Review

Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes

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Soils contain a multitude of fungi with vastly divergent lifestyles ranging from saprotrophic to mutualistic and pathogenic. The recent release of many fungal genomes has led to comparative studies that consider the extent to which these lifestyles are encoded in the genome. The genomes of the symbiotic fungi *Laccaria bicolor* and *Tuber melanosporum* are proving especially useful in characterizing the genetic foundation of mutualistic symbiosis. New insights gleaned from these genomes, as compared to their saprotrophic and pathogenic cousins, have helped to redefine and shape our understanding of the nature of the symbiotic lifestyle. Here we detail the current state of research into this complex relationship and discuss avenues for future exploration.

Genomes of mutualistic fungi: towards an understanding of the molecular basis of symbiosis

It is often hard to grasp the complex community structures and incredible species diversity that abound beneath our feet in soil ecosystems. Thousands of species of animals, plants, insects, fungi and bacteria coexist in a complex web of life that is responsible for the cycling of water, carbon and nutrients that support the continued productivity of our soils. To understand fully the workings of this complex ecosystem one must not only regard organisms as individuals, but also as members of a larger community that reflects the interplay and communication between each individual within a population. The developing picture of systems biology would suggest that the environment and community with which an organism associates can affect the function of an individual genome [1]. Although some broad generalities behind the trophic workings in these soil ecosystems have been characterized, little of the fine detail of the molecular crosstalk between individual species is understood [1]. With many sequenced bacterial, fungal, plant and animal genomes becoming available, the data encoded therein can now be applied to multi-member systems in order to understand the functional potential of any one organism within its community.

One emerging model for such studies is the mutualistic symbiosis between soil-borne fungi and tree roots. A number of different classes of beneficial symbiotic fungi inhabit forest soils and form mycorrhizal structures with their plant hosts [2]. These fungi play an essential role in nutrient and carbon cycling in forest soils.

Ectomycorrhizal (ECM) fungi (e.g. truffles, bolets, amanitas, chanterelles) form symbiotic relationships with the roots of most tree species and support increased forest health and productivity [2–4]. Although ECM fungi are only able for form a relationship with approximately 3% of seed-bearing plants, these lineages of the *Betulaceae, Cistaecae, Dipterocapaceae, Fagaceae, Pinaceae, Myrtaceae, Salecaeceae* and *Fabaceae* dominate boreal, temperate, Mediterranean and subtropical forests and woodlands [5]. Phylogenically, ECM fungi are found in the phyla Basidiomycota and Ascomycota (Glossary) along with non-mutualistic saprotrophic fungi (Figure 1). They do not share a common symbiotic ancestor, but instead have

Glossary

Apoplastic space: the free diffusional extracellular space in between plant cell walls.
Ascomycota: a subdivision of fungi whose spores are contained in a sac-like tissue called an ascus. This is an ancient, large, diverse division of fungi that include pathogens (e.g. *Aspergillus fumigatus*), saprotrophs (e.g. *Neurospora crassa*) and mutualistic fungi (e.g. *Tuber melanosporum*).
Basidiomycota: a subdivision of fungi whose spores are borne on basidia. As with the Ascomycota, this division of fungi contains a large number of pathogens (e.g. *Cryptococcus neoformans*), saprotrophs (e.g. *Coprinopsis cinerea*) and mutualistic fungi (e.g. *Laccaria bicolor*).
Ectomycorrhizal (ECM) fungi: mutualistic fungi, belonging to the Basidiomycota and Ascomycota, whose hyphae surround plant roots to form a mantle and grow within the apoplastic space of the plant root (forming the Hartig net; below).
Filamentous fungi: eukaryotic fungi that form colonies growing in the form of multicellular filaments. These organisms do not produce their own nutrients, but instead rely on absorption of complex organic substances from their surrounding environment. This group includes pathogenic, saprotrophic and mutualistic fungi.
Hartig net: the complex structure of ECM fungal hyphae that reside within the apoplastic space of host roots. It is across the large surface area of contact between fungal hyphae and plant roots within the Hartig net that the fungus exchanges nutrients (e.g. nitrogen) for symbiotically-derived carbon in the form of glucose.
Hyphae: a single filament of a fungal colony consisting of long, thin cells attached end to end.
Hypaphorine: indole-alkaloid produced by fungi that is an auxin antagonist.
Kinome: an inventory of each unique protein kinase gene or gene family within the genome of an organism.
Mantle: thick, multi-layered sheath of fungal hyphae that surround plant host roots.
Mycorrhizal-induced small secreted protein (MiSSP): any fungal small secreted protein that shows a higher level of expression during the formation or maintenance of the symbiosis between ECM fungi and their host plant.
Mycorrhizal root tip: plant lateral roots colonized by ECM fungi. Morphologically these roots are surrounded by the fungal mantle and contain a developed Hartig Net.
Mycelium: the entirety of the interwoven, multi-branched vegetative fungal hyphae.
Saprotroph: organisms that rely on nutrients absorbed from the decomposition of dead organic substrates.
Small secreted protein (SSP): any protein secreted by an organism that is less than 300 amino acids in length.
Trophic: of, or relating to, the feeding relationship between various organisms within an ecosystem.

