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Symbiotic plant-fungi interactions stripped down to the root

Luca Venturini & Massimo Delledonne

Mycorrhizal fungi live in the roots of host plants and are crucial components of all forest ecosystems. A large-scale study of fungal genomics provides new insights into the evolution of mycorrhizae and a deep exploration of mycorrhizal diversity that helps to uncover the molecular and genetic details of fungal symbiotic relationships with plants.

Evolution of mycorrhizal symbiosis

The evolutionary origins of mycorrhizal associations have long fascinated plant scientists, especially given the ecological and agronomic importance of symbiosis for terrestrial ecosystems. Forest trees are particularly dependent on ectomycorrhizal symbioses, which are characterized by the localization of the mycelium exclusively on the root epidermis. This association is so beneficial for the participants that it appears to have emerged multiple times since the Jurassic^{1,2}, but the molecular basis of this convergent evolutionary process has thus far eluded researchers². Francis Martin and colleagues³ addressed this challenge with intergenomic phylogenetic analysis of mycorrhizal fungi and found that the emergence of mycorrhizal symbiosis has required similar genetic adaptations across multiple clades.

By selecting 31 fungal genomes representing both saprophytic and symbiotic lifestyles and supplementing this panel with the *de novo* sequencing and assembly of the genomes from 18 further species (Fig. 1), Kohler *et al.* obtained a collective gene catalog of 611 single-copy gene families with members present in greater than 15 species and generated a reconciled evolutionary tree. Such families of orthologous genes that are devoid of any duplication event are valuable for phylogenomic studies because their divergence closely follows that of the species^{4,5}. In line with previous studies^{1,2},

molecular clock analysis on the reconciled tree suggested that ectomycorrhizal fungi evolved fairly recently from multiple lineages fewer than 200 million years ago, far later than the appearance of the last common ancestor of white- and brown-rot fungi 300 million years ago. This result supports the long-standing hypothesis that ectomycorrhizal fungi evolved polyphyletically from multiple saprophytic species.

Interaction-specific remodeling of fungal genomes

The story told by these gene families also clarifies the more chaotic evolutionary

history of plant cell wall-degrading enzymes (PCWDEs). The saprotrophic relatives of mycobionts, which cause white and brown rot, have evolved a large catalog of enzymes to degrade the components of plant cell walls⁶. The most important of these are the carbohydrate-active enzymes (or CAZymes), which degrade cellulose and hemicellulose, and the fungal class II peroxidases (or PODs), which degrade lignin. Mycobionts and ectomycorrhizal species in particular lack the ability to degrade lignocellulose with the efficiency of their predatory relatives². This may reflect the evolutionary loss of genes encoding PCWDEs,

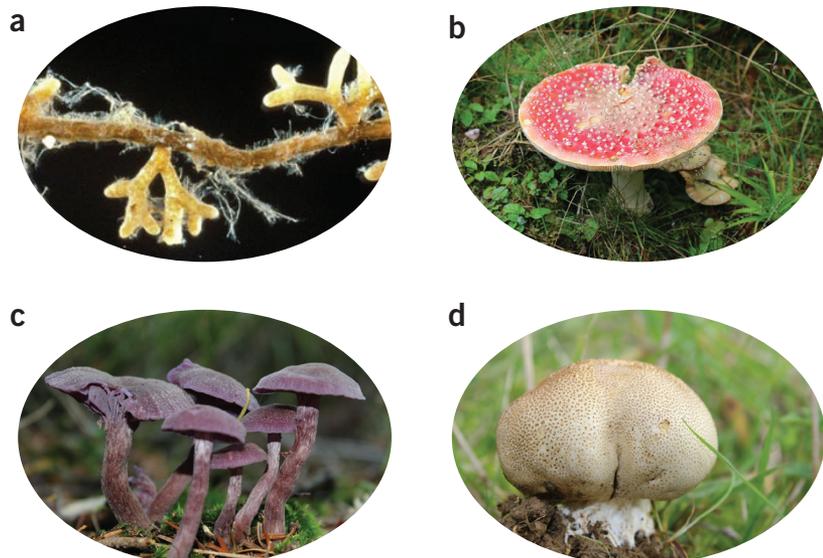


Figure 1 Ectomycorrhizal fungi diversity. (a) Ectomycorrhizal fungi colonize the exterior of tree roots (reprinted with the permission of Wiley from ref. 12) and often develop visible fruiting bodies. (b–d) Kohler *et al.* sequenced the genome of 13 of these symbiotic species, including *Amanita muscaria* (fly agaric) (b), *Laccaria amethystina* (amethyst deceiver) (c) and *Scleroderma citrinum* (common earthball) (d).

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as suggested by the paucity of such genes in the first two sequenced ectomycorrhizal genomes, *Tuber melanosporum*⁷ and *Laccaria bicolor*⁸. To assess whether ectomycorrhizal lineages have a reduced complement of PCWDEs compared to ancestral white-rot wood decayers, Kohler *et al.* analyzed the evolution of 16 gene families associated with plant cell wall degradation across the lineages in the panel. Their results unequivocally showed that all symbiotic fungi have experienced substantial gene loss in these families. However, the process has not been uniform for all genes but rather has primarily concentrated on genes necessary for the degradation of lignin. This convergent selection suggests that the ectomycorrhizal fungi have shed the most threatening weapons in their arsenal to interact successfully with plant roots. Intriguingly, this loss was typical of ectomycorrhizal fungi but not of other fungal symbionts. The endomycorrhizal ericoid and orchid fungi included in the panel maintain an extensive repertoire of PCWDEs, suggesting that these enzymes might still be needed for the initial penetration and colonization of root cells.

Gene loss explains only half the story. Another pressing question concerns the nature of the genes that have evolved specifically to establish symbiosis. Kohler *et al.*

investigated this part of the puzzle by comparing gene expression in free-living mycelia and established mycorrhizae for eight symbiotic species (including five ectomycorrhizal organisms)³. In line with available data on *L. bicolor*, which expresses its few remaining PCWDEs during the first phases of interaction with its hosts⁹, they found that most of the PCWDEs present in ectomycorrhizal fungi were expressed during host interaction. Remarkably, they also observed that a large proportion of the up- and downregulated genes in mycorrhizal roots were taxonomically restricted, 'orphan' genes. Many of these lineage-specific genes do not have a functional annotation, but a large proportion of the genes induced in symbiosis appear to encode relatively short proteins with a secretory signal peptide, suggesting that, in contrast to convergent loss of PCWDEs, the establishment of the symbiotic relationship spurred intense positive selection and gene birth. The orphan genes encoding small, secreted effector-like proteins are prime targets for future investigations of mycorrhizal symbioses, as many of these are likely used to modulate plant immunity during the massive colonization of root tissues by the fungus.

The extensive process of gene birth and loss observed in the evolution of fungal symbionts

might be paralleled by analogous genomic adaptations in host plants. Research on this front has been hindered by the difficulties in reconstructing the extremely large and repetitive genomes of many forest plant hosts. This situation is constantly improving, however, as the recent release of the *Pinus taeda* genome demonstrates^{10,11}. The increasing number of available plant host genomes might soon lead to a replication of the experiments performed by Kohler *et al.* from a plant perspective, allowing us to discover the putative counterparts to the regulators and effectors discovered in fungal symbionts.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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