that suppresses jasmonate-related defence mechanisms by binding to JAZ6, which prevents its recognition by JA–Ile–COI1 and thus its proteasomal degradation[67], thereby maintaining the inhibition of MYC2 and the repression of jasmonate-responsive genes. The identities of these genes are not yet known, and nor has the duration and spatial range of the suppression of these genes been determined. Other than fungi, effectors from *Pseudomonas syringae*, which is a biotrophic plant-pathogenic bacterium, are also known to modulate jasmonate signalling in host plants[94–96]. BLH, basic helix–loop–helix transcription factor; Jas, carboxy-terminal jasmonate-associated domain; NT, amino-terminal domain; Uq, ubiquitin; ZIM, zinc-finger protein expressed in inflorescence meristem (also known as TIFY).

of ectomycorrhizal fungi. It should thus be kept in mind that the mechanisms that have been described for the development of model ectomycorrhiza may not be true for all ectomycorrhizal associations.

Together, the findings of the recent studies that are described in this article enable the development of a model for the molecular mechanisms that entail the development of ectomycorrhizal symbioses. Such a model would comprise several steps. First, the host plant has a restricted set of genes that are induced during the pre-infection phase and during the colonization of the apoplastic space[22,23,62]. Second, ectomycorrhizal fungi can alter root metabolism so that the intruding hyphae are accommodated, similarly to what has been observed for plant pathogens and arbuscular mycorrhizal symbionts[53,54]. Before physically contacting the plant root, colonizing ectomycorrhizal hyphae alter endogenous auxin metabolism, signalling and responses in root cells, through the use of mechanisms that may include a range of diffusible chemical signals (such as fungal and plant auxins, and fungal sesquiterpenes), such that more short roots are produced, thereby providing a larger surface area to colonize[51,56–60]. Third, attenuated expression of genes that function