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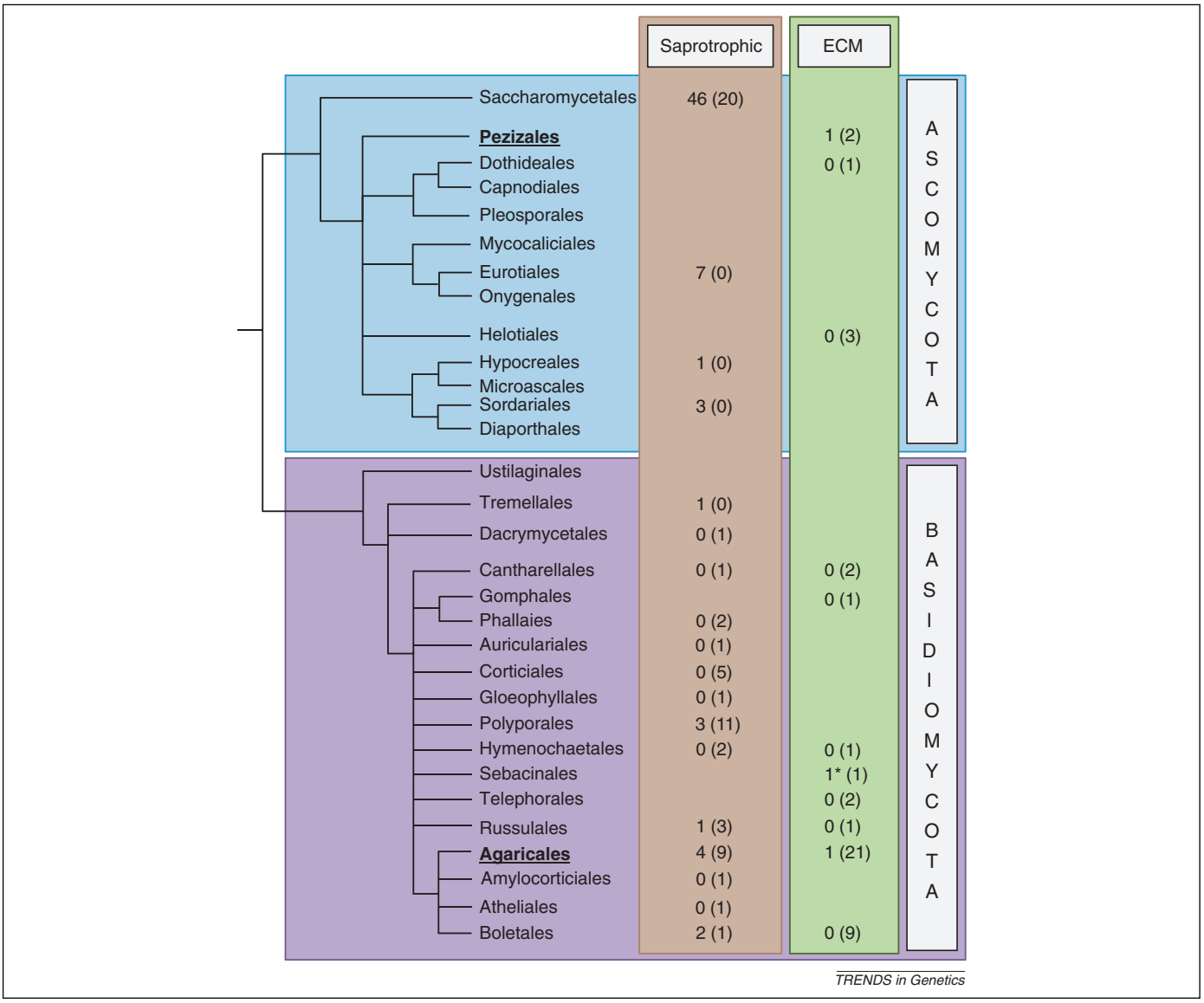

Figure 1. Phylogenetic distribution of basidiomycete and ascomycete saprotophic and ectomycorrhizal fungal orders with genomes sequenced or scheduled for sequencing. Because the ECM lifestyle has arisen independently several times from saprotrophic ancestors within the ascomycota (blue box) and the basidiomycota (purple box) [6], the genomes of ECM and saprotrophic fungi from many diverse orders are currently being sequenced within the JGI MycoCosm project to understand better the genetic basis of mutualistic symbiosis. The number of genomes currently available (with upcoming genomes in parentheses) are found in the brown column for sapotrophic and wood decay species and in the green column for ECM species. Orders with sequenced ECM fungi discussed in the text are highlighted in bold and underlined. Additional orders with no sequenced species have been included for completness. *Oidiodendron maius*, an ascomycete of the Leotiomycete class, has not been included because it does not have a defined order at the time of publication. *, *Piriformospora indica*, of the Sebacinales order, is a growth-promoting endophyte of *Arabidopsis thaliana* and is not a true mycorrhizal fungus. Figure adapted from [76,77].

