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Evolution of the Mode of Nutrition in Symbiotic and Saprotrophic Fungi in Forest Ecosystems

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Abstract

In this review, we highlight the main insights that have been gathered from recent developments using large-scale genomics of fungal saprotrophs and symbiotrophs (including ectomycorrhizal and orchid and ericoid mycorrhizal fungi) inhabiting forest ecosystems. After assessing the goals and motivations underlying our approach, we explore our current understanding of the limits and future potential of using genomics to understand the ecological roles of these forest fungi. Comparative genomics unraveled the molecular machineries involved in lignocellulose decomposition in wood decayers, soil and litter saprotrophs, and mycorrhizal symbionts. They also showed that transitions from saprotrophy to mutualism entailed widespread losses of lignocellulose-degrading enzymes; diversification of novel, lineage-specific symbiosis-induced genes; and convergent evolution of genetic innovations that facilitate the accommodation

of mutualistic symbionts within their plant hosts. We also identify the major questions that remain unanswered and propose new avenues of genome-based research to understand the role of soil fungi in sustainable forest ecosystems.

1. FUNGI IN FOREST ECOSYSTEMS: KEY PLAYERS IN NUTRIENT AND CARBON CYCLING

The health, productivity, biodiversity, and sustainability of forest ecosystems are shaped by below- and aboveground microbial communities. In woodlands and forests, microbes associated with trees and understory plants play multiple roles, including transformation and translocation of essential soil nutrients, protection from plant pathogens and predators, and mitigation of environmental stresses. It is clear that tree species, like other plants, are highly dependent on their associated microbial communities, and in turn, they shape the microbial assemblages (referred as to the microbiome) proliferating in their rhizosphere, phyllosphere, and endosphere by activating either plant defense responses that limit microbial infection or symbiotic responses that facilitate colonization and in planta accommodation. Trees are colonized by bacterial and fungal communities that coregulate many of their most important physiological and developmental processes (Gottel et al. 2011, Wullschleger et al. 2012, Hacquard & Schadt 2015, Trivedi et al. 2020). Therefore, identifying the factors that drive the distribution, population dynamics, and metabolism of plant-associated microorganisms is integral to understanding the biology of trees and the present and future functioning of forest ecosystems. It is critical to decipher the multiple microbe–plant interactions at the molecular level as well as the effects of biotic and environmental changes on their balance. From this, scientists should be able to anticipate the consequences of future global climate change on the forest microbiome and mitigate problems before they arise, allowing for the preservation of our important forest resources.

The largest terrestrial carbon pool is formed by soil organic carbon, with the top 100 cm of soil containing twice the amount of carbon stored in the atmosphere and vegetation together. A major part of that soil organic carbon is stored in forest biomes as wood and soil organic matter (SOM). Decomposition of these biomaterials is mainly driven by fungal wood decayers and soil and litter saprotrophs (**Figure 1**) (Baldrian et al. 2012). Thus, these organisms play a major role in recycling and storing carbon, as well as other nutrients, from plant litter and dead bacterial, fungal, and animal compounds. However, as forest trees form mutualistic mycorrhizal associations with root-inhabiting fungi (**Figure 1**), knowledge of how the mycorrhizal symbiosis impacts SOM decomposition and regulates the input of plant-related carbon compounds into the soil microbial communities is critically important to understand nutrient cycling in forests (Clemmensen et al. 2013, 2015; Peay et al. 2016; Frey 2019).

Limited availability of macronutrients in forest soil pools has driven tree species into mutualistic mycorrhizal symbioses, with taxonomically diverse clades of arbuscular mycorrhizal fungi, ectomycorrhizal fungi, or ericoid mycorrhizal fungi (Strullu-Derrien et al. 2018) (see the sidebar titled The Different Types of Mycorrhizal Symbioses). Ectomycorrhizal symbiosis dominates temperate forests, in which seasonally cold and dry climates inhibit SOM decomposition, and is the predominant type of mutualistic association at high latitudes and elevations. Ectomycorrhizal symbiosis arose repeatedly across multiple lineages of Mucoromycotina, Ascomycota, and Basidiomycota from saprotrophic ancestors (Brundrett & Tedersoo 2018). By contrast, trees establishing symbiosis with arbuscular mycorrhizal fungi dominate in seasonal, warm tropical forests and occur with ectomycorrhizal trees in temperate and subtropical biomes, in which seasonally warm and wet climates enhance decomposition (Brundrett & Tedersoo 2018).

Figure 1

The major guilds of fungi in forest ecosystems. Saprotrophic fungi (white-rot fungi, brown-rot fungi, and soil and litter decomposers), mutualistic fungi (ectomycorrhizal fungi and arbuscular mycorrhizal fungi), and pathogens are shown. Photo of the *Gigaspora* spores provided by Guillaume Bécard.

THE DIFFERENT TYPES OF MYCORRHIZAL SYMBIOSES

Based on characteristic morphological structures of the symbiosis and the host plant, several types of mycorrhizal symbioses are recognized (Martin et al. 2016), with the two major associations being the endocellular arbuscular mycorrhiza and the intercellular ectomycorrhiza. In arbuscular mycorrhiza, fungal hyphae colonize the host roots to form intracellular arbuscules and vesicles. In ectomycorrhiza, the network of colonizing hyphae remain in the intercellular, apoplastic space forming a symbiotic interface known as the Hartig net. These hyphae do not penetrate the root cells. Additionally, the ericoid mycorrhiza is restricted to ascomycetous Helotiales interacting with Ericales species, such as *Vaccinium* and *Calluna*. Ericoid fungi form hyphal coils in outer cells of the narrow hair roots of plants in the family Ericaceae. They can be abundant in boreal forest understory. All orchids are myco-heterotrophic at some stage during their lifecycle and form orchid mycorrhizas with a range of basidiomycete fungi (e.g., *Tulasnella*). The mycobiont forms coils of hyphae within roots or stems of orchidaceous plants. This type of mycorrhiza is unique because the endophytic fungus supplies the plant with carbon during the heterotrophic seedling stage of orchidaceous plants. Arbutoid mycorrhizal associations are variants of ectomycorrhiza found in certain plants in the Ericaceae in the genera *Arctostaphylos* and *Arbutus* characterized by hyphal coils in epidermal cells.

More than 20,000 species of ectomycorrhizal basidiomycetes and ascomycetes have established symbioses with ~6,000 tree species, such as eucalypts, oaks, and pines, whereas arbuscular mycorrhizal glomeromycetes have established symbioses with ~200,000 plant species, including poplars, eucalypts, and some gymnosperms (Van der Heijden et al. 2015, Brundrett & Tedersoo 2018). Ectomycorrhizal trees represent only 2% of all plant species, but ectomycorrhizal associations represent the dominant tree symbiosis. Steidinger et al. (2019) estimated that ectomycorrhizal fungi interact with approximately 60% of the three trillion tree stems on Earth. Outside of tropical biomes, the estimate for the relative abundance of ectomycorrhizal symbiosis increases to approximately 80% of trees (Steidinger et al. 2019). It should be kept in mind that ectomycorrhizal fungi inhabit a dual ecological niche in the soil and the host root. Similar to their saprotrophic ancestors, ectomycorrhizal fungi therefore have access to mineral and organic nutrients in soil layers that are efficiently taken up by the perennially absorbing mycelial network and partly translocated to the host root. The so-called wood wide web of mycorrhizal hyphae radiate out from tree roots in the upper soil profiles and leaf litter (Lindahl et al. 2007) and acquire a large array of macronutrients, including inorganic and organic nitrogen compounds and inorganic and organic phosphate compounds that are not bio-available to plants (Nehls & Plassard 2018). However, ectomycorrhizal fungi have lost much of the saprotrophic ability required to efficiently acquire carbohydrates through the decomposition (mineralization) of lignocellulose that accumulates in wood and SOM (Koide et al. 2008, Lindahl & Tunlid 2015, Pellitier & Zak 2018, Zak et al. 2019).

