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Metarhizium: an opportunistic middleman for multitrophic lifestyles

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Metarhizium spp. mediate multiple interactions that are usually positive with respect to their long-term plant environment, and negative with respect to short-lived hosts. In particular, their ability to kill a wide range of insects maximizes protection to the plants and provides a resource of nitrogen that the fungus trades with the plant for carbon. Here, we highlight emerging concepts underlying *Metarhizium*–plant–insect interactions. Experiments on model systems have provided detailed mechanistic knowledge of how these fungi interact with plants and insects, and a greater understanding of the evolutionary forces driving these interactions. However, further integration of studies at the ecological and mechanistic level is needed to evaluate the importance of *Metarhizium*'s multitrophic interactions to the structuring of natural communities.

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Metarhizium: a ubiquitous Jack of all trades

The ascomycete genus *Metarhizium* is among the most abundant fungi, often reaching 10^6 colony-forming units g^{-1} in grassland soils [1,2]. As well as being widely used for insect pest control, and as models for genetic engineering projects [1], many are also beneficial root endophytes [2]. Furthermore, *Metarhizium* forms a monophyletic clade with *Pochonia chlamydosporia*, a root-colonizing nematode egg pathogen that diverged from *Metarhizium* about 180 MYA (Figure 1), coincident with the appearance of many other root-colonizing lineages [2]. The *Pochonia–Metarhizium* clade arose independently from other insect/nematode pathogens, probably from saprophytes that first become endophytes after attraction to roots by exudates, as still occurs for extant *Metarhizium* spp [3,4].

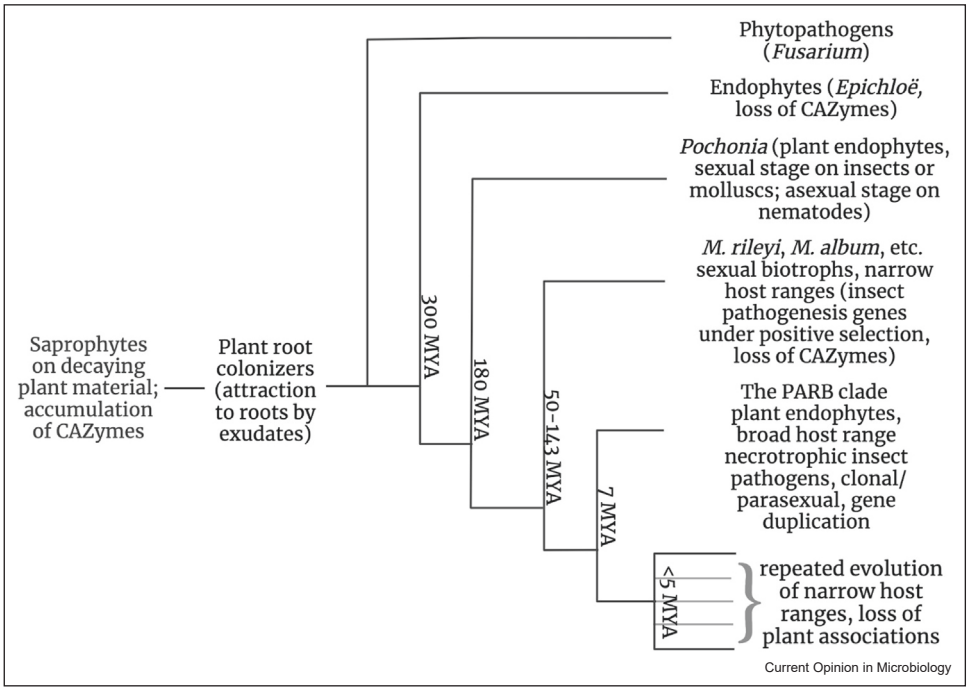
P. chlamydosporia var. *chlamydosporia* and var. *catenulate* produce sexual morphs on snail eggs and beetle larvae, respectively, and assuming sexuality preceded asexuality, pathogenicity to insects (or mollusks) may be ancestral to their asexual nematode pathogenicity [2]. Asexual *Metarhizium* lineages can also be nematocidal [6]. Based on molecular phylogenies [2,7], the earliest-derived (basal) *Metarhizium* lineage is the root- colonizing saprophyte/mushroom pathogen *M. marquandii*, consistent with an ancestral *Metarhizium* lifestyle as soil-dwelling root colonizers. The oldest sequenced entomopathogen lineages have narrow host ranges to insects that live aboveground, thus separating the fungus from its ancestral root habitat, for example, the commercially produced locust control agent *M. acridum*. Recent intensive and focused sampling has identified many new *Metarhizium* species that have narrow host ranges, and that unlike generalists frequently retain a sexual cycle [9]. Genomic data suggest that these specialists have a larger number of rapidly evolving genes than broad host-range *Metarhizium* spp, reflecting evolutionary arms races with their specific hosts [2].