arisen independently at least eight times with angiosperms, and between six and eight times with gymnosperms from saprotrophic ancestors [6]. In the ECM fungus–plant symbiotic interaction, fungal hyphae surround plant lateral roots forming a mantle and penetrate between root cells to form a complex web of fungal and plant cells (called the Hartig net; Figure 2). The symbiotic tissue is referred to as a mycorrhizal root tip. It is across the large surface area of the Hartig net that nutrients from the fungus (e.g. nitrogen, phosphorus, and sulfur compounds) are exchanged for photosynthetically derived sugars [7]. Given the important role of the fungus in plant nutrient acquisition, when considering the factors regulating tree growth it is impossible to look solely to plant genomes for answers. Because plants and fungi have been associated since the first colonization of land [5,8], their genomes have coevolved, developing complementary traits (e.g. loss of plant cell-wall-degrading enzymes by ECM fungi and a decreased plant defense response to colonizing ECM hyphae) [9]. Thus they are best considered together to gain a holistic understanding of the information and signaling highways that are present.

It is unknown if all classes of symbiotic fungi share a common core set of genes required for the formation of symbiosis (hereafter referred to as the symbiotic 'toolbox'), or if the genetic mechanisms required for mutualism were reinvented each time it developed in evolutionary history. The study of symbiosis between ECM fungi and trees has seen some significant advances in the past 2 years with the availability of the sequenced genomes of model ECM fungi as well as of plant hosts such as poplar. To date, the genomes of two purely ECM fungi have been sequenced,

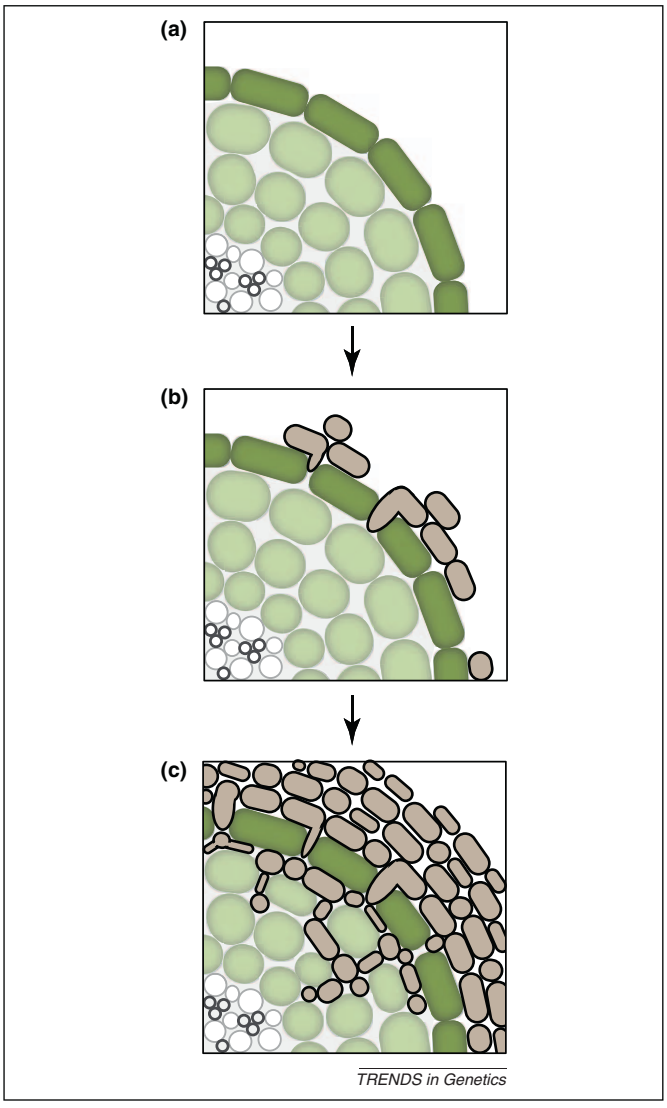

Figure 2. Schematic representation of a transverse cross-section of a root undergoing colonization by an ECM fungus. **(a)** Representation of a transverse cross-section of a plant lateral root before ECM fungal colonization. **(b)** During the initial contact between the root (green cells) and ECM fungal hyphae (brown cells) the fungus begins by attaching to the root surface. The attachment process causes the fungus to secrete proteins, phytohormones and metabolites that cause restructuring of the root that allows fungal hyphae to penetrate into the root apoplastic space. **(c)** Representation of a transverse cross-section of a mature mycorrhizal root tip. At this stage of colonization the fungus has completely wrapped around the entire root surface forming a thick, multi-layered 'mantle' constructed from individual hyphae (cells outlined in black). A number of fungal hyphae have also invaded between the plant cells of the root, forming a structure called the Hartig net. It is in the Hartig net that nutrient exchange between the fungus and the plant takes place.