It is through the mycorrhizal interface that up to 20% of all the carbohydrates photosynthesized by trees enters soils (Högberg et al. 2001), while as much as 80% of the host nitrogen and phosphate can be supplied by the cortege of mycorrhizal fungal partners (Nehls & Plassard 2018). The mechanisms regulating the uptake, assimilation, translocation, and off-loading of these nutrients in the apoplastic space of mycorrhizal root tips are poorly known and have been studied in only a few model associations (e.g., *Populus–Laccaria*, *Betula–Paxillus*, *Eucalyptus–Pisolithus*) (Martin et al. 2016, Nehls & Plassard 2018). Most importantly, it is not yet known whether the mechanisms driving the bidirectional plant–fungus fluxes of metabolites and mineral elements and those that are described for the adaptation to a symbiotic lifestyle are shared by all independent lineages of mycorrhizal fungi.

Thanks to several ongoing large-scale research initiatives, we can now use unparalleled genomic resources to reconstruct the evolutionary history of saprotrophic and mycorrhizal fungi inhabiting forests and woodlands and the genomic patterns that underlie the diversity of fungal modes of nutrition. In addition, individual genomes can be used to investigate species-specific traits and the mechanisms underlying symbiosis development and/or SOM decomposition. Identifying whether different types of mycorrhizal fungi forming different morphological symbiotic structures with contrasting biology and ecology differed in their gene set and symbiosis-related gene networks is one of the main goals of large-scale genome sequencing of mycorrhizal fungi (Martin et al. 2011).

In this review, we highlight the main insights that have been gleaned from recent developments in large-scale genomic initiatives involving fungal saprotrophs and mycorrhizal symbiotrophs (including ectomycorrhizal, orchid, and ericoid fungi), focusing on (*a*) the evolution of the lignocellulose decomposition machinery in wood decayers and soil/leaf litter decomposers, (*b*) the evolutionary relationships between saprotrophic and symbiotrophic species and the loss of the saprotrophic ability in ectomycorrhizal symbionts, and (*c*) the acquisition of symbiosis-related genes that facilitate the accommodation of mutualistic symbionts within their plant hosts. The key evolutionary processes driving the convergent evolution of the mycorrhizal lifestyle throughout the tree of life of Fungi are discussed. We conclude by discussing how these results pose new and

exciting questions regarding the ecological strategies underlying nutrient cycling in forest soils and proposing research avenues to answer these questions and understand the role of forest fungi in sustainable forest ecosystems.

2. THE CURRENT STATE OF GENOMICS OF SAPROTROPHIC AND MYCORRHIZAL FUNGI

A major step toward unlocking the similarities and differences between mycorrhizal fungi has involved the cooperative effort of several international collaborators and the US Department of Energy Joint Genome Institute (JGI) to sequence the genomes of ectomycorrhizal, arbuscular mycorrhizal, ericoid mycorrhizal, and orchid mycorrhizal fungi within the framework of the Mycorrhizal Genomics Initiative (Martin et al. 2011). These studies have shed light on the genetic similarities between mycorrhizal fungi and their saprotrophic cousins and identified key genes in the regulation of symbiosis (Martin et al. 2016, 2017).

The publication of the genome sequences of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Hydnangiaceae, Agaricales) (Martin et al. 2008) and ectomycorrhizal ascomycete *Tuber melanosporum* (Tuberaceae, Pezizales) (Martin et al. 2010) provided a number of novel insights into genomic features of ectomycorrhizal symbionts. Compared to their (distant) saprotrophic relatives, genomes of these two mutualistic species exhibit common genetic trends: proliferation of repetitive elements, suggesting a fluid genome; reduction of gene families coding for secreted degradative enzymes acting on plant cell-wall polysaccharides; and a lack of gene clusters for secondary metabolism pathways involved in toxin biosynthesis. Several of these genomic features were further identified in the genome of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and other arbuscular mycorrhizal fungi (Tisserant et al. 2013, Morin et al. 2019). However, the findings obtained from the *L. bicolor* and *T. melanosporum* genomes and symbiosis-related transcriptomes suggested that ectomycorrhizal ecology entails variable genomic traits and that these mutualistic species share fewer functional similarities in their molecular symbiotic toolboxes than anticipated (Plett & Martin 2011). Sequencing of the *L. bicolor*, *T. melanosporum*, and *R. irregularis* genomes and the subsequent development of genetic, transcriptomic, and proteomic resources have solidified the roles of these species as model systems for molecular studies in mycorrhizal biology research (for reviews, see Martin & Selosse 2008, Kües & Martin 2011, Martin et al. 2016, Martin et al. 2017, Reinhardt et al. 2021).

These early studies also highlighted the need to have sequence data for more than one representative of each lineage of mycorrhizal fungi in order to characterize the wide range of functional and phenotypic traits of mycorrhizal symbionts. To benefit from a more systematic effort, our community proposed a large-scale initiative with a core mission of producing a much larger set of mycorrhizal genomes, reflecting the broad phylogenetic and ecological diversity of these symbiotrophs. Under the auspices of the JGI Fungal Genomics Program (Grigoriev et al. 2014), the Mycorrhizal Genomics Initiative began in 2011 with the JGI community science program (CSP) entitled Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis in Woody Shrubs and Trees. The mycorrhizal symbionts targeted for sequencing in this project have been selected based on their phylogenetic diversity, their ability to establish different types of mycorrhizal symbiosis, their prominence in ecological settings, their host specificity, their ability to promote growth of model trees with sequenced genomes (*Populus*, *Eucalyptus*), and their role as a model system to understand the development and functioning of the various mycorrhizal symbioses. The combined analysis of a first set of 13 genomes of mycorrhizal fungi was published by Kohler et al. (2015). This set of sequenced species included several Boletales, an order that contains a major concentration of ectomycorrhizal forms (e.g., the porcini

and the gasteromycetes *Pisolithus tinctorius* and *Pisolithus microcarpus*); the first ectomycorrhizal Atheliales (*Piloderma croceum*); the first orchid mycorrhizal fungi (*Sebacina vermifera* in Sebacinales and *Tulasnella calospora* in Cantharellales); and the first ericoid mycorrhizal fungus (*Oidiodendron maius*).

