Insect. Soc. 53 (2006) 300–306
0020-1812/06/030300-7
DOI 10.1007/s00040-006-0872-y

Insectes Sociaux

Research article

Behavioral ecology and natural history of *Blepharidatta brasiliensis* (Formicidae, Blepharidattini)

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Received 20 December 2005; revised 1 March 2006; accepted 7 March 2006.

Abstract. Fungus-growing ants (Attini, Formicidae) originated about 45–65 million years ago when forging a mutualistic association with basidiomycete fungi (Lepiotaceae). Here we use information on the biology of the non-leafcutting fungus-growing ants and their close relatives in the genus *Blepharidatta* to evaluate hypotheses for the evolutionary origin of fungus-growing behavior in attine ants. Observations on the natural history, ecology, and behavior of the Amazonian species *Blepharidatta brasiliensis* are reported here for the first time. Like most attine species, *B. brasiliensis* and the great majority of species in the tribe Blepharidattini are inhabitants of moist tropical rainforest, suggesting a rainforest habitat also for the ancestral attine ant. The ancestral attine was probably a leaf litter dweller, building small to medium sized nests (e.g., 20–200 workers) either between leaves in the litter or in decaying wood on the rainforest floor.

Keywords: Attini, colony demography, fungivory, nearest neighbor analysis, nest architecture.

Introduction

The symbiotic relationship between attine ants and basidiomycete fungi is the defining behavioral characteristic of the approximately 210 described fungus-growing ant species in the monophyletic, Neotropical tribe Attini (Schultz and Meier, 1995; Schultz, 1998; Wetterer et al., 1998; Brandão and Mayhé-Nunes, 2001). Most attine species build nest chambers in the soil or leaf litter where the fungus gardens are cultivated. The fungus cultivar is grown on organic matter collected by the ants; in return, the ants feed on the fungus' mycelia or on specialized nutrient-rich hyphal structures.

The origin of the attine ant-fungus mutualism has been dated to about 45–65 million years ago in the tropical rainforest of South America (Mueller et al., 2001), but many details about selective forces and ecological setting at the origin of the fungus-growing behavior remain unresolved. Mueller et al. (2001) reviewed seven hypotheses that had been suggested in the literature for the origin of attine fungiculture, each different with respect to the substrate used by ancestral attine ants for fungal cultivation. Sánchez-Peña (2005) recently added an eighth hypothesis. All living attine species exhibit obligate associations with fungi and no transitional states such as facultative fungiculture occur in any of the extant attine species. Therefore, testing of hypotheses about the origins of fungiculture cannot be simply done by comparing the diets of existing species. However, some clues may be obtained by asking how close relatives of attines respond to attine fungal cultivars.

To gain a better understanding of attine evolution, we report here the first observations on the natural history, ecology, and behavior of a close relative of attine ants, *Blepharidatta brasiliensis*. In contrast to the savannah-dwelling species *B. conops* (Diniz et al., 1998), *B. brasiliensis* inhabits the tropical rainforests of South America, the presumed habitat of the origin of the attine ant-fungus symbiosis (Weber, 1972; Mueller et al., 2001). Mueller et al. (2001) suggested the study of *Blepharidatta* ecology and behavior in order to "provide clues to the ant-fungus association that preceded the origin of fungiculture in the Attini".

The myrmicine genus *Blepharidatta* has a strictly Neotropical distribution and consists of four species, *B. brasiliensis*, *B. conops*, and two undescribed congeners (Diniz et al., 1998; www.antweb.org). Studies of larval mouthparts (Schultz and Meier, 1995) and worker morphology (Diniz et al., 1998) identified the genus *Blepharidatta* to be a close relative of the attine ants. Schultz's (1998) molecular phylogeny suggested a close relationship between the Attini and the monophyletic tribe Blepharidattini, which includes the two genera *Blepharidatta* and *Wasmannia* (Wheeler and Wheeler, 1991; Bolton, 1995; Bolton, 2003; see also Wetterer et al., 1998). Except for the widespread *W. auropunc-*

tata (Ulloa-Chacon and Cherix, 1988, 1990) the biology of ants from both genera and particularly from *Blepharidatta* is largely unknown. *Blepharidatta* ants are rarely collected because of their cryptic nesting habits, and even more rarely studied (Wheeler, 1915; Wheeler and Wheeler, 1991; Diniz et al., 1998). Diniz et al. (1998) were the first to report information on the natural history and feeding biology of the savannah dwelling species *Blepharidatta conops*. Here we describe the natural history and behavioral observations of the cryptic rainforest dwelling species *B. brasiliensis* and discuss the possible implications of these observations for the origin of fungiculture in attine ants.

