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Molecular adaptations to advanced fungus farming in leaf-cutting ant symbiosis

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About 50 million years ago a single ancestor of today's more than 230 species of fungus-growing ants committed herself irreversibly to farming fungi for food instead of being a hunter-gatherer. The most derived genera within this group, the *Atta* and *Acromyrmex* leaf-cutting ants, have realized the key innovation that they use fresh leaves as substrate for their fungus gardens, which allowed them to tap into tremendously abundant resources and to evolve enormous societies. Assumingly this innovation was accompanied by specific adaptations in the ants and their fungal symbionts to be able to ameliorate the effects of defence compounds found in live plant tissues. A promising venue for identifying such adaptations at the molecular level is the fecal fluid of the ants, which contains many fungal enzymes that pass through the ant digestive system unharmed to end up in the fecal fluid that the ants mix with the fresh leaves and deposit on top of their gardens. To understand the adaptive value of this phenomenon, we have used state of the art proteomics and high-throughput genome and transcriptome sequencing to identify the proteins found in the ant fecal droplets. Enzyme assays for selected fecal proteins showed that they functionally disappeared from the fecal droplets when the ants were deprived of their fungal symbiont. We further used qPCR to establish that many of these proteins are more highly expressed in the hyphal tips (gongylidia) of the fungus on which the ants feed, than in normal mycelium, suggesting that they have been actively selected to be ingested by the ants. Of particular interest is the finding of a polyphenol-oxidizing laccase enzyme that shows signs of positive selection in the higher attine ant symbionts, and may be an important prerequisite for the ability to cope with the polyphenols present in living plant tissues.