2.1. Genomic Features of Ectomycorrhizal Fungi

This first large-scale study of mycorrhizal genomes revealed that ancestors of ectomycorrhizal fungi were genetically and ecologically diverse, being white-rot fungi, brown-rot fungi, or soil and litter saprotrophs. It also showed that the polyphyletic evolution of the ectomycorrhizal mode of nutrition was marked by convergent losses of different plant cell wall–degrading enzymes (PCWDE) (e.g., cellulases, ligninases) of the ancestral decomposition apparatus of their saprotrophic ancestors. Most of them lack ligninolytic class II peroxidases (PODs), endocellulases and cellobiohydrolases of the glycoside hydrolase (GH) 6 and 7 families, and cellulose-binding motif 1 (CBM1), considerably restricting their ability to degrade lignocellulolytic compounds accumulated in SOM and the plant cell walls of their hosts. Nevertheless, many of the investigated species have kept a unique array of PCWDEs, including GH5 endoglucanases, pectinases, and oxidoreductases/laccases, thus suggesting that they possess diverse abilities to scavenge plant detritus from the soil and litter (see Section 4). As stated in the title of Lindahl & Tunlid (2015), "[e]ctomycorrhizal fungi [are] potential organic matter decomposers, yet not saprotrophs."

2.2. Genomic Features of Orchid Mycorrhizal Fungi

In contrast to ectomycorrhizal fungi in Atheliales, Boletales, and Agaricales, the sequenced orchid-related symbionts showed a robust enzyme arsenal for the degradation of crystalline cellulose, particularly *Tulasnella calospora*, which has 7 GH6, 27 GH7, and 33 lytic polysaccharide monooxygenase (LPMO) genes (even more than its putatively saprotrophic sister taxon, *Botryobasidium botryosum*). Phylogenetic reconciliation analyses suggested that the divergence of Cantharellales and Sebacinales occurred before the diversification of ligninolytic POD in Agaricomycetes and the origin of white rot (Kohler et al. 2015). The well-developed capacity to attack crystalline cellulose in these early-diverging orchid mycorrhizal lineages may reflect a primitive mode of ecology relying on the saprotrophic ability to decay nonwoody substrates with modest lignin content.

2.3. Genomic Features of Ericoid Mycorrhizal Fungi

Of note was the observation that the genome of the ericoid fungus *Oidiodendron maius* encodes multiple copies of PCWDE genes, such as GH6, GH7, and LPMO, explaining its saprotrophic ability in *Sphagnum* peat. Subsequently, this very large repertoire of carbohydrate-active enzymes (CAZymes), including PCWDEs, has been confirmed by the sequencing of other ericoid fungi: *Meliniomyces bicolor*, *Meliniomyces variabilis*, and *Rhizoscyphus ericae* in the Leotiomycetes (Ascomycota) (Martino et al. 2018). Given the more recent appearance of ericoid mycorrhizal symbiosis, ericoid fungi were speculated to have retained this efficient saprotrophic machinery because, unlike ectomycorrhizal symbionts, they are still in a transitional evolutionary stage between saprotrophy and mutualism.

Over the last 5 years, dozens of additional mycorrhizal species have been sequenced within the framework of the JGI 1000 Fungal Genomes project (1KFG) Deep Sequencing of Ecologically Relevant Dikarya (CSP #1974). As of September 2021, 185 genomes of mycorrhizal fungi are available at the JGI MycoCosm database (**https://mycocosm.jgi.doe.gov**) (Grigoriev et al. 2014).

A combined analysis of 135 fungal genomes from 73 saprotrophic, endophytic, and pathogenic fungal species and 62 mycorrhizal fungal species has been carried out (Miyauchi et al. 2020). This study sampled major clades of mycorrhizal symbionts, for which there were previously no ectomycorrhizal genomes available, including Russulales, Thelephorales, Phallomycetidae, and Cantharellales. These species were selected because they are ecologically dominant in most boreal and temperate forests (Russulales and Thelephorales), represent early diverging lineages for which no ectomycorrhizal genomes were previously available (Phallomycetidae and Cantharellales), and include clades that arose before (Cantharellales) or after the origin of class II ligninolytic PODs that typify the white-rot nutritional strategy.

Knowing that ectomycorrhizal fungi are independently derived from multiple lineages of saprotrophs (Matheny & Hibbett 2009, Kohler et al. 2015), clades from taxonomically related saprotrophic species investigated within the framework of other large-scale JGI sequencing projects (Eastwood et al. 2011; Floudas et al. 2012, 2020; Nagy et al. 2016) were included in the comparative analysis. This allowed the genetic bases of the multiple transitions between saprotrophism and mutualism, i.e., the expansion or contraction of PCWDE, to be deciphered. Insights into the origins of lignocellulose decomposition in wood decayers and soil and leaf litter decomposers are provided in Section 3.

3. INSIGHTS INTO THE ORIGINS OF LIGNOCELLULOSE DECOMPOSITION CAPABILITIES

3.1. A Wide Range of Saprotrophic Fungi in Forest Soils

In forests, the topsoil litter layer is composed of a cocktail of complex plant polysaccharides, such as cellulose, hemicelluloses, pectin, and phenolic lignans. Dead fungal material includes ß-1,3- and ß-1,6-glucans and chitin, among other components (Brabcová et al. 2016). This complex matrix occurs as a range of organic compounds continuously processed into smaller molecules by microorganisms through the production of extracellular hydrolytic and oxidative enzymes. The spatially heterogeneous local environment is a critical driver of SOM decomposition, as it determines its physicochemical accessibility to extracellular enzymes and modulates microbial metabolism (Dungait et al. 2012). Saprotrophic fungi play a critical role in wood and SOM decomposition processes (**Figure 1**) (Baldrian & Valášková 2008). They have evolved several strategies for decomposing lignocellulose and other compounds that accumulate into organic matter, such as chitin, lipids, and proteins. Various guilds of molds (e.g., *Penicillium*, *Trichoderma*, *Mortierella*) are abundant in forest litter, where they likely use simple substrates (Algora Gallardo et al. 2021). The majority of fungi decomposing wood, wood-related materials, and decaying plant detritus are mushroom-forming Agaricomycetes, which exhibit two main modes of plant cell-wall (wood) decomposition: white rot, in which all plant cell-wall components (i.e., lignin, cellulose, hemicellulose, pectins) are degraded, and brown rot, in which lignin is modified but not appreciably degraded. Evolution of these molecular machineries was one of the most ecologically important innovations in fungi, giving them access to gigatons of carbon sequestered in wood materials (Floudas et al. 2012).

As mentioned in Section 2, the evolutionary origins of lignocellulose decomposition in fungi have been investigated by sequencing hundreds of saprotrophic species (white-rot wood-decaying fungi, brown-rot wood-decaying fungi, and soil and litter saprotrophs) belonging to Agaricales, Atheliales, Auriculariales, Boletales, Cantharellales, Dacrymycetales, Gloeophyllales, Polyporales, Russulales, Sebacinales, and Trechisporales (Floudas et al. 2012, Hori et al. 2013, Nagy et al. 2016, Sipos et al. 2017, Floudas et al. 2020, Ruiz-Dueñas et al. 2020). As of today, most Basidiomycota genomes deposited in the MycoCosm database (611 in March 2021) belong to saprotrophic or

ectomycorrhizal fungi inhabiting forest ecosystems, including Agaricales (181 genomes), Polyporales (83 genomes), Boletales (74 genomes), and Russulales (39 genomes).

