

gus gardening. Prior to departing from her natal nest to mate and found a new colony, an attine daughter queen stores a nucleus of fungus from the maternal garden in her infrabuccal pocket, a filtering device located at the bottom of her mouth. Following mating, the foundress queen establishes a suitable nesting location (usually by herself, i.e., by haplometrosis, the condition in most but not all attines and, indeed, in most ants, RISSING & POLLOCK 1988), where she expels the fungal pellet and uses it to start her own garden (HUBER 1905). Cultivars are propagated vegetatively, i.e., as asexual clones, from parent to offspring nests and within nests. It has been noted that, under a scenario of within-garden genetic variability of mycelial strains, this generational subsampling and subculturing of gardens provides the opportunity for the ant-mediated equivalent of "artificial selection" of fungal-cultivar clones (MUELLER 2002). In all attines, the foundress queen cares for the garden and raises the first brood and, in most attines, the foundress forages for garden substrate (i.e., semi-claustral nest foundation). The primary chamber where the foundress will spend her time as she rears her first brood tends to be small and shallow in all attines, likely minimizing energetic costs to the queen. A typical attine worker takes approximately 40 - 60 days to develop from egg to adult (WEBER 1972, TORRE-GROSSA & al. 1982). Upon emergence, the first brood begins foraging, brood care, and excavation duties, and when a sufficient work force is achieved, the queen permanently relinquishes all but egg-laying duties to her workers.

Brood care, one of the most important tasks performed by the workers, is somewhat different in the attines relative to other ants. The eggs and larvae of fungus-growing ants are typically embedded in the garden and covered with mycelium (WEBER 1972, SCHULTZ & MEIER 1995, LOPES & al. 2005). Attine larvae feed exclusively on cultured fungal hyphae (WEBER 1955, MURAKAMI & HIGASHI 1997). In fact, oral trophallaxis of liquid between workers and larvae, which is common in most myrmicines (WILSON 1971, HÖLDOBLER & WILSON 1990), is extremely rare in the Attini (WEBER 1972, LITLEDYKE & CHERRETT 1976, QUINLAN & CHERRETT 1979, BESHES & TRANIELLO 1996, MURAKAMI & HIGASHI 1997, LOPES & al. 2005). Unlike other ant larvae, attine larvae are densely covered with spinules on the labrum, mandibles, and labium; these spinules undoubtedly function in the mastication of fungal hyphae (WHEELER & WHEELER 1976). (Interestingly, the larvae of the non-attine social parasites and agro-predators in the genus *Megalomyrmex*, which are known to feed on attine fungi, do not have such mouthparts, ADAMS & al. 2000.) In addition, attine larvae, with only a few exceptions (*Myocepurus*, *Sericomyrmex*, and some *Acromyrmex* species), lack hairs on the dorsal and lateral surfaces of their bodies. The near-universality of this condition in attine larvae, in contrast to the nearly universal presence of hairs in all other ant larvae, suggests an unknown biological function likely connected with fungal cultivation. Hairs do consistently occur in the region of the attine larval mouthparts, where they clearly function as "baskets" for anchoring fungal hyphae fed to them by workers. Likewise, a distinctive ring of hairs surrounds the anal region of all attine larvae (SCHULTZ & MEIER 1995). These hairs hold droplets of anal secretions that are imbibed by workers for unknown reasons, suggesting that larvae may play an important but

entirely unexplored role in nutrient flow through attine colonies (BESHES & TRANIELLO 1996, SCHNEIDER 2000, LOPES & al. 2005).

Unlike attine larvae, adult attine ants are not obviously modified for fungus cultivation except for: (I) the presence in some attine species of cuticular crypts hypothesized to support the growth of bacteria that suppress the growth of garden pathogens (CURRIE & al. 2006) and (II) the enlarged heads and thickened mandibles of the larger worker subcastes in the leafcutting genera *Acromyrmex* and *Atta*, which are obviously modified for leaf- and grasscutting (as well as, in the case of *Atta* soldiers, for defense).

Natural history of attine fungal cultivars and *Escovopsis* pathogens

To understand the attine agricultural symbiosis, we must understand the natural history of the attine fungi. All attine fungal cultivars are members of the order Agaricales (Basidiomycota), a large and diverse clade characterized by the production of spore-bearing "mushroom" fruiting bodies. Mushrooms rarely occur in attine nests, however, because the ants suppress fruiting by constantly cropping the mycelium and disrupting hyphal connections, and perhaps through other, as-yet unknown mechanisms. Because traditional mycological taxonomy largely depends on characters of fruiting bodies, their absence in the attine system impeded progress for many years (but see MÖLLER 1893, HEIM 1957, WEBER 1957, HERVEY & al. 1977). We now know that the vast majority of attine ants cultivate fungi in the tribe Leucocoprineae, which consists of two genera, *Leucoagaricus* and *Leucocoprinus*, in the family Lepiotaceae (MUELLER & al. 1998, JOHNSON 1999). In a striking and unique exception to this rule, members of the *Apterostigma pilosum* group, derived within the genus *Apterostigma*, cultivate a distantly related basidiomycete fungus belonging to the coral-mushroom family Pterulaceae (VILLESEN & al. 2004, MUNKACSI & al. 2004, DENTINGER & al. 2009).

Because attine fungal cultivars are vertically transmitted from parent to daughter nests by foundress queens, and because the closest free-living relatives of the cultivars remained unknown, for many years it was assumed that attine fungi were ancient clonal lineages that were obligately associated with their ant hosts (WEBER 1972). This assumption was challenged by the first molecular-phylogenetic studies of attine fungi in which it was discovered that the attine cultivars do not form a clade exclusive of free-living forms (CHAPELA & al. 1994, HINKLE & al. 1994). It was challenged again with the discovery that the lower attine cultivars are polyphyletic, representing a minimum of three separate lineages within the Leucocoprineae (MUELLER & al. 1998). Perhaps the best evidence against ancient clonality was the discovery of the same fungal species and strains living both within the attine symbiosis and freely outside of it, occupying the typical lepiotoid niche of growing in the leaf litter (MUELLER & al. 1998, VO & al. 2009). Other studies within genera and species of attine ants and their associated fungi have further demonstrated incongruence between fungal and ant lineages that can only be explained by frequent switching in ant-fungal associations over evolutionary time periods, either due to recruitment of free-living fungi into the symbiosis, trading of cultivars between nests, or both (MUELLER & al. 1998, MUELLER & al. 2001, GREEN & al. 2002). This pattern, in which some lower-attine and yeast-