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Report

Evolution of Filamentous Plant Pathogens: Gene Exchange across Eukaryotic Kingdoms

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Summary

Filamentous fungi and oomycetes are eukaryotic microorganisms that grow by producing networks of thread-like hyphae, which secrete enzymes to break down complex nutrients, such as wood and plant material, and recover the resulting simple sugars and amino acids by osmotrophy. These organisms are extremely similar in both appearance and lifestyle [1] and include some of the most economically important plant pathogens [2, 3]. However, the morphological similarity of fungi and oomycetes is misleading because they represent some of the most distantly related eukaryote evolutionary groupings, and their shared osmotrophic growth habit is interpreted as being the result of convergent evolution [3–5]. The fungi branch with the animals, whereas the oomycetes branch with photosynthetic algae as part of the Chromalveolata [6–10]. In this report, we provide strong phylogenetic evidence that multiple horizontal gene transfers (HGT) have occurred from filamentous ascomycete fungi to the distantly related oomycetes. We also present evidence that a subset of the associated gene families was initially the product of prokaryote-to-fungi HGT. The predicted functions of the gene products associated with fungi-to-oomycete HGT suggest that this process has played a significant role in the evolution of the osmotrophic, filamentous lifestyle on two separate branches of the eukaryote tree.

Results and Discussion

Multiple HGTs Have Occurred between Fungi and Oomycetes

Comparative analysis of a large number of microbial genome sequences has begun to reveal the extent and evolutionary significance of HGT among prokaryotic species and between prokaryotes and eukaryotes [11–15]. The importance of HGT among eukaryotic species is, however, far less clear. We set out to explore the evolutionary history of the 11,109 predicted genes in the genome of filamentous ascomycete plant pathogenic fungus *Magnaporthe grisea* [16], the causal agent of rice-blast disease. During our analyses, we detected 11 *M. grisea* genes that had a significantly higher level of sequence similarity (shown by BLASTp) to sequences from the oomycete genus *Phytophthora* than to any fungal sequences used in the primary genome-comparison analysis (see Table S1 in the Supplemental Data available online). These results are contrary to predicted gene similarities given the number of evolutionary branches that exists between fungi and oomycetes (Figure 1) and therefore suggest the possibility of HGT. To explore this idea, we carried out phylogenetic analysis, which revealed that for four of the 11 candidate HGT genes, the oomycete sequence was clearly within a clade of fungal gene sequences, branching with the filamentous ascomycetes (Figure 1). These specific phylogenetic relationships were consistently supported by at least one node with high posterior probabilities in Bayesian analysis and, importantly, by two distinct bootstrap methods (PHYML and ML distance, with 1000 replicates) with support values in excess of 85% (Figures 2A, 3A, 4A, and 4C; see also Figures S1A, S1B, S2A, and S2B). Because bootstrapping is generally considered to be a more conservative indicator of phylogenetic resolution [17], these values confirmed that the relationships were particularly robust. In the case of two of the potential HGTs (*AraJ* and *CodB*), we repeated the phylogenetic experiments by using alignments with distantly related genes removed and altered character sampling to exclude long-branch and outgroup attraction problems (Figures 2A and 3A), but we consistently recovered topologies where the oomycete sequences were specifically embedded within a clade of the fungi as a sister branch to the filamentous ascomycetes (fungal and oomycete paraphyly). Such a phylogenetic pattern is strongly indicative of HGT. To pinpoint the branching position of the oomycetes within the fungi radiation, we then performed additional phylogenetic analyses that focused on increased fungal sampling and reduced the outgroup being sampled. This allowed us to confirm sisterhood of the oomycete and the filamentous ascomycete sequences (Figures 2B, 3C, 4A, and 4C). Figure 1 summarizes this pattern of HGT and shows the most likely branching position of the fungi and the oomycetes in the eukaryotic tree.

Osmotrophy-Related Gene Functions Are Predicted among the Fungi-to-Oomycete HGT Candidates

Of the four strongly supported candidate HGTs, the first gene putatively encodes a sugar transporter (PFAM classification—pfam00083) of the multifacilitator superfamily. This sugar transporter possesses an AraJ arabinose permease-like domain (COG2814) based on interrogation of the conserved domain database (CDD) [18]. The transfer of a multifacilitator sugar-transporter-encoding gene could potentially increase the accessibility of sugar