3.2. Evolution of White-Rot Fungi and Brown-Rot Fungi

The current evolutionary scenario posits that early Agaricomycotina were saprotrophic or parasitic species, and the extant species that have retained this ancestral mode of nutrition include osmotolerant molds (class Wallemiomycetes), parasites (class Tremellomycetes), and brown rots (class Dacrymycetes, such as *Calocera viscosa*), which all have limited repertoires of lignocellulolytic enzymes. The expansion of gene families encoding secreted enzymes that degrade crystalline cellulose, such as the GH6 and GH7 families and LPMO, occurred early in the evolution of species in the class Agaricomycetes and in the orders Sebacinales and Cantharellales. Extant descendants of Cantharellales and Sebacinales have retained the ancestral cellulolytic apparatus and include species that are saprotrophs, ectomycorrhizal, or orchid mycorrhizal fungi. They, however, lack key oxidoreductases involved in lignin decomposition, such as the ligninolytic PODs. The diversification of these PODs and other ligninolytic PODs gave rise to extant descendants that include diverse white-rot saprotrophs in the orders Auriculariales (such as *Auricularia auricula-judae*), Hymenochaetales (such as *Inonotus hispidus*), Polyporales, and the subclass Phallomycetidae. The analysis of an expanded sample of early-diverging Agaricomycetes genomes by Nagy et al. (2016) suggested that the first white-rot species may have been the most recent common ancestor (MRCA) of Auriculariales and more derived clades that evolved in the Carboniferous. Brown-rot fungi have several independent origins, each of which is associated with the loss of ligninolytic PODs, heme dye-decolorizing PODs, heme-thiolate POD/peroxygenases (HTPs), cellulolytic enzymes (GH6, GH7, LPMO), and the carbohydrate-binding module CBM1. These fungi degrade wood polysaccharides with only a partial modification of lignin by releasing Fenton-generated hydroxyl radicals in the colonized material (Baldrian & Valášková 2008). Extant species of brown-rot fungi that are derived from white-rot lineages include wood-decaying fungi in Gloeophyllales (such as *Gloeophyllum sepiarium*), Polyporales (such as *Fomitopsis pinicola*), and Boletales (such as *Coniophora puteana* or *Serpula lacrymans*).

3.3. The Lignocellulolytic Ability of Soil and Litter Decomposers in Agaricales

In Agaricales, three main ecophysiological groups are recognized in the saprotrophic guild, i.e., wood-decaying species (such as *Gymnopilus junonius*), buried wood–decaying species (such as *Gymnopus fusipes*), and leaf litter–degrading species (such as *Lepista nuda*). The latter soil and litter saprotrophs have adapted to a wide range of habitats in both forest and grassland ecosystems. Their habitats in SOM are often shared with ectomycorrhizal roots and their external web of hyphae, raising questions about how these fungal networks compete and/or cooperate in their exploitation of SOM (Bödeker et al. 2016, Frey 2019). For example, chemical inhibition and the competition for limiting resources, such as nitrogen compounds, between saprotrophic and mycorrhizal fungi in SOM has long been hypothesized to suppress decomposition rates, resulting in greater sequestration of carbon in forest soils, which is known as the Gadgil effect. However, Fernandez & Kennedy (2016) questioned this effect of ectomycorrhizal fungi on litter and SOM decomposition and showed that the results are much more variable than previously recognized, calling for additional experiments in both broadleaf and conifer forests.

Based on the reconstruction of ancestral enzyme repertoires, a wood white-rot species was predicted to be the MRCA of the Agaricomycetes class (including the Agaricales, Polyporales, Russulales, Boletales, Amylocorticiales, and Atheliales orders), confirming previous predictions (Ruiz-Dueñas et al. 2020). This ancestral mode of nutrition, dated to 192 Mya in the early Jurassic,

was maintained in the MRCAs of Agaricales (169 Mya), Polyporales (150 Mya), and Russulales (152 Mya), but it changed to brown rot in the MRCA of Boletales (134 Mya) in the early Cretaceous. In the latter three orders, the diversifying lignocellulolytic apparatus supported a dominant ecology based on carbon acquisition from hardwood and conifer woody materials. By contrast, Ruiz-Dueñas et al. (2020) suggested that the observed expansions, contractions, and diversification of the lignocellulolytic enzyme repertoires during the last 170 million years led ancestral species of the Agaricales to shift their growth preferences toward a wider diversity of lignocellulosic substrates. These shifts in their PCWDE arsenal converged multiple times in different saprotrophic (grass litter, forest litter, and decayed wood) and ectomycorrhizal lifestyles along the evolution of this order, with saprotrophic species gaining, and ectomycorrhizal ones losing, PCWDE genes.

The genomes of saprotrophic Agaricales encode a larger PCWDE repertoire (112 to 338 genes) than any other Agaricomycetes order (Polyporales, 89–212 genes; Russulales, 160–253 genes; and Boletales, 100–148 genes) (Floudas et al. 2020, Ruiz-Dueñas et al. 2020) (**Figure 2**). This distinct repertoire of PCWDE likely explains their known ability to decompose the multiple states of decaying wood and SOM. Cellulose is degraded by a combination of exocellobiohydrolases and endoglucanases (GH5, GH6, GH7, and LPMO), while lignin degradation is catalyzed by lignin, Mn, and versatile PODs acting together, with flavo-oxidases and copper-radical oxidases providing the H_2O_2 required by PODs, and laccases acting directly and in the presence of redox mediators.

Although both litter decomposers and white-rot fungi share their enzymatic arsenal for the plesiomorphic enzymatic network involved in crystalline- and amorphous-cellulose decomposition, they differ with respect to hemicellulose and lignin degradation (Floudas et al. 2020, Ruiz-Dueñas et al. 2020). The evolution of soil and litter decomposers is associated with a variable decrease or expansion of lignocellulolytic enzymes and copper-radical oxidoreductases, such as laccases and LPMOs, possibly associated with the formation of humus and degradation of humic materials by these fungi.

3.4. The Expansion of Gene Families Coding for Ligninolytic Oxidoreductases

The expansions of gene families coding for oxidoreductases involved in lignin degradation (such as PODs and laccase-type enzymes) and/or contributing to the catabolism of lignin-derived compounds (unspecific peroxygenases and aryl-alcohol oxygenases) are among the major evolutionary events in forest-litter decomposers. Families and subfamilies of ligninolytic PODs diversified strikingly during the evolution of Agaricales in forest ecosystems. So-called long Mn PODs (MnPs), ligninolytic PODs, versatile PODs (VPs), and several new POD types evolved from ancestral short MnPs (Ruiz-Dueñas et al. 2020). The acquisition of a Mn(II)-binding site, formed by two glutamate and one aspartate residues, allowed for the novel MnPs to catalyze the oxidation of lignin phenolic groups via Mn^{3+} chelates. Among the new POD types, MnP-ESD, which has a Glu/Ser/Asp Mn^{2+} oxidation site, appeared before the diversification of Russulales and Agaricomycetidae, remaining only in Agaricales (and Russulales) with very different lignocellulose-decaying lifestyles, whereas MnP-DGD, which has an Asp/Gly/Asp Mn^{2+} oxidation site, arose directly in an ancestral species of the order Agaricales, and it is specific to grass-litter and decayed-wood decomposers. The lignocellulolytic apparatus of leaf-litter decomposers is thus characterized by a diversification of ligninolytic PODs and an enrichment in laccases sensu stricto and HTP genes. The expansion of HTP genes that has been documented in *Agaricus bisporus* has been suggested as a possible adaptation to humic environments (Morin et al. 2012).