Table 1. Sequenced and upcoming ectomycorrhizal fungal genomes

Completed genomes	
Ascomycota	
Tuber melanosporum [11]	
Basidiomycota	
Laccaria bicolor [10]	
Re-sequencing	
Basidiomycota	
Laccaria bicolor (12 strains)	*Laccaria proxima*
Laccaria laccata	*Laccaria tortillis*
Ongoing sequencing	
Ascomycota	
Tuber magnatum	
Basidiomycota	
Hebeloma cylindrosporum	*Piloderma croceum*
Pisolithus microcarpus	*Laccaria amethystina*
Upcoming genomes	
Ascomycota	
Cenococcum geophilum	*Oidiodendron maius*
Meliniomyces bicolor	*Rhizoscyphus ericae*
Meliniomyces variabilis	*Terfezia boudieri*
Basidiomycota	
Amanita muscaria	*Pisolithus tinctorius*
Boletus edulis	*Ramaria formosa*
Cantharellus cibarius	*Rhizopogon salebrosus*
Coltricia cinnamomea	*Scleroderma citrinum*
Cortinarius glaucopus	*Sebacina vermifera*
Gymnomyces xanthosporus	*Suillus luteus*
(formerly *Hydnangium*	*Thelephora terrestris*
carneum var. *xanthosporum*)	*Tomentella sublilacina*
Lactarius quietus	*Tricholoma matsutake*
Paxillus involutus	*Tulasnella calospora*
Paxillus rubicundulus	
Piloderma croceum	

that of the Basidiomycete *Laccaria bicolor* (bicolored deceiver) [10] and that of the Ascomycete *Tuber melanosporum* (perigord black truffle) [11]. Other ECM genomes are currently being sequenced (Table 1). *L. bicolor* has a 64.9 Mb genome with the largest complement of predicted proteins for any fungus, whereas *T. melanosporum* has the largest fungal genome at 125 Mb with one of the smallest complements of predicted proteins in any filamentous fungal genome sequenced to date. Analysis of these genomes has given great insights into the mechanisms driving eukaryotic symbiosis.

The very nature of a mutualistic relationship implies that both partners benefit from the interaction. However, the extent to which a given relationship is considered equal and what factors drive the initiation and continuation of that relationship are less clear. In ECM fungus–plant symbiosis, these are pertinent questions to be addressed. The study of ECM fungal genomes can shed light on some of the factors that drive symbiosis. A number of interesting predictions concerning the evolution of the ECM lifestyle, as well as the role of many secreted proteins in promoting symbiosis, have been made based on the *L. bicolor* genome [7]. With the newly released *T. melanosporum* genome we can now address if these predictions still hold. Together, these genomic analyses could better define the role of each partner in the symbiotic interaction. Is it truly a relationship of equals, or does one partner hold the balance of power? We consider below some of the lessons learned from the contrasting genomes of *L. bicolor* and of *T. melanosporum* in nutrition, signaling and in mediating symbiosis.

Factors driving nutrient transfers between symbiotic partners

In a mutualistic relationship both partners must benefit from the interaction, a criterion that ECM fungi fulfill [12,13]. One factor that predisposes the plant to accept a symbiotic interaction is the ability of ECM fungi to utilize nitrogen and phosphorus sources not easily bioavailable to plants [7,14–21]. This is of particular importance to the plant in soils that are nutrient-poor. In this case it is highly beneficial to the plant to give up carbon in return for the growth-limiting nutrients (Figure 3a) [22].