With the exception of the independently evolved tundra-adapted *M. frigidum*, *Metarhizium* genotypes with broad host ranges (the PARB clade, *M. pingshaense*, *M. anisopliae*, *M. robertsii*, and *M. brunneum*) have diverged comparatively recently (Figure 1). Most PARB strains retain the ancestral root association, and have clonal population structures with parasexuality within each biotype potentially combining adaptive mutations that arise in spores of each lineage into one genome [2]. The significance of sexual, parasexual, and asexual life histories to *Metarhizium* biology and evolution has been recently reviewed [2]. Here, we highlight emerging general concepts underlying *Metarhizium*–plant–insect interactions.

How do *Metarhizium* spp interact with insects?

Metarhizium strains with narrow host ranges exhibit less physiological adaptability than generalists, and require the specific physical and chemical features of their host cuticle to stimulate infection processes [2]. Host-range choices involve transmembrane G-protein-coupled receptors (GPCRs) that show an expanded repertoire in

Figure 1



A phylogenomic tree with the estimated time of divergence for sequenced *Metarhizium* species and related fungi estimated from a multigene phylogeny compiled from genome sequences [2]. The text indicates major transitions in the evolution of *Metarhizium* species.

generalists compared with specialists [10,11]. Different GPCRs have different roles in responding to host-related recognition signals and differentially activate the major Hog1–MAPK, Slt2–MAPK, and Fus3–MAPK signaling cascades [12]. Thus, *M. robertsii* mediates the transition from plant symbiont-to-insect pathogen by modulating production of a membrane protein, MrOPY2 (via alternative transcription start sites), which activates the Slt2–MAPK pathway that in turn regulates AFTF1 (appressorial-formation transcription factor 1) [13]. The transition from cuticle penetration to hemocoel colonization is mediated by transcription factors COH1 and COH2 (colonization of hemocoel 1 and 2). Penetrating the cuticle requires extensive production of cuticle-degrading enzymes [2], and is choreographed by COH2. Once the fungus enters the hemocoel, a reduction in epigenetic repression upregulates COH1, which deactivates COH2 turning of genes for cuticle penetration and upregulating genes for immune evasion and nutrition [14].

How do *Metarhizium* species interact with plants?

A pattern of gain and loss of carbohydrate-active enzymes is a feature of the *Metarhizium* clade and reflects the extent of their ongoing interactions with plants, with *P. chlamydosporia* and generalist *Metarhizium* strains having the most and specialist *Metarhizium* spp. and the endophytic close relation of *Metarhizium*, *Epichloe festucae*, the fewest [2]. *P. chlamydosporia* is a better plant-root colonizer than many *Metarhizium* spp. [15], but even so its root rather than insect associations that maintain field populations of *M. robertsii* [16]. Unlike *Metarhizium* spp, *P. chlamydosporia* retains GH6 and GH7 endocellulases that may explain why *P. chlamydosporia* more frequently penetrates into plant cells than *Metarhizium*, which instead usually grows between cortical root cells [2,17].

The PARB species may have been selected principally to soil and plant-root habitat rather than host insects. Specific associations between *Metarhizium* spp. and plants have been reported in field studies in Canada and the United States [18,19], but not in Denmark [20] and Japan [21]. The most abundant plant and *Metarhizium* species differed in these studies, so it remains to be determined how plant and *Metarhizium* communities affect each other, and whether the fungi differ in their associations with plant species or are ecologically equivalent. However, experimentally, multiple species of *Metarhizium* can colonize the roots of many plant species, and *M. robertsii* can also grow systemically in aboveground tissues [22].

Endophytic colonization by *Metarhizium* promotes growth in many plant species [22]. In addition to direct

entomophagous activities, endophytic *M. robertsii* also suppresses insect growth probably by production of metabolites within plants that deter feeding [22]. *M. robertsii* also protects roots from the related fungus *Fusarium solani* [23] perhaps because it produces volatiles that repress nematode, fungal, and bacterial competitors for rhizospheric resources [24,25]. *Metarhizium* species' more direct growth-promoting effects include production of indole-3-acetic acid (IAA), which stimulates root development [26], solubilizing rock phosphorus in soil, making it more accessible to plants [27], and transfer of nitrogen by hyphae connecting insect cadavers and plant roots [28]. The benefits to the plant will be conditional on soil fertility; when carbon and nitrogen sources are abundant, then nitrogen transfer from *Metarhizium* to insects is reduced [28]. A fungus colonizing an insect presumably has nitrogen in excess of its immediate requirements, and it would clearly increase opportunities for nutrition if the colonizing endophyte could exploit diverse insects, which potentially could have selected for the broad host range characteristic of endophytes [2].