It should be noted that the most recent large-scale genome comparisons (Floudas et al. 2020, Ruiz-Dueñas et al. 2020) confirmed that the recognized nutritional categories, such as white-rot

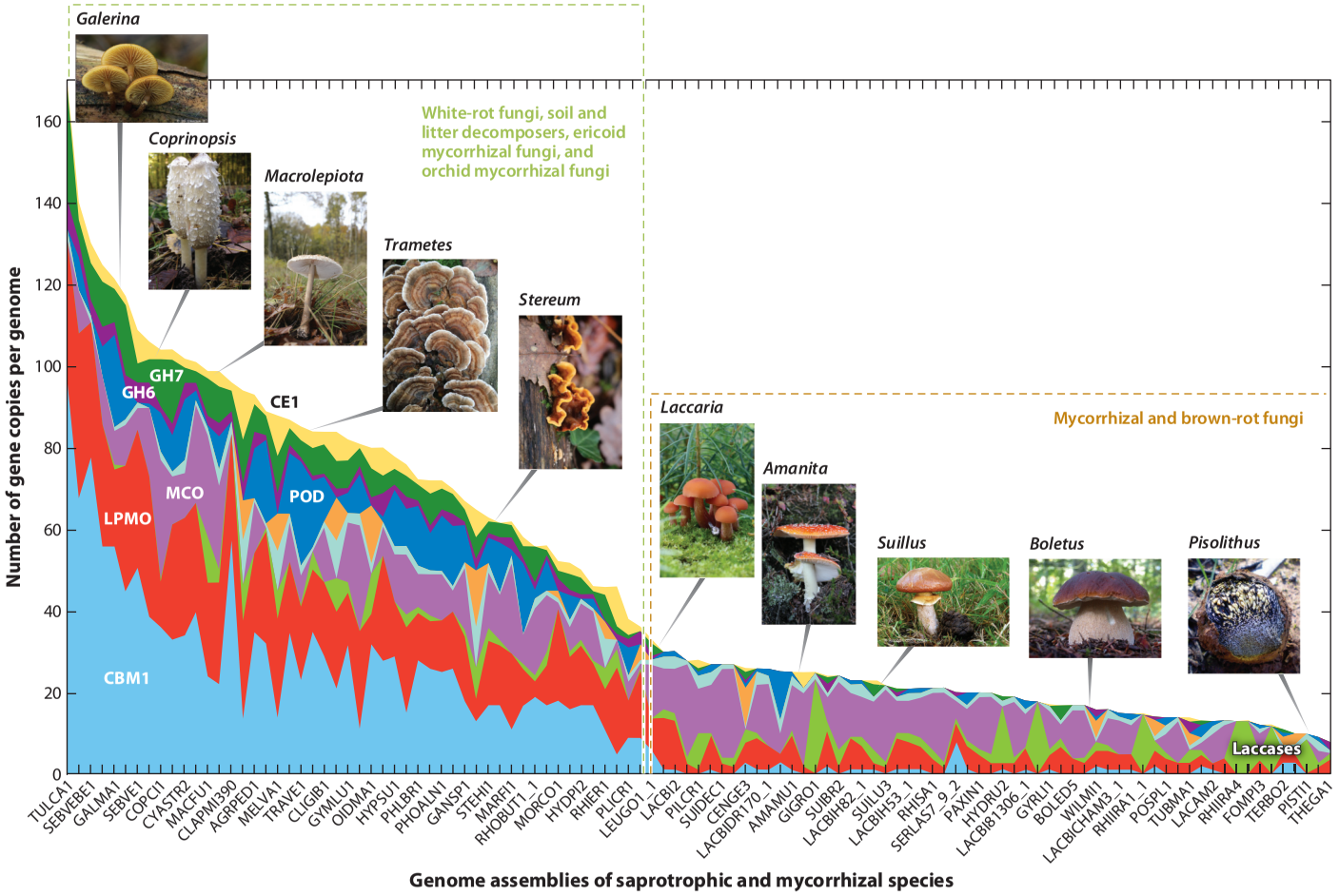


Figure 2

Distribution of genes coding for key secreted PCWDEs and CBM1 in 110 representative saprotrophic and mycorrhizal fungi. Listed genes include those coding for CBM1 (cellulose-binding modules), LPMO (cellulose-acting lytic polysaccharide monooxygenases), MCO (multicopper oxidases), laccases, POD (class II lignolytic peroxidases), GH6 (cellobiohydrolases), GH7 (reducing end-acting cellobiohydrolases), and CE1 (feruloyl esterases). CAZyme gene sets were retrieved from published genomes at JGI MycoCosm. Abbreviations: CAZyme, carbohydrate-active enzyme; CBM1, carbohydrate-binding module family 1; PCWDE, plant cell wall–degrading enzyme; POD, peroxidase. Only a selection of genome assemblies is shown on the x-axis; for a full list of the 110 assemblies, see Miyauchi et al. (2020).

fungi and litter decomposers, mask a much larger functional diversity across mushroom-forming fungi (Riley et al. 2014) (**Figures 2** and **3**). The as-yet poorly described nutritional strategies likely reflect the mosaic of overlapping microhabitats generated by the degradation of wood-related material from freshly fallen trees to the ultimate stage of degradation, the humus or soil (Bhatnagar et al. 2018, Algora Gallardo et al. 2021).