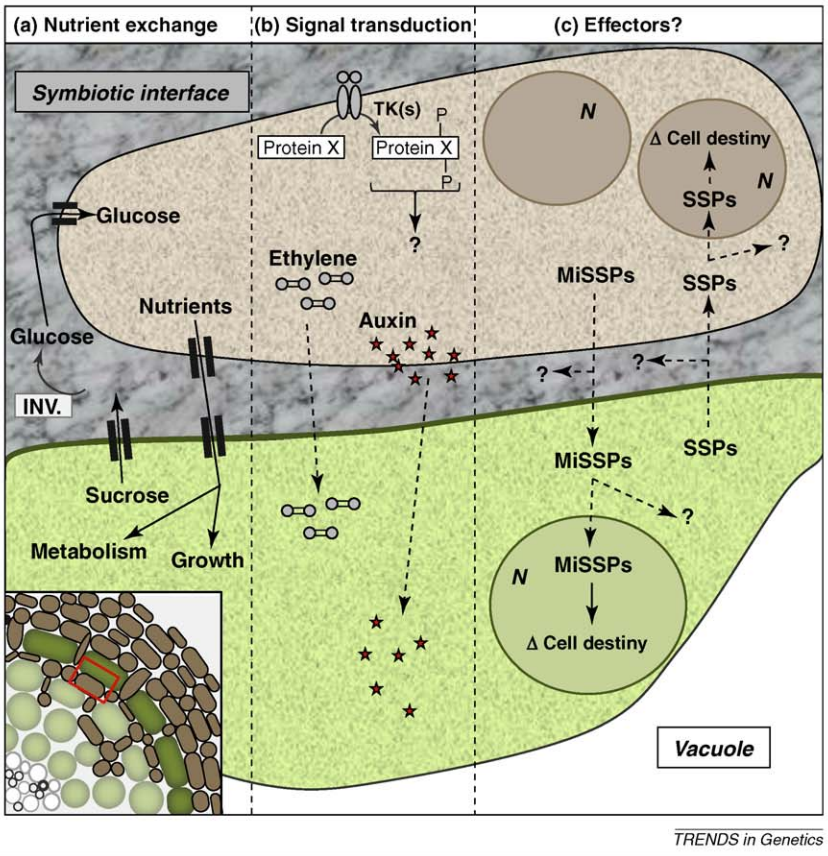

Figure 3. Summary of three key levels of control in the symbiotic interaction between ECM fungi and plant cells. High magnification schematic representation of a hyphal cell (brown) and a plant cell (green) from a transverse cross-section of the Hartig net (see inset for orientation, red box). **(a)** The main common feature of all plant–ECM fungus mutualistic symbiotic interactions is reciprocal nutrient exchange. Photosynthetically-derived sucrose from the plant is cleaved by invertases (INV) into glucose and fructose in the symbiotic interface. Hexose transporters in the hyphae uptake this glucose which is used by the fungus as its primary carbon source. In return, the fungus releases nutrients (e.g. nitrogen, phosphorus) into the apoplastic space of the root, after which high-affinity plant nutrient importers take these nutrients into the root cell where they are used to support plant growth and metabolism. **(b)** To establish symbiosis, ECM fungi must have novel signaling pathways, as compared to their saprotrophic cousins, to negotiate a mutualistic relationship with the plant root. Suggested novel signaling pathways are those controlled by tyrosine and tyrosine-like kinases (TKs) that activate signaling cascades within the fungal cells. The signaling pathways controlled by these kinases are unknown. Diffusible elements such as ethylene and auxin have also been implicated in the development of symbiosis between the two partners. **(c)** A second set of novel control pathways in ECM fungi are those governed by MiSSPs. These secreted proteins are thought to act as effectors to control the fate of plant cells and disrupt plant cell defenses in both the apoplastic space of the root as well as in root cells, although this has yet to be proven. The plant partner is unlikely to be silent in the symbiotic relationship. As has been demonstrated in bacterial symbiosis with plant roots, ECM host plants probably release a fleet of SSPs which, like MiSSPs, are destined to control fungal development within the root. Solid arrows represent known pathways active in ECM root tips; dashed arrows represent hypothesized signaling relays at work during symbiosis. N, nucleus.

Enabling the fungus to exploit soil resources is a group of 116 different secreted proteases including fungalysin metalloproteases, aspartyl proteases, and serine proteases in *L. bicolor* that are implicated in liberating nitrogen from decaying plant and animal sources in the soil [10,14,23]. In addition, the recent discovery of both low- and high-affinity phosphorus transporters in *Hebeloma cylindrosporum, L. bicolor* and *T. melanosporum,* and the thorough analysis of membrane transporters in *L. bicolor,* could soon change our understanding of the uptake of nitrogen and phosphorus from the soil by the ECM fungus [10,11,24–26]. Therefore, whereas ectomycorrhizal fungi are very poor degraders of wood or fresh leaf litter as a nutrient source, as compared to white- or brown-rot saprotrophic fungi, the presence of such proteases and transporters allows these fungi to mine the material released during decay to support the nutrient needs of the ECM colony and the plant.

Analysis of upregulated genes in ECM symbiotic tissues has identified several different gene families that are consistently upregulated during symbiosis [10,11,27,28]. These include major facilitator superfamily (MFS) transporters, aquaporin-related major intrinsic proteins, and amino acid permeases. For example, in nitrogen transfer nitrogenous compounds are transported into the host apoplastic space by mycorrhiza-upregulated amino acid transporters and ammonium transporters [29–35]. Reassimilation of the nitrogen by the fungus is then prevented by reduced expression of high-affinity ammonium importers normally found in the extramatrical mycelium [35]. Disturbance of nitrate assimilation results in the disruption of symbiosis, confirming the key role played by nitrogen transfer [36]. It is interesting that, despite many differences seen between the genomes of *L. bicolor* and *T. melanosporum*, one of the rare commonalities between these two ECM fungi is a common core set of symbiosis-induced metabolic transcripts related to nutrient cycling [11]. The end result – provision of nutrients to the plant host – is a key factor driving and maintaining symbiosis.