Material and methods

Field observations were conducted by CR and MV from February to July 2003 at the experimental field sites of the EMBRAPA-Amazônia Ocidental near Manaus, Brazil (2°53´S, 59°59´W) in a terra firme lowland rainforest; and by UGM in February–March 2000 at BDFFP Camp 41 (2°27´S, 59°45´W) and August 2000 at BDFFP Fazenda Dimona (2°20´S, 60°05´W), approximately 80 km north of Manaus, Amazonas, Brazil. The terra firme forest in this area is characterized by a median canopy height of 35–40 m, with single trees standing above the main canopy and an understory of predominantly 2–3 m high Arecaceae palm trees (*Attalea attaleoides*) (Ribeiro et al., 1999).

At the EMBRAPA site, densities of *B. brasiliensis* nests were investigated in eight areas of 12.5 m^2, yielding a total survey area of 100 m^2 in primary forest. Areas where nest densities were mapped were chosen randomly, but had a minimum distance of 100 m between each other. Samples from different sites are therefore regarded as independent. A minimum distance of 100 m was also kept from the border of the forest to preclude edge effects. The lower vegetation was removed in each plot to facilitate observations, and leaf litter and soil were carefully searched for ant nests. Nest locations were mapped in each plot, and the nests constructed in twigs or nuts were opened to census nest contents. Each area was observed and mapped for 20 h on consecutive days. We assume that most, if not all, nests were discovered during this extensive observation period.

Nest distribution patterns within the eight 12.5 m^2 plots were analyzed with the nearest neighbor measure, R (Clark and Evans, 1954). The value of R can range from 0 (aggregated) to 2.1492 (regular); random patterns are indicated by a value of 1. R was tested for statistical significance using the Z-transformation; if $|Z|$ is less than 1.96, the null hypothesis at $\alpha = 0.05$ was accepted (i.e., nests were dispersed at random). Because no boundary strip was included in the study area, the Clark and Evans test is biased in favor of finding normal distributions, so that aggregated distributions can be erroneously judged random, and random patterns can be erroneously judged uniform. We therefore used the Donnelly modification of the Clark and Evans test (Donnelly, 1978; see also Krebs, 1989) to preclude such errors.

In order to investigate diel activity patterns of *B. brasiliensis,* two colonies (UGM000819-01 and UGM000819-02) were monitored for a 24-hour period starting just before dusk at 18:30 h on August 17th, 2000, and ending at 18:30 h on August 18th. Foraging activity was monitored by closely observing each colony for 10 min every 1.5 h and counting the number of workers that left or entered each colony.

After mapping and observing colonies of *B. brasiliensis* in the field, we collected them and brought them intact in branches to the laboratory to census nest contents. Nests were collected by UGM and CR & MV in 2000 and 2003, respectively. UGM located nests by baiting with cream-of-rice and following foragers to their nests, whereas CR & MV located and followed foragers without baiting. Nests in the 2000 collections were only censused for the numbers of workers, reproductives, and alates, but not brood, whereas nests from the 2003 collections were carefully censused for both adult ants and brood. Nest dimensions were recorded, as well as any prey items found in the nest chamber. Prey remains found in a nest or in the nest midden were identified to the lowest taxonomic level possible.

Eleven colonies (six in February/March 2000 and five in August 2000) were kept alive in separate observation chambers (7 × 7 cm chamber of transparent plastic) for further observations of feeding behavior. To test if *B. brasiliensis* is a facultative fungivore, colonies were presented with 125 mm^3 fragments of attine fungus gardens. Garden fragments were taken from attine colonies of four genera (*Apterostigma auriculatum, Cyphomyrmex faunulus*, *Mycetarotes acutus* and *Myrmicocrypta sp.*) collected near the same locations where *B. brasiliensis* colonies had been collected. During feeding experiments, activities of *B. brasiliensis* workers were observed sporadically over a 24-hour period through a 10x magnifying lens. When colonies were not observed, they were kept in the dark. Interactions of workers with fungus gardens were recorded, particularly any behavior that might reveal consumption of fungus or fungiculture by *Blepharidatta* workers, such as antennation of the garden fragment, addition of debris to the garden fragment, or spatial associations of the *Blepharidatta* colony with the garden fragment in the observation chamber.

Results

Nest distribution

Nest distribution patterns of *B. brasiliensis* within the 12.5 m^2 plots were random (Table 1). In plot C (7 nests) the distribution tended towards a clumped pattern ($R = 0.95$; $Z = -0.23$ corrected after Donnelly, 1978) whereas in plot D (5 nests) the distribution tended towards a uniform pattern ($R = 1.39$; $Z = 1.51$ corrected after Donnelly, 1978), however, because $|Z| < 1.96$, we accepted the null hypothesis that *B. brasiliensis* nests were randomly spaced in our study plots (Table 1). The spatial arrangement of *B. brasiliensis* nests among all eight 12.5 m^2 plots showed a non-homogenous distribution pattern. Five of the eight total mapping plots contained one to seven *B. brasiliensis* colonies, three plots contained no *Blepharidatta* nest at all (Table 1).

Nesting biology

Blepharidatta brasiliensis occupied two kinds of nest sites within the leaf litter stratum, rotting branches and natural spaces between leaves. Of the total of 26 colonies discovered in 2000 (n = 10) and 2003 (n = 16), 81 % (n = 21) utilized cavities in rotting wood and branches and 19 % (n = 5) nested between leaves (Fig. 1).

B. brasiliensis appears to take advantage of pre-existing cavities, which were only slightly modified by them. The shape of the main chambers was amorphous ellipsoid to round and had an inner dimension of 4 cm length by 1.5 cm width. If pre-existing cavities in branches were too large or the nest was located between leaves (Fig. 1), the ants had reduced the nest volume by constructing chamber walls, built from a mixture of soil, vegetable debris, and insect parts in order to seal off the nest chamber.

B. brasiliensis lived in small to medium size colonies with up to 358 workers (Table 2). The average *B. brasiliensis*

Study plot	# *B. brasiliensis* nests	R-value	Z-transformation	Probability (two-tailed test)	Aggregation pattern within one plot
A	1	N/A	N/A	N/A	N/A
B	2	N/A	N/A	N/A	N/A
C	7	0.95	–0.23	> 0.4	Random
D	5	1.39	1.51	> 0.1	Random
E	1	N/A	N/A	N/A	N/A
F	0	N/A	N/A	N/A	N/A
G	0	N/A	N/A	N/A	N/A
H	0	N/A	N/A	N/A	N/A

Table 1. Distribution patterns of *Blepharidatta brasiliensis* nests within eight sampling plots. R-value is the Clark and Evan's dispersion index and Z-transformation is the value of the test statistic; both modified after Donnelly (1978). Nearest neighbor statistics could not be applied (indicated by "N/A") in plots with less than three nests.

colony ID	workers	females	males	larvae	pupae	total brood
CR 030429-01pw3	150	5	0	77	45	122
CR 030429-03pw3	149	4	0	111	33	144
CR 030429-04pw3	207	6	1	124	34	158
CR 030429-05pw3	35	1	0	84	4	88
CR 030429-11pw3	13	0	0	5	3	8
CR 030429-01pw4	102	10	0	47	38	85
CR 030501-02pw4	253	2	0	98	39	137
CR 030501-03pw4	358	0	0	97	45	142
UGM 000815-03	55	0	0	N/A	N/A	N/A
UGM 000815-04	31	2	0	N/A	N/A	N/A
UGM 000819-01	88	1	0	N/A	N/A	N/A
UGM 000819-02	184	5	0	N/A	N/A	N/A
RMMA 000819-11	91	11	1	N/A	N/A	N/A
Mean ± SD	**132 ± 95.63**	**3.62 ± 3.54**	**0.15 ± 0.36**	**80.36 ± 35.79**	**30.13 ± 15.91**	**110.5 ± 45.82**

Table 2. Demographic structure of *B. brasiliensis* colonies. Values present counts of individuals. Brood was not censused in the five colonies collected in 2000, which is indicated by "N/A".

colony contained 132 workers (SD = 95.63). Colonies were polygynous with one to eleven ergatoid queens. Ergatoids were characterized by their worker-like, wingless appearance (Peeters, 1991). Nests contained large numbers of brood, which were kept lying on the bottom of the nest chamber. If a colony was disturbed, workers picked up the immatures and held them between their mandibles. On average, the number of brood, including larvae and pupae, was comparable to the number of workers per colony. Approximately 25 % of the brood were pupae and 75 % were larvae. Males were encountered in only two colonies, once in late April and once in mid August. In both cases only a single male was found per colony.

Figure 1. Leaf litter nest of *Blepharidatta brasiliensis*. The nest was found enveloped between leaves on the rainforest floor, and the cover leaf was removed to expose the colony. Towards the left margin, the ants constructed a wall-like structure from debris and insect corpses to seal off the nest chamber in between the enveloping leaves. The smaller white particles in the centre of the colony are mostly ant brood, but the larger white particles are rice fragments used as bait to locate the colony by following foragers returning to their nest. Photo: U.G. Mueller.

Foraging behavior

Foragers of the two observed *B. brasiliensis* colonies foraged predominantly at night (Fig. 2). While some foragers left the colony and other returned with prey items, a few workers appeared to guard the nest entrance. During the morning hours, a few workers were still guarding the nest entrance,