4. THE EVOLUTIONARY RELATIONSHIPS BETWEEN SAPROTROPHIC AND SYMBIOTROPHIC FUNGI

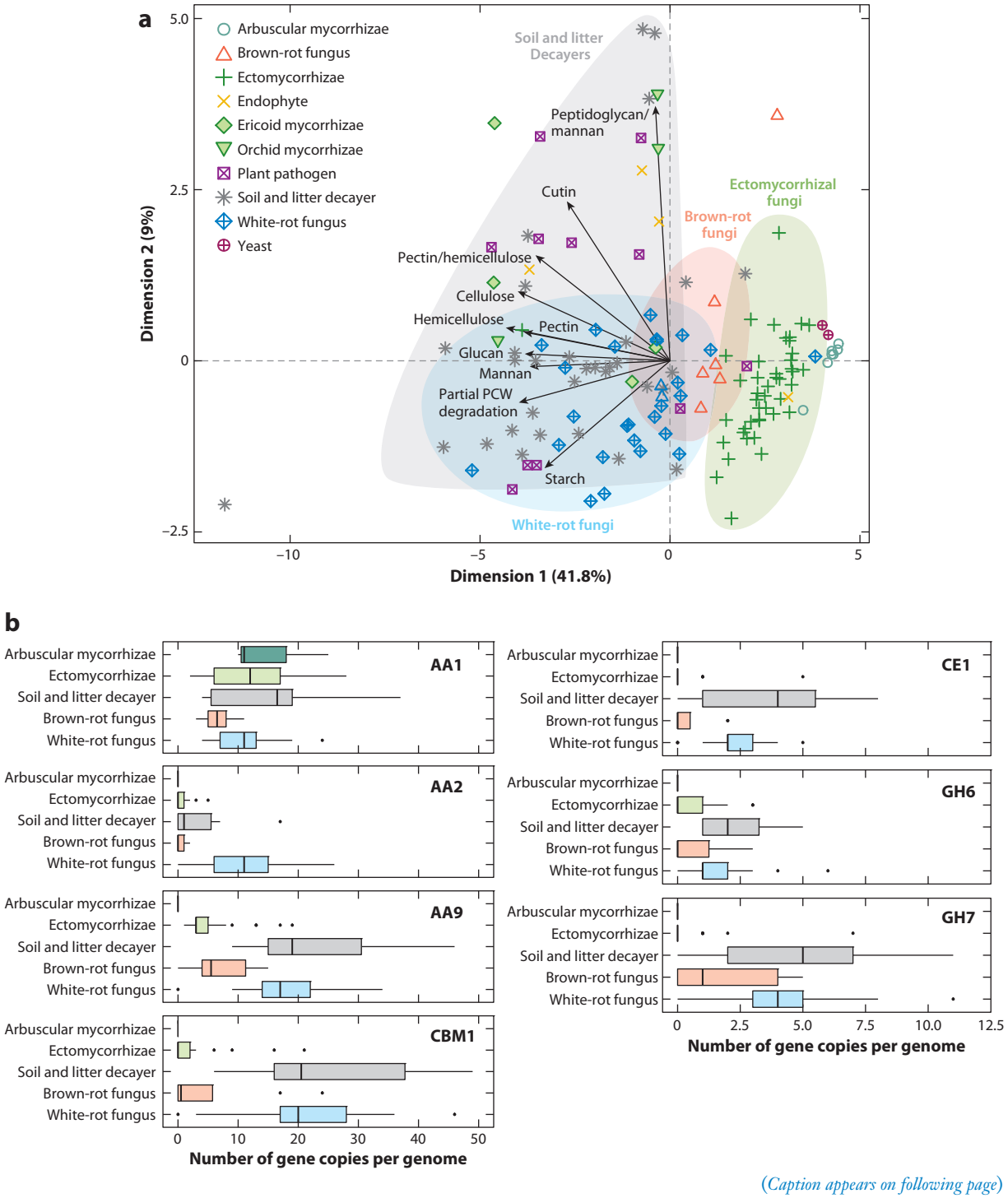
4.1. Evolution of the Ectomycorrhizal Lifestyle

In all fungal lineages, the ectomycorrhizal lifestyle is thought to have first arisen subsequent to the establishment of Pinaceae hosts (~200 Mya) (Martin et al. 2017, Strullu-Derrien et al. 2018). Since then, ectomycorrhizal symbiosis has arisen independently and persisted perhaps 80 times or more across multiple lineages of Mucoromycotina (Endogonales), Ascomycota, and especially Basidiomycota (Matheny & Hibbett 2009, Tedersoo et al. 2010). In the most comprehensive phylogenetic analysis of the evolution of ectomycorrhizal fungi in Agaricomycetes carried out so far, 36 origins of ectomycorrhizal lineages were found and possibly several reversals to pathogenic nutritional modes (Sánchez-García et al. 2020). Of note, across the entire 8,400-species phylogeny, diversification rates of ectomycorrhizal lineages were no greater than those of saprotrophic lineages. However, some ectomycorrhizal lineages have elevated diversification rates compared to their nonsymbiotic sister clades, suggesting that the evolution of ectomycorrhizal symbioses may act as a key innovation at local phylogenetic scales.

Ectomycorrhizal fungi derived from diverse ancestors that include white-rot fungi, soil- or litter-decaying fungi (such as species in the order Agaricales), and brown-rot saprotrophs (in the order Boletales). The erosion of the gene set coding for degradative enzymes that are associated with a saprotrophic lifestyle has occurred in parallel in each ectomycorrhizal lineage that is derived from these ancestors (Wolfe et al. 2012, Kohler et al. 2015, Peter et al. 2016, Miyauchiet al. 2020, Looney et al. 2021). Despite the general pattern toward loss of PCWDEs in ectomycorrhizal clades throughout their phylogenetic breadth, there is a wide diversity in the decomposing ability of ectomycorrhizal symbionts (**Figures 2** and **3**). For example, the genome of *Tricholoma matsutake* (Agaricales) encodes two GH7 cellobiohydrolases, consistent with its known facultative saprotrophic ability during fruiting (Kusuda et al. 2008). Notably, *Cortinarius glaucopus* possesses twelve recently duplicated copies of ligninolytic MnP-ESD genes. Copies of MnP-ESD genes have also been found in the ectomycorrhizal *Hebeloma cylindrosporum* (Kohler et al. 2015) and several *Russula* species (Looney et al. 2021). MnP-ESD peroxidases have been suggested to be involved in bleaching of soil litter (Bödeker et al. 2014), but they can also be used during mushroom fruiting on well-decayed logs and are thought to associate with roots within the wood (Mäkipää et al. 2017).

Ectomycorrhizal symbionts that evolved from brown-rot fungi in Boletales, such as *Paxillus involutus*, appear to have adapted the Fenton oxidative system from their saprotrophic ancestors in order to modify lignin and thereby scavenge nitrogen compounds sequestered in decaying SOM (Op De Beeck et al. 2018, Nicolás et al. 2019). Secreted proteases, LPMOs, and laccases encoded in the genome of *Paxillus* species may facilitate this oxidative decay of SOM. In addition, some of the remaining secreted PCWDEs, such as the GH5-CBM1 endoglucanase and LPMO-like copper protein X325 of *Laccaria bicolor*, play a role in cell-wall remodeling that takes place during colonization of the root apoplastic space (Zhang et al. 2018, Labourel et al. 2020).

Among the newly sequenced species, ectomycorrhizal Phallomycetidae and Cantharellales showed highly variable suites of PCWDEs (**Figure 2**). Most striking was the ectomycorrhizal *Gautieria morchelliformis* (Gomphales, Phallomycetidae), which has 25 MnPs that may be involved



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Comparison of the distribution of CAZymes (including PCWDEs) in the genomes of 141 species of forest fungi with contrasted ecology along the saprotrophism–mutualism–pathogenesis continuum. (*a*) Principal coordinate analysis of secreted PCWDEs grouped by their predicted soil organic matter substrate (e.g., cellulose, glucan, pectin). Each symbol represents a genome or species. Ecology/lifestyle (e.g., ectomycorrhizae, white rot) is indicated by different colored symbols as shown in the legend. The main variables contributing to the construction of the axis are represented by arrows. Note the divergent distribution of ectomycorrhizal fungi and soil and litter decayers. Shading identifies the divergent distribution of CAZymes for white-rot fungi (*light blue*), brown-rot fungi (*red*), soil and litter decayers (*gray*), and ectomycorrhizal fungi (*green*). (*b*) The distribution of a series of PCWDEs that are critical for lignocellulose decay, such as LPMO (AA9 family) and GH6/GH7 cellulases, in 117 saprotrophic and mycorrhizal genomes. The boxes represent the median and the upper and lower quartiles, with whiskers showing minimal and maximal values and dots representing outliers. The PCWDEs are grouped into the following families: AA1 (laccase, *p*-diphenol:oxygen oxidoreductase, ferroxidase), AA2 (manganese peroxidase, versatile peroxidase, lignin peroxidase), AA9 (copper-dependent lytic polysaccharide monooxygenases acting on cellulose), CBM1 (carbohydrate-binding module family 1), CE1 (acetyl xylan esterase), GH6 (cellobiohydrolase), and GH7 (cellobiohydrolase: reducing end-acting cellobiohydrolase). CAZyme gene sets were retrieved from published genomes at JGI MycoCosm (Kohler et al. 2015, Nagy et al. 2016, Peter et al. 2016, Murat et al. 2018, Floudas et al. 2020, Miyauchi et al. 2020, Hage et al. 2021, Looney et al. 2021, Marqués-Gálvez et al. 2021). Abbreviations: AA, auxiliary activity; CAZyme, carbohydrate-acting enzyme; CBM1, carbohydrate-binding module family 1; CE1, carbohydrate esterase family 1; GH, glycoside hydrolase; PCW, plant cell wall; PCWDE, plant cell wall–degrading enzyme.