ECM genomes reveal a loss of degradative enzymes

Saprotrophic fungi are able to use dead plant tissue found in soil litter as a carbon source and produce cellulases and

hemicellulases that are able to deconstruct and hydrolyze plant cell wall (PCW) materials [37–39]. Necrotrophic and hemi-biotrophic fungal pathogens (e.g. *Magnaporthe grisea*) use similar carbohydrate-cleaving enzymes to digest, or rot, living tissues. These features make it difficult if not impossible for both classes of fungi to enter into a symbiotic relationship with plants because these enzymes would damage the host and elicit a plant defense response. Because ECM fungi are very poor saprotrophs and do not predate host cells, it was hypothesized that ECM fungi could have a decreased contingent of PCW-degrading enzymes [10]. This was found to be true in the genomes of both *L. bicolor* and *T. melanosporum,* where the gene families showing the largest number of losses or extinctions, as compared to extant species of saprotrophic fungi, were those involved in degrading cell walls [10,11,40]. Similarly, a recent comparison of ECM *Amanita* species to saprotrophic *Amanita* species also found that the ECM members exhibited loss of several key cellulase genes, thus reducing their ability to survive on plant carbon sources ([41], B. Wolfe, personal communication). There are, however, distinct differences between the two genomes with regard to which PCW-degrading families were lost. Although both ECM fungi still encode a small group of enzymes able to degrade components of plant cell walls, those that have been conserved provide insights into how differently these two fungi behave when prospecting the soil as well as during symbiosis. *L. bicolor* encodes a number of enzymes expressed in prospecting hyphae that allow it to act as a weak saprotroph, and thus survive in soil litter when not in symbiosis with a plant root [10]. Conversely, during root invasion *L. bicolor* expresses very few PCW-active enzymes, and instead focuses on the secretion of expansin-like proteins that aid in plant cell remodeling during hyphal penetration. *T. melanosporum* does not encode the PCW-active enzymes necessary to confer the same saprotrophic capabilities as *L. bicolor* when prospecting the soil for nutrients – potentially making it much more dependent on its plant host than *L. bicolor*. *T. melanosporum*, however, is much more aggressive in its colonization of root tissue, expressing high levels of transcripts coding for a laccase, a tyrosinase and a lipase as compared to *L. bicolor* [11]. The higher expression of these enzymes is thought to aid in breaking down the connections between plant cells, aiding the penetration of fungal hyphae and the establishment of the Hartig net. Interestingly, a single GH5 cellulase is expressed by the two ECM fungi during symbiosis development. The role played by this enzyme in root colonization remains to be determined.

Although these results would suggest two very different symbiotic toolboxes, one of the novelties of ECM genomes that promote symbiosis is the loss of PCW-degrading enzymes. This change makes the ECM fungi more dependent on the plant for photosynthate as a carbon source while being less of a threat to the integrity of the plant cell.

Novel and expanded signaling pathways could control symbiosis

During the establishment of symbiosis the fungus must go through a change in growth habit, from that of free-living mycelium to being hosted in the plant root. This step requires signal relays that maintain enough plasticity to adapt to changing conditions. The genomes of both *L. bicolor* and *T. melanosporum* contain novel and expanded gene families that could play a role in signaling during these different life-stages [10,11,42].

The initial contact between fungal hyphae and a plant lateral root is mediated by a variety of chemical signals from both partners, including the hormones auxin and ethylene (Figure 3b). ECM fungi overproducing auxin have an increased percentage of mycorrhizal root tips [43,44]. Diffusible signal(s) from *L. bicolor* also alter the homeostasis and transport of endogenous plant auxins very early in the communication between the fungi and the plant [45], leading to emergence of lateral roots at higher density. It has been proposed that this effect on lateral root induction is due to altered localization of the PIN-formed (PIN) auxin-transport facilitators in the plant [45]. Fungal indolic auxin-signaling agonists, such as tryptophan betaine (hypaphorine) secreted by the ECM fungus *Pisolithus microcarpus*, have also been found to control the development of short roots [46].

After initial contact, as ECM fungi grow in the root apoplastic space, the fungus must continue to communicate with the plant in order to be accommodated within the plant root. Transcriptomic analysis of ECM root tips has identified over 100 fungal genes induced by symbiosis [10,11]. Of these, a number code for secreted proteins that might act within the apoplastic space to divert plant proteases (e.g. mycocypin/clitocypin proteins) or to restructure the plant fungal interface (e.g. lectins, RGD-motif acidic proteins and hydrophobins) [6,7]. This chemical-based inhibition of plant defenses is crucial in the establishment of symbiosis, and is closely linked to internal signaling within the fungal cell itself.