As befits an ancient association, there is evidence that sophisticated and subtle signaling underlie plant–*Metarhizium* interactions. Colonization by *Metarhizium* lowers plant production of several hormones and defense responses, showing the plant is acutely aware of the fungus, whereas pathogenic colonization by *Fusarium* species increases defense responses [29,30]. The net outcome of interactions is thus likely to be complicated and depend on how colonizing *Metarhizium* affects the plants' defensive potential to pathogens, *Metarhizium*'s own interactions with pathogens, and physiological trade offs. Thus, increased nutrient content in the plant could increase resistance expression or conversely make a plant more attractive to pathogens. The cell wall of *M. robertsii* may have means to avoid recognition by both plant [31] and insect immune systems [32], suggesting commonalities in *Metarhizium*'s strategy.

Metarhizium as a model for multitrophic lifestyles

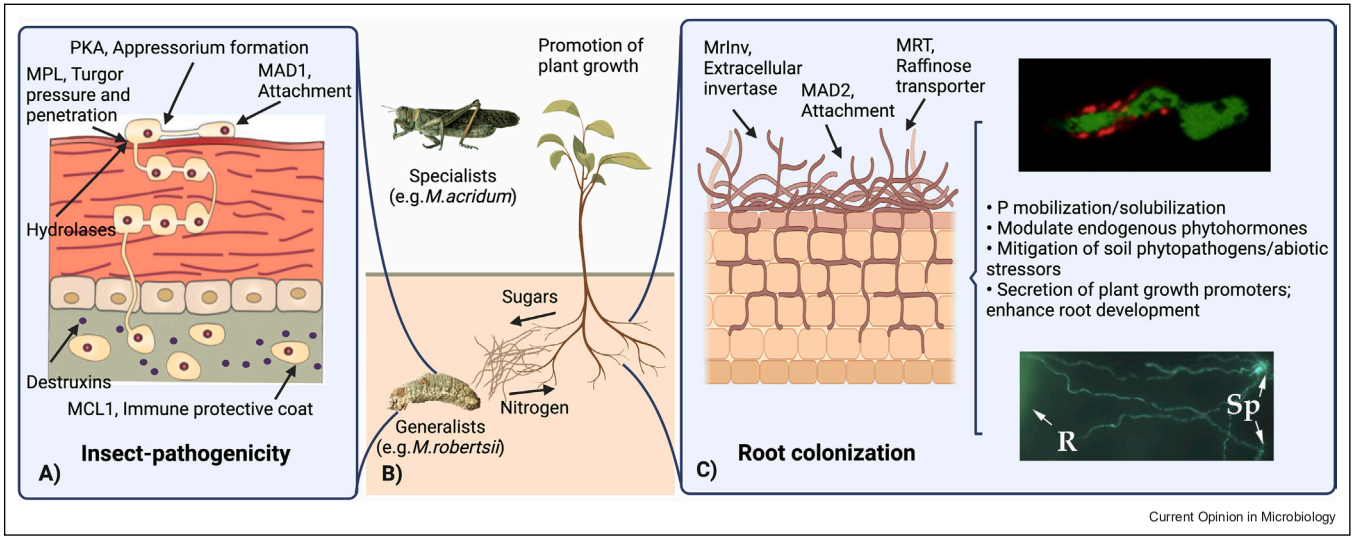
The prolific production of enzymes and secondary metabolites (SMs) by *Metarhizium* species is linked to their broad lifestyle options, and an extremely flexible metabolism that enables them to live in various environmental conditions, and in the presence of compounds lethal to other microbes [2]. The capacity for evolution of new lifestyles displayed by the *Metarhizium* clade and other fungi could depend on them expressing molecules that act upon a wide range of organisms. *M. robertsii*, the opportunistic human pathogen *Aspergillus fumigatus* and the plant pathogen *Haematonectria haematococca* secrete a range of enzymes on host polymers that are common toxic components of reptile and invertebrate venoms [33].