in the decay of SOM, as well as numerous enzymes that act on cellulose and hemicellulose. These *G. morchelliformis* MnPs are members of a gene family that underwent expansion after the divergence of Auriculariales and other Agaricomycetes. In contrast, Cantharellales evolved prior to the diversification of class II ligninolytic PODs (Floudas et al. 2012, Kohler et al. 2015, Nagy et al. 2016), and none of its species—saprotrophic or mycorrhizal—possess these ligninolytic enzymes. The iconic golden chanterelle (*Cantharellus cibarius anzutake*) and terracotta hedgehog mushroom (*Hydnum rufescens*) are ectomycorrhizal Cantharellales that lack not only class II PODs but also many GHs, LPMOs, and CBM1 motifs that are needed for lignocellulose decomposition (**Figure 4**). In this regard, they resemble some of the most derived ectomycorrhizal Agaricomycetidae with highly reduced saprotrophic capabilities, such as species of Boletales. The ectomycorrhizal Russulales (Looney et al. 2021), Thelephorales (Miyauchi et al. 2020), and truffles

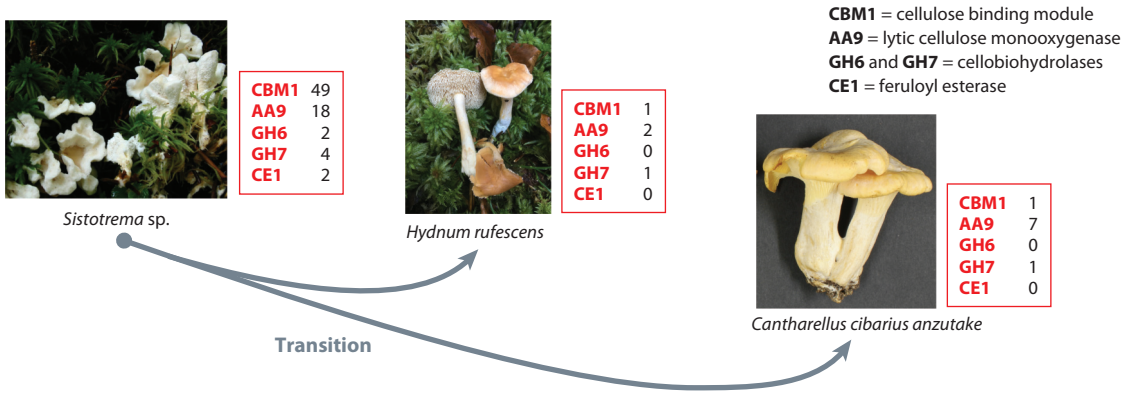


Figure 4

The evolutionary transition from soil saprotrophism to ectomycorrhizal symbiosis in the Cantharellales. The number of PCWDEs in key families (CBM1, AA9, GH6, GH7, and CE1) is shown for the saprotrophic *Sistotrema* sp. and the ectomycorrhizal symbionts, *Hydnum rufescens* and *Cantharellus cibarius anzutake* (see Miyauchi et al. 2020). Abbreviations: AA, auxiliary activity; CBM1, carbohydrate-binding module family 1; CE1, carbohydrate esterase 1; GH, glycoside hydrolase; PCWDE, plant cell wall–degrading enzyme.

(*Terfezia*, *Tirmania*, *Tuber*) (Murat et al. 2018, Marqués-Gálvez et al. 2021) that have been sequenced also conform to this model of a greatly reduced saprotrophic apparatus in symbiotic fungi.

A major goal of Miyauchi et al. (2020) was to find species transitioning to symbiosis that still had some saprotrophic genes in their genome. In addition to the abovementioned *G. morchelliformis*, the ectomycorrhizal ascomycete *Acephala macrosclerotium* (Leotiomycetes) may represent a transitional step from pure saprotrophy toward pure ectomycorrhizal symbiosis. In these species, the mycelial web exploring topsoil litter could be able to partly decompose decaying organic matter. As suggested for ericoid mycorrhizal fungi, which encode among the largest PCWDE arsenal (Kohler et al. 2015, Martino et al. 2018), a dual saprotrophic/mutualistic lifestyle may allow for greater ecological flexibility and fitness under specific environmental conditions. Most interestingly, Miyauchi et al. (2020) showed by using RNA sequencing transcriptome profiling that *A. macrosclerotium* PCWDE genes were repressed at the transcriptional level during this fungi's symbiotic interaction with pine roots. They suggested that downregulation of PCWDE genes is needed to avoid triggering plant defense reactions during host-root colonization. If this gene regulation pattern is confirmed in other symbionts, this would suggest that loss of genes encoding PCWDE is a consequence of but not a requirement for the evolution of ectomycorrhizal mutualisms. Ectomycorrhizal symbionts, as prime users of sucrose and glucose released by root cells in the apoplastic space, have no need to keep the decay apparatus necessary to decompose SOM. As shown in *Tuber melanosporum*, useless genes coding for PCWDEs, such as GH6 cellulases, then tend to accumulate nucleotide mutations and give rise to pseudogenes and eventually gene remnants (Murat et al. 2018). DNA decay is probably the main inactivation mechanism specifically driving the loss of PCWDEs.

4.2. Evolution of the Ericoid Mycorrhizal Lifestyle

The evolutionary pathway that led to emergence of ericoid mycorrhizal symbioses is less clear. Phylogenomic analysis placed the MRCA of the sequenced ericoid fungi, *O. maius*, *M. bicolor*, *M. variabilis*, and *R. ericae*, at ~118 Mya (Martino et al. 2018). This is the same age as the Ericaceae family (~117 Mya) (Schwery et al. 2015), suggesting that the ancestral ericoid fungal and plant partners diversified simultaneously. Ericoid fungi appear less dependent on plants than other mycorrhizal types, because of their higher saprotrophic capabilities; their arsenal of PCWDEs is so large that they have been hypothesized to be facultative symbionts representing recently recruited lineages of soil-decomposer fungi (Martino et al. 2018). Their arsenal of PCWDE enzymes is certainly under tight regulation during root colonization. To assess the level of expression of fungal genes during symbiosis, *Vaccinium myrtillus* seedlings were inoculated with the sequenced ericoid fungi, *O. maius*, *M. bicolor*, and *R. ericae*. The expression of several genes coding for secreted CAZymes was upregulated during the interaction with *V. myrtillus*. The most highly induced PCWDE genes encoded for secreted PCWDEs targeting cellulose, pectin, and hemicellulose. Sustained expression of PCWDEs in colonized roots might be used to penetrate the thick outer plant cell walls to establish intracellular structures inside the epidermal root cells of ericaceous hosts (Martino et al. 2018).