The genomes of *L. bicolor* and *T. melanosporum* also contain genes coding for several hundred small secreted proteins. Interestingly, ten of these genes in *L. bicolor* that are highly regulated in symbiotic tissue, dubbed mycorrhiza-induced small secreted proteins (MiSSPs), bear some resemblance to pathogenic effector proteins (Figure 3c) [10]. Fungal pathogens, such as *Ustilago maydis* (order Ustilaginales, Figure 1), use small secreted effector proteins (<300 aa) to subvert plant cell defenses and colonize plant tissues [47–49]. These effector proteins act to alter plant signaling relays either within the apoplatic space or within the plant cell [50,51]. Pathogen-like effectors have also been discovered to be important in symbiotic interactions between *Rhizobia* bacteria and legumes [52]. The precise use of MiSSPs in *L. bicolor* symbiosis is under investigation; however, it is interesting to note that the transcriptome of *T. melanosporum* does not contain any MiSSPs. If MiSSPs prove to be important, it is evident that *T. melanosporum* must utilize other unknown signals in their place.

In filamentous fungi, a large portion of signaling is controlled by phosphorylation of proteins by kinases. These phosphorylation events occur more commonly during periods of major differentiation in the fungal lifecycle, such as the production of fruiting bodies [53]. Although kinases are found ubiquitously in all living organisms, comparison of the kinomes between organisms demonstrates that the

types of kinases present are highly diverse [54]. As a result, novel or different subfamilies of kinases could be expected to exist in ECM fungi due to their particular lifestyle. Analysis of 12 subgroups of kinases in 30 fungal taxa recently revealed that *L. bicolor* has a higher than average number of tyrosine-like kinases (25 genes as compared to saprotrophic basidiomycetes which encode up to four genes) [54]. Tyrosine and tyrosine-like kinases affect a number of different cellular processes, but they are especially interesting because of their involvement in cellular differentiation and proliferation. Their over-representation could be a fingerprint of an ECM symbiotic genome because the genome of *T. melanosporum* was also found to have an over-representation of tyrosine kinase genes (42 genes distributed between two families) that exhibit the highest rate of expansion as compared to other saprotrophic and pathogenic fungal genomes [11]. Of these 42 genes, three are unique to *T. melanosporum.* These gene families were determined to be expanding (as opposed to contraction, extinction or stasis) using the CAFE (computational analysis of gene family evolution) program [55]. This program makes phylogenetic inferences on changes in family size using a maximum likelihood estimation to model the random birth and death process of genes in each family. Given these data, phosphorylation-controlled signaling is likely to play a role in the establishment of symbiosis, and it is possible that tyrosine and tyrosine-like kinases control symbiosis specific pathways (Figure 3B). Further characterization of this family of genes will be needed before conclusions can be made with regard to their role as mediators in symbiosis.

The role of the plant in symbiotic establishment

When considering ECM symbiosis as a whole, it appears that much of the control over the initiation of symbiosis is wielded by the fungus. This is not surprising because the fungus generally has more to gain from symbiosis than the plant [56]. Many symbiotic fungi are unable, or have reduced ability, to harvest their own carbon, making them dependent on plant colonization [10,11,57–59]. The arsenal of signaling molecules and proteins used by the fungus to promote symbiosis is therefore crucial to its survival. Although the plant receives nutrients from the fungus, this comes at the expense of 10–50% of its carbon budget [60–65]. In well-fertilized soils the benefit of symbiosis to the plant is less evident. Although mycorrhizal symbiosis has often been considered as the classic example of mutualism, depending on the abiotic and biotic conditions in the environment and the resulting impact on plant fitness, the interaction could also be classified as parasitic [9,22,56,66]. The reality probably exists somewhere in between mutualism and parasitism, constantly shifting with environmental conditions and organism life stages. What controls then, if any, does the plant have to avoid exploitation? We feel that the plant is not a silent partner in this exchange and that it also exerts a measure of control over the fungus. Experimental evidence, in fact, suggests that either partner is able to terminate symbiosis should the nutrient supply stop (be it plant or fungal supplied) [67,68]. The genetic basis of this control, however, is unknown – although there are hints from both ECM studies and from other symbiotic interactions as to how this control could occur.

First, in the context of ECM symbiosis, it was discovered that the genome of *L. bicolor* lacks an invertase to hydrolyze plant sucrose. Host trees to this fungus, however, do encode an invertase in their roots. This is an important distinction because tree roots secrete sucrose at the symbiotic interface, but *L. bicolor* is only able to import glucose [10,40,69], making it heavily reliant on host invertase activity (Figure 3a). This is not unique to *L. bicolor* because other mycorrhizal fungi, including *Amanita muscaria* and *Hebeloma crustuliniforme*, have been shown to be completely dependent on the invertase activity of their host [70]. Indeed, a recent study of 46 ectomycorrhizal fungal species in the Basidiomycota found that only one species contained an invertase gene [71]. This is in contrast to plant pathogenic fungi, which generally encode multiple invertases or are able to directly import sucrose [71,72]. The dependence of ECM fungi on plant invertases produces one possible avenue for the plant to regulate the amount of carbon being given to the fungus. In the case where the symbiosis is no longer beneficial, lowered expression of plant invertases in the root apoplast or at the symbiotic interface would starve the fungus and end the interaction. This level of control by the plant is probably not suitable for all ECM fungal species because *T. melanosporum* encodes a symbiotically expressed invertase [11].