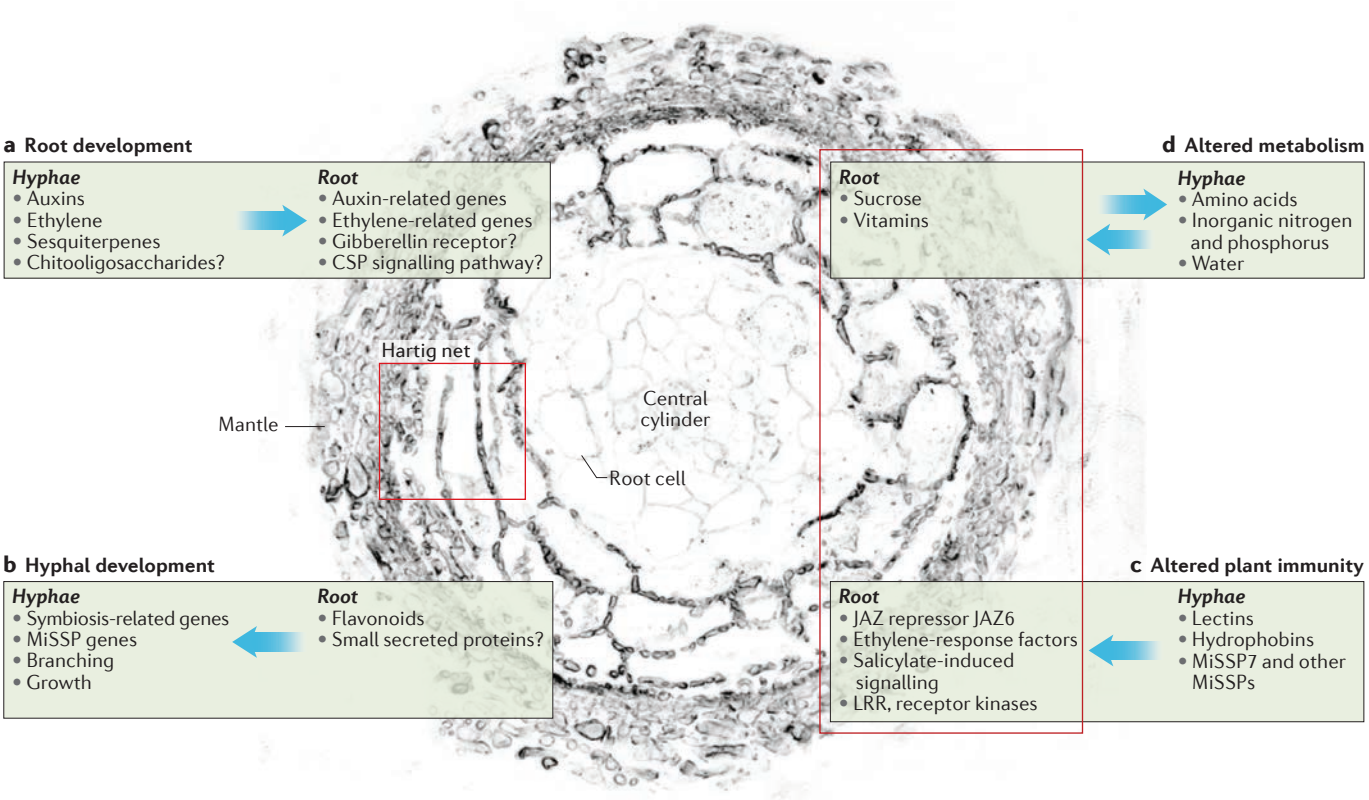


Figure 5 | **A model for the establishment of ectomycorrhizal symbioses.** Insights gleaned from genomic and molecular studies enable the formulation of a conceptual summary of the signalling pathways, and key genes and proteins, that are involved in the development and function of ectomycorrhizal symbioses, at least for the model organisms that have been studied. The model can be formulated as four steps that must be achieved by the fungus: modulation of host root development; hyphal development; suppression of plant defences; and modulation of the metabolism of host root cells. It is important to note that it is not known whether the plant and/or the fungus, or both, initiate ectomycorrhizal interactions; once established, the model assumes that these interactions involve reciprocal feedback. **a** | Diffusible non-specific molecules, such as auxins and sesquiterpenes, that are secreted by ectomycorrhizal fungi trigger an increase in the formation of short roots[16,51,56–60,74] and arrest of growth at the short root meristem[56]. It is also speculated that secreted fungal mycorrhiza-induced small secreted proteins (MiSSPs; see below) interact with auxin, gibberellin and salicylate receptors to alter root development. However, increased concentrations of host ethylene and jasmonate, which are hormones that have functions in root development, repress hyphal colonization of the apoplastic space and thus can have an effect on the development of the ectomycorrhizal rootlet[58]. **b** | Host roots release diffusible molecules, such as the flavonoids rutin and quercetin, that are sensed by ectomycorrhizal fungi, which leads to the synthesis and secretion of effector proteins, such as MiSSPs[97]. **c** | Some MiSSPs, such as MiSSP7, are targeted to the host nucleus after cellular uptake[66]; others, such as hydrophobins, are localized to the symbiotic interface[79,80]. Interactions between MiSSP7 and JASMONATE ZIM DOMAIN (JAZ) proteins suppress host defences[67]. The early phase of symbiosis development probably also includes partner recognition steps that involve lectins, nucleotide binding site (NBS)–leucine-rich repeat (LRR) proteins and receptor kinases and yet unknown ligands[62]. **d** | The molecular mechanisms that control nutrient exchange in ectomycorrhizal symbioses[63] remain largely unknown. However, genes that encode a large set of membrane transporters and primary metabolism enzymes are induced in both host plant roots and fungal hyphae during the establishment of ectomycorrhizal symbioses[44,63,98–102]. CSP, common symbiosis pathway. Background photo of a *Cenococcum geophilum–Pinus sylvestris* ectomycorrhizal root, courtesy of M. de Freitas Pereira and C. Miquel-Guennoc, French National Institute for Agricultural Research (INRA).

in chemical-based and hormonal defence pathways occurs in the host plant during the initial steps of the fungal invasion of plant tissue, during which MiSSP effectors are secreted[3,56,58,62,67,74]. Genome-based studies of symbiosis development suggest that fungal effector proteins, such as MiSSP7, target conserved proteins, such as JAZ proteins, that form protein network 'hubs' in plant defence pathways, which enables the ectomycorrhizal fungus to colonize the root while escaping and/or subverting plant defences. This is reminiscent of microbial pathogens for which analogous protein hubs that are targeted by virulence effectors have been identified[75]. Molecular studies have shown that these effector proteins are secreted into the host plant cell and translocated to the nucleus, where they are able to suppress the expression of defence pathway genes by physically interacting with their 'hub' protein targets[76]. This mechanism of weakening plant defences is probably crucial for enabling hyphal penetration into the root apoplastic space[74,77]. However, the host plant may

respond to the developing ectomycorrhizal interaction by secreting its own effector-like proteins and chemical signals, which might, in turn, control the secretion of fungal effectors[77]. Fourth, fungal effectors, such as symbiosis-upregulated PCWDEs that are upregulated during symbiosis, modify cell-to-cell attachments and plant cell wall rigidity to enable further hyphal penetration into the root tissues[43,78]; however, ectomycorrhizal fungi have a decreased number of PCWDEs, and thus this set of enzymatic effectors is of limited size[43]. Finally, once in the apoplastic space, and following the establishment of the bi-directional transport of nutrients with the host plant, the intruding hyphae must continually protect themselves from detection by plant defences, which is probably achieved through the use of masking proteins (such as hydrophobins[79–81]) and decoys (such as MiSSPs) as diversionary tactics.