5. EVOLUTION OF OTHER IMPORTANT SYMBIOTIC TRAITS

The predominant mechanism for the transition from saprotrophy to ectomycorrhizal symbiosis involves the restricted secretion of PCWDEs and secondary metabolites through the loss of genes or transcriptional gene repression. In addition, novel genes, such as effector-like mycorrhiza-induced small secreted proteins (MiSSPs), are also required for symbiosis development and have

evolved in every ectomycorrhizal lineage (Martin et al. 2008, 2010; Kohler et al. 2015; Pellegrin et al. 2015; Miyauchi et al. 2020). Arrays of ectomycorrhiza-induced genes are unique to each specific clade with between 14 and 39% of symbiosis-upregulated genes being species-specific genes with no known function (Kohler et al. 2015, Miyauchi et al. 2020). The dampening of the host plant immunity by lineage-specific symbiotic effectors, such as MiSPP7 and MiSSP7.6 (Plett et al. 2011, Kang et al. 2020), was likely a necessary step for in planta accommodation (for a discussion, see Martin et al. 2016). Silencing these genes by RNA interference precluded the formation of the intraradicular Hartig net, confirming their key role in symbiosis development (Plett et al. 2011, 2014; Kang et al. 2020).

A large proportion of genes that are upregulated in ectomycorrhizas have been coopted from saprotrophic ancestors for the symbiotic lifestyle (Miyauchi et al. 2020). Important ecological traits, such as nitrogen and phosphorus acquisition (e.g., secreted organic nitrogen- and phosphorus-degrading enzymes) were already present in free-living saprotrophic ancestors of ectomycorrhizal symbionts. No expansion of gene families coding for secreted phosphatases and phytases, secreted proteases, and nutrient transporters was found in ectomycorrhizal genomes despite their major role in the acquisition of organic nitrogen and transport of nutrients through the symbiotic interface (Miyauchi et al. 2020).

6. BEYOND THE GENOMES

In conclusion, the ever-increasing number of sequenced genomes of mycorrhizal species released over the last 5 years provides a rich foundation for future studies to elucidate the unique features of these ubiquitous plant mutualistic symbionts. However, many long-term challenges remain for the application of genomics and other -omics tools to enhance our understanding of the evolution, development, functioning, and ecology of mycorrhizal symbioses. Firstly, the current set of sequenced mycorrhizal genomes represents less than ~0.4% of the 50,000 species of mycorrhizal fungi identified so far in European and North American forests. Thousands of additional genomes should be sequenced to unearth the true genetic and functional diversity of this fungal guild. We foresee that sequencing genomes of mycorrhizal species from tropical or arctic biomes may reveal specific gene sets involved in adaptation to these biomes but also unexpected transitions from saprotrophism to mutualism in poorly explored mycorrhizal lineages, such as the ectomycorrhizal Heliotales or Sebacinales. Characterizing the distribution of the lignocellulose decay enzymes, signaling pathways, and effector-like MiSSPs (i.e., the symbiosis molecular toolbox) in these lineages provides novel information on the multiple evolutionary pathways leading to mycorrhizal symbioses.

Despite substantial advances in recent years, gaps remain in our understanding of basic biological and ecological processes that underlie how mycorrhizal individuals, populations, and communities withstand environmental changes such as host switching, nutrient depletion, or drought stress. Although comparative genomics of fungal genomes has led to a burst of important discoveries, as highlighted in this review, it also has some important limitations. The availability of hundreds of new genomes from mycorrhizal fungal species facilitates functional studies (e.g., characterization of symbiosis signaling pathways, regulation of nutrient fluxes in symbiotic tissues, molecular factors driving host specificity) on nonmodel but ecologically relevant species. For instance, the emergence of genes (or gene families) may contribute to certain metabolic and cellular pathways or morphological features (e.g., the ectomycorrhizal mantle, Hartig net, and extraradical hyphal web), but these genes need to operate in the context of other genes to do so. Even though comparative genomics has identified hundreds of new gene families in each lineage

of saprotrophic or symbiotrophic species, this approach does not provide much information about how these gene families relate to known pathways or cellular processes. Approaches such as transcriptomic-based coexpression analysis, proteomics, and protein–protein interaction studies (interactomics) that can link genes (or gene families) to existing biological processes (SOM decay, symbiosis) may be beneficial for predicting evolutionary relationships. Phylostratigraphic analyses can be used to identify the evolutionary period, the so-called phylostratum, in which genes and gene families (e.g., symbiosis-related genes) appeared, as shown in Miyauchi et al. (2020). This approach can be used to determine when in the history of a specific fungal order (or family) specific gene modules appeared and how they changed.

A more reductionist approach would aim to identify protein structures coded by conserved and species-specific symbiosis-regulated genes with unknown function, such as MiSSPs, by using high-throughput protein-folding computation and directed high-throughput automated protein crystallization and X-ray characterization of protein structures. This should facilitate the characterization of the function of proteins playing a key role in symbiosis development.

Genetic diversity holds the key to ecological adaptation. The access to reference genomes for several species within a single fungal genus (e.g., *Tuber*, *Russula*, *Trametes* species) has greatly accelerated our knowledge of the genetic variation within lineages (Murat et al. 2018, Hage et al. 2021, Looney et al. 2021) and its links with important ecological traits. However, it is increasingly evident that a single reference genome per species is insufficient to fully capture the diversity within a lineage, as large amounts of both sequence and genes from a single reference genome have not been found in the genomes of other individuals in the species. For example, a comparison of Russulaceae species revealed that over 30% of genes did not have homologs in the alternative genome (Looney et al. 2021). Reliance on a single reference genome also limits the capacity to identify structural sequence variants including insertions, deletions, duplications, inversions, and translocations, even though these variants certainly play critical roles in the genetic determination of ecological traits and genome evolution. Sequencing the genome of numerous geographical isolates within a species is therefore urgently needed to appreciate the ecological plasticity of SOM decomposers or mycorrhizal symbionts.

In addition to the genomics of single model species, environmental genomics has emerged as a rapidly expanding research field, and this new field is likely to help link genes to ecological traits in forest ecosystems. Metatranscriptomics of decaying wood, forest soils, and mycorrhizal roots (Liao et al. 2018) benefit from the increasing number of available genomes from ecologically relevant species for precise taxonomic and functional annotations of anonymous environmental sequences generated by metatranscriptomic profiling of forest soils. Environmental RNA sequencing provides information on community composition (through phylogenetic affinity of the sequence) and on the environmental physiology of each component in the community. By coupling genome sequencing of prominent fungal species inhabiting a specific experimental site and RNA sequencing of mycorrhizal roots and soil and litter samples, we should be able to assess the specific gene expression of each of the dozens of mycorrhizal partners associated with a single root system to gain insight into the functional relationships between symbiotic neighbors. This would allow us to question whether multiple symbiotic species can coexist on the same host tree by using the same resources and performing identical ecological function(s) (i.e., high functional redundancy) or by using different resources and providing distinct functions (i.e., limited functional redundancy). In highly complex forest ecosystems involving many interacting fungal guilds and tree/plant communities, we believe that a genome-to-ecosystem strategy provides a better understanding of the respective roles of the thousands of species of wood decayers, soil and litter decomposers, and mycorrhizal species flourishing in forest biomes.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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