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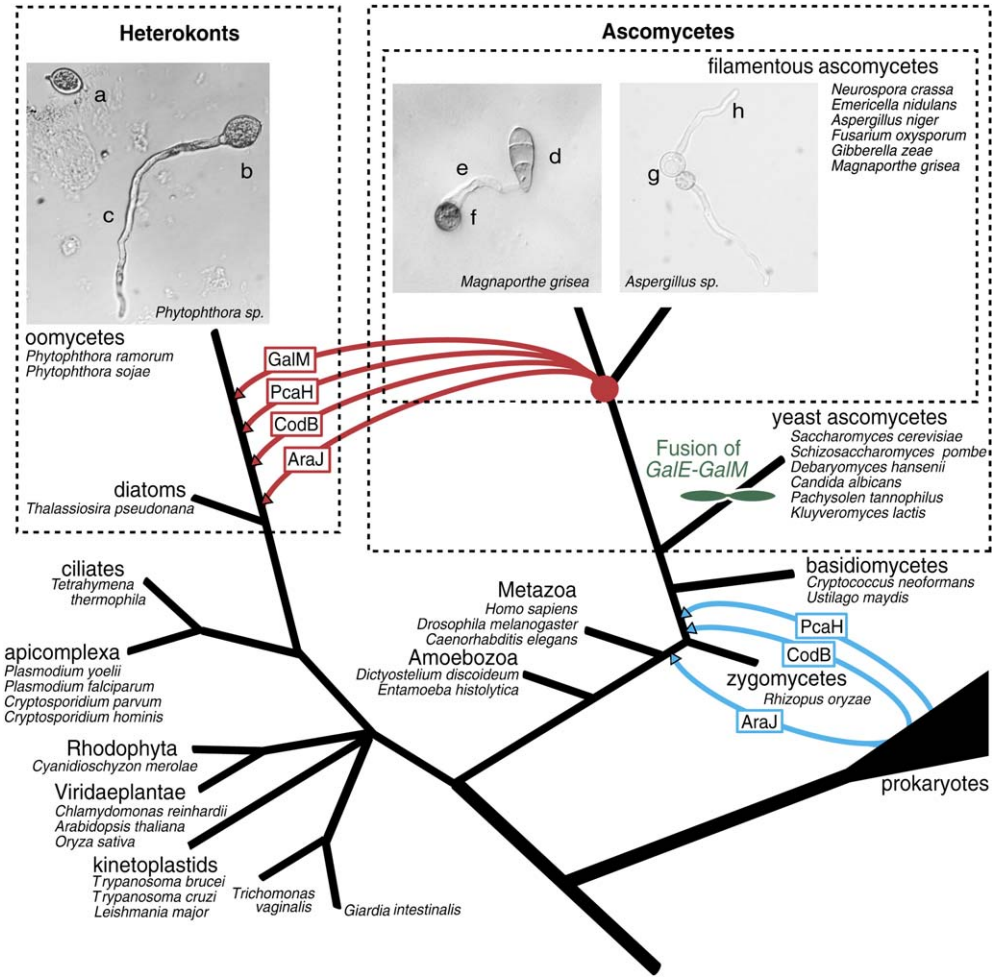

Figure 1. Schematic Representation of Eukaryotic Phylogeny, Indicating Oomycete and Fungi HGT Events and Demonstrating Superficial Morphological Similarities between the Fungi and the Oomycetes

The tree topology shown serves as a hypothesis of eukaryote tree topology for contrasting with the gene phylogenies reported here. Only eukaryotic groups with genome project representation, those that were searched during this study, are placed within the tree. Candidate prokaryote-to-fungi HGTs are shown in blue, and candidate fungi-to-oomycete HGTs are shown in red. The *GalE-GalM* fusion gene used to root the ascomycetes is indicated (see Figure 4E). Pictures show typical and similar filamentous characters exhibited by both oomycetes and fungi: Lowercase letters a and b indicate oomycete sporangia that can germinate to produce motile zoospores or to form germ tubes as indicated by lowercase letter c. The letter d indicates asexual conidium; e indicates germ tube; f indicates specialized infection cell known as an appressorium; and g indicates asexual conidia that have germinated to produce germ tubes (marked by h).

substrates to an osmotrophic microorganism. Phylogeny of the *AraJ* HGT was supported by three nodes (1/60/90%, 1/100/98%, and 1/100/100% support), which specifically grouped the oomycetes within the fungi and the ascomycete radiation (Figure 2A). The three phylogeny support values are listed, here and subsequently, in the order Bayesian posterior probability, % PHYML bootstrap value, and % ML-distance bootstrap value.

The second HGT candidate putatively encodes a permease protein containing a *CodB* cytosine/purine, uracil/thiamine/allantoin permease domain, identified with CDD [18] (COG1457). This gene phylogeny also demonstrated a HGT event from the filamentous ascomycetes to the oomycetes (resolved with 1/100/100% and 0.88/80/78% phylogeny support values—Figure 3A and 0.99/85/87% phylogeny support values—Figure 3C). The budding yeast *Saccharomyces cerevisiae CodB* gene encodes a broad specificity permease for purine uptake [19]. Thus, acquisition of *CodB* represents a potential means by which oomycetes could access nucleotide substrates.

The third gene reported putatively encodes a protocatechuate 3,4-dioxygenase β-subunit (3,4-*PCD*) annotated as *PcaH* (COG3485) in CDD. Phylogenetic analysis of the homologs of the *PcaH* gene family demonstrated tree topologies consistent with fungi-to-oomycete HGT and with 1/94/86% support values (Figure 4A). *PcaH* encodes an enzyme involved in degradation of aromatic compounds as part of the β-ketoadipate pathway [20].

Finally, phylogenetic analysis of the aldose-1-epimerase (*GalM*) gene family (COG2017), demonstrated *Phytophthora ramorum* and *P. sojae* sequences grouping within the fungi (1/99/100% support), specifically as a sister group to the filamentous ascomycete *GalM* homologs (1/100/100% support—Figure 4C). The *GalM*-encoded aldose-1-epimerases can demonstrate broad substrate specificity [21] and are present in an evolutionary diverse selection of eukaryotes. The *GalM* protein of