For purposes of brevity, we will focus on SMs as exemplars of molecules known to have targets in hosts belonging to different kingdoms [2]. Of the known infection-promoting factors, many are toxins that directly target the most conserved cellular components such as the cytoskeleton (e.g. cytochalasins) or cellular membranes (e.g. destruxins), and potentially could function against diverse hosts. Many biosynthetic pathways have been uncovered by *Metarhizium* genome sequences with generalist *Metarhizium* species usually possessing a greater potential for the production of SMs than specialist strains, or indeed almost all other ascomycetes [34]. The core genome of *Metarhizium* species is represented by about 60% of the genes in the whole genome, with the remainder consisting of variably represented genes [10]. This pan-genome typifies species that colonize multiple environments and have multiple ways of exchanging genetic materials. Some SM gene clusters such as destruxins that are exclusive to broad host-range *Metarhizium* were probably acquired by horizontal gene transfer (HGT) from other fungi [35]. Several HGT-acquired genes encoding proteins involved in breaching cuticular barriers also contributed to host-range expansion, implicating HGT both from close fungal relatives and from bacteria, plants, and insects as a mechanism for global plasticity and the emergence of new pathogenic fungi [36]. Another characteristic of generalist *Metarhizium* strains is that they have lost repeat-induced point mutation along with sexuality, allowing extensive gene duplications and subsequent functional divergence [2]. Thus, duplication and divergence of a polyketide (Pk) gene cluster in *Metarhizium* species produced Pks2 and Pks1 involved in infection-structure formation and conidial pigmentation, respectively [37]. Similar duplication events followed acquisition of a terpene synthase gene by HGT from a bacteria [38].

Pathways identified in genomes include those responsible for other known *Metarhizium* chemistries and pathways with candidate products not yet known in *Metarhizium*. Experimental studies have validated some predictions based on genomic sequence data, for example, certain *Metarhizium* species produce ergot alkaloids (but only during insect colonization) [39]. Some other pathways in *Metarhizium* genomes are so unique that the molecules they produce cannot yet be predicted [34]. Work-arounds to quickly remedy this deficiency include using transcriptional regulation as a guide to gene-cluster functionality, for example, identifying gene clusters specifically expressed in iron-deficient conditions [40].

Many capabilities appear unique to *Metarhizium* isolates but may preadapt them for various habitats, for example, *Metarhizium*'s unique ability to hydrolyze the environmentally dangerous nonylphenol [41]. Likewise, scientists looking for compounds for the treatment of

Figure 2



Overview of interactions between *Metarhizium* and the insect cuticle (A), between generalist *Metarhizium* spp. and plants (*M. acridum* is included as an example of a specialist) (B), and between generalist *Metarhizium* spp. and roots (C). The top inset in C shows a germling of *M. robertsii* expressing GFP; the red is secreted IAA. The bottom inset shows germlings of *M. robertsii* growing toward a root following a concentration gradient of raffinose [3]. R = root, Sp = spore.

skin disorders found that *Metarhizium* produces 2-hydroxytyrosol, a powerful inhibitor of phenoloxidases, and previously only known as a synthetic compound [42]. These unusual chemistries likely evolved in *Metarhizium* to defend against the melee of toxic melanizing reactions produced by insect defenses. *M. robertsii* has many adaptations to resist this toxicity, including producing a metalloprotease that degrades host phenoloxidases [43].

Conclusions

From the perspective of *Metarhizium*, its beneficial associations with plants and virulence to insects are simply means of establishing a nutritional relationship with these hosts [2]. Recent studies have aimed to identify additional general concepts underlying *Metarhizium*–insect–plant interactions through a detailed knowledge of mechanistic aspects. To date, most of these studies have focused on highly controlled interactions between single (insect, fungal, and plant) species in the laboratory. These have been extremely valuable for assessing the role of fungal, insect, and plant components, and the concomitant signaling pathways involved in *Metarhizium* interactions with plants and insects, but they may lack ecological realism. Additional studies are required that provide insight into the effect of interactions by different *Metarhizium* species or strains in a community context. The extent to which individual *Metarhizuim* strains can specialize to particular plants has not been determined, but interactions between *Metarhizium* species and plants seem ubiquitous, and connect with many other ubiquitous soil organisms increasing the complexity of interactions in as yet poorly understood ways. It is also likely that individual plant–*Metarhizium* combinations have independently evolved idiosyncratic interactions that will necessarily add to a huge diversity of possible outcomes. Quantitative effects will also need to be considered when trying to find general patterns; thus, studies have focused on how densities of *Metarhizium* impact insects feeding on plants, and comparable studies are needed on disease resistance of plants. Only when these studies are performed will we have a comprehensive view of the impact of *Metarhizium*–plant–insect interactions on natural ecosystems, and be in a position to realize the full potential of *Metarhizium* strains in plant protection (Figure 2).

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Authors' contributions

H.S, P.J.M. data analysis and research. R.J.S. conceptualization, paper preparation. All authors reviewed and edited the paper.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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