A second mechanism of control by the plant could be similar to that found in certain legume–*Rhizobium* symbioses. *Rhizobium* constitutes a genera of mutualistic bacteria that enter into symbiosis with the roots of leguminous plants. These bacteria are able to fix atmospheric nitrogen, some of which is released to the plant in return for plant-derived sugars. Although it was known that this symbiosis is favored by the release of signaling molecules (nodulation factors) by the bacteria, more recently it was demonstrated that several plants encode small secreted protein (SSP) required for proper development of the symbiotic nodules [73,74]. In this example, a cysteine-rich secreted protein from the plant was shown to initiate terminal differentiation in symbiotic *Rhizobium,* thus limiting unrestrained proliferation of the bacteria within plant tissue. The role of differentiation of the bacteria during symbiosis is presently unknown, but it demonstrates a degree of control over the symbiont partner by the plant. Similar to *Rhizobium*–legume symbiosis, it is logical to assume that ECM host plants, like leguminous plants [73,74] and *Arabidopsis thaliana* [75], might also produce SSPs that are essential for the proper establishment and control of symbiosis (Figure 3c). The roles of these hypothetical plant-based SSPs are likely to be diverse. For example, they might limit the depth of hyphal invasion by the ECM fungus into the root so as to maintain integrity of the root structure. They could also favor the uploading of nutrients by the fungus into the plant apoplastic space; there are also many other possibilities. Although no plant-based SSPs regulated by the interaction between roots and ECM fungi have been identified in tree species to date, this would be an interesting avenue of research in coming years towards an understanding of the controls that govern the establishment of mutualism between tree roots and ECM fungi.

Concluding remarks and future perspectives

Despite debate over the exact nature of ECM fungus–plant symbiosis, its overall benefit and importance in the forest ecosystem is indisputable. For this reason it is an important topic for research because we seek to exploit this relationship to maximize forest and crop productivity and sustainability. One crucial step in mycorrhizal research is the production of sequenced genomes, both of a variety of ECM fungi and their plant hosts. Two ECM genomes have been sequenced to date: those of *L. bicolor* and *T. melanosporum*. It would appear, based on the different molecular 'toolboxes' of these two ECM genomes, that the evolution of the ECM lifestyle is quite divergent. Although commonalities exist (Figure 3), such as a reduction in PCW-degrading enzymes, the presence of common nutrient transporters, and a large complement of tyrosine-like kinases, large differences are also seen, such as the secretion of MiSSPs that are present only in *L. bicolor*. To understand if the differences between these two species are due to their origins in the Ascomycota, versus the Basidiomycota, the genomes of more ECM fungi are currently being sequenced to serve as a comparison (http://genome.jgi-psf.org/programs/fungi/index.jsf; Table 1). To understand the genomic variation within a species and within a genus, the *Laccaria* Pan-Genome project is also underway which will sequence the genomes of 12 *Laccaria bicolor* strains from geographically diverse origins as well as four other *Laccaria* species (Table 1). This resource will allow mycologists to extract valuable information about adaptive mutations that are most likely to be important to symbiosis in a well-investigated symbiotic clade.

Because ECM fungi derive from saprotrophic ancestors it is interesting to note the many differences between the genomes of ECM fungi and those of modern saprotrophs. A number of these differences are in areas of nutrient use and transportation and strongly favor a mutualistic lifestyle. For example, the inability of most ECM fungi to use carbon sources in the soil effectively, or even to hydrolyze sucrose donated by the plant, are adaptations that make them highly dependent on their host plant. What factors drove these evolutionary changes and how did they occur? Certainly the evolutionary pressure from the plant host cannot be ignored because symbiotic fungi coevolved with plants.

An emerging field in ECM research is a consideration of the signaling pathways that are used to initiate contact between the fungus and the host plant and that regulate the symbiosis. The role of tyrosine and tyrosine-like kinases in signaling, as well as MiSSPs in the case of *L. bicolor*, warrants further investigation. Other crucial questions that need to be addressed in greater detail are: do these different levels of regulation act together simultaneously, or do they act in discrete stages in a perfectly timed sequence of signaling events? Can interruption or augmentation of these signaling pathways be used to halt or favor the establishment of symbiosis? Also of importance is the contribution of plant signaling in the establishment and maintenance of this interaction. As the ability to genetically manipulate different fungi, both symbiotic and non-symbiotic, progresses and as data concerning the timing of gene expression during plant colonization become available these questions will be more easily tackled.

The study of ECM fungi is still in its infancy relative to other systems such as pathogenic fungi. With recent advances in genome sequencing the study of mycorrhizal fungi is gaining new momentum. We await with anticipation and eagerness to see what progress and discoveries into the subtleties of this complex system will be made in the coming years.

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