The several independent lifestyle transitions from saprotrophism to mutualism that have given rise to ectomycorrhizal lineages in fungal evolution (FIGS 2,3), as revealed by phylogenetic and phylogenomic analyses, suggest that litter-decaying fungi and brown-rot wood-decaying fungi that proliferate in the upper layers of the soil require only a switch from a free-living to a symbiotic lifestyle, without a change in niche, to acquire ectomycorrhizal traits; although an evolutionary intermediate with both saprotrophic and biotrophic capacities, as has been observed in extant orchid and ericoid symbionts, may be required for this transition. As stressed above, the transition to a symbiotic lifestyle requires an ability to control plant defences, which is achieved through the secretion of protein effectors, such as MiSSP7, and the restricted release of damage-associated molecules and toxins that would trigger defence reactions in the plant[43]. The observed convergence in the evolution of ectomycorrhizal fungi, arbuscular mycorrhizal fungi and some pathogenic biotrophic fungi (that is, powdery mildew and rusts) supports an evolutionary scenario in which these features of plant–fungus interactions are required for the formation of ectomycorrhizal symbiosis[17]. However, although the number of genomes of ectomycorrhizal fungi that have been sequenced has increased substantially in recent years, representative genomes of most of the estimated 66 lineages of independent origin have not yet been sequenced[20]. Continued genome sampling, guided by phylogenetic analyses, is required to assess the generality of the mechanisms that are described based on model systems. This should enable us to answer pressing questions, such as: do evolutionarily distant ectomycorrhizal fungal species (for example, species in the orders Boletales and Agaricales) use effectors that target similar host proteins (such as JAZ signalling hubs) to broker ectomycorrhizal symbioses? Did these effector proteins evolve from saprotrophic ancestors, such as brown-rot fungi and/or litter-decaying fungi, or did they evolve *de novo* to enable *in planta* colonization[82]? Expanded phylogenomic analyses are also required to assess the timing of the evolution of ectomycorrhizal symbioses and their relationship to the diversification of land plants.

Important questions also need to be addressed in regard to the signalling pathways that mediate the development of ectomycorrhiza and the complex molecular crosstalk that occurs between ectomycorrhizal partners[51,56,57,74,83]. What are the regulatory mechanisms and signalling pathways that orchestrate the expression of fungal and plant signalling effectors that guide ectomycorrhizal interactions? What are the molecules — such as receptors, transcription factors and microRNAs — that sense and relay these signals? Why are some ectomycorrhizal species able to colonize diverse hosts, whereas other species have a more restricted host range (for example, *L. bicolor* and *Amanita muscaria* are generalists that can colonize a wide range of hardwood and conifer species, whereas *Suillus* spp. are specialists that only colonize members of the Pinaceae family)? Finally, population genomic analyses of ectomycorrhizal fungal species are required to understand the evolution of orphan genes that are upregulated in symbiosis and to measure the speed with which they are modified.

In the context of the fungus in the symbiosis, a better molecular understanding may be achieved through the identification of MiSSPs in natural settings (such as in the complex networks of hyphae in forest soils), which can be achieved using soil metatranscriptomics[65] and genome sequencing of ecologically relevant fungal species[19]. Furthermore, the role of chitin oligomers, such as lipo-chitooligosaccharides and chitooligosaccharides, that are released by ectomycorrhizal fungi is not yet known[84]. In the context of the plant in the symbiosis, the signalling pathways that are stimulated by ectomycorrhizal fungi to enable symbiosis have not yet been identified. With the exception of *REDUCED ARBUSCULAR MYCORRHIZATION 2* (*RAM2*), the common symbiosis pathway (CSP) genes that are required for arbuscular mycorrhizal symbioses[18,85,86] are missing from species in the Pinaceae[85], which suggests that the establishment of the ectomycorrhizal symbiosis, at least in members of the Pinaceae, does not rely on the CSP. However, as mentioned above, the independent origins of ectomycorrhizal lineages means that it is unlikely that all ectomycorrhizal associations involve the same signalling pathways, and therefore it is possible that the CSP is required for ectomycorrhizal symbioses for other host plants, especially those that use this pathway for arbuscular mycorrhizal symbioses.

We hope that answering these outstanding questions will lead to an improved understanding of the role of ectomycorrhizal fungi in forest ecosystems. By reconstructing how these fungi have adapted to environmental changes during the past more than 150 Mya of evolution, we may be able to predict how they are likely to adapt to future anthropogenic climate changes. Finally, elucidating the true functional potential of trees depends on understanding the complex relationships that are formed with symbionts such as ectomycorrhizal fungi, which demonstrates the importance of developing genomics-based science for application to multi-organism systems.

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Competing interests statement
The authors declare no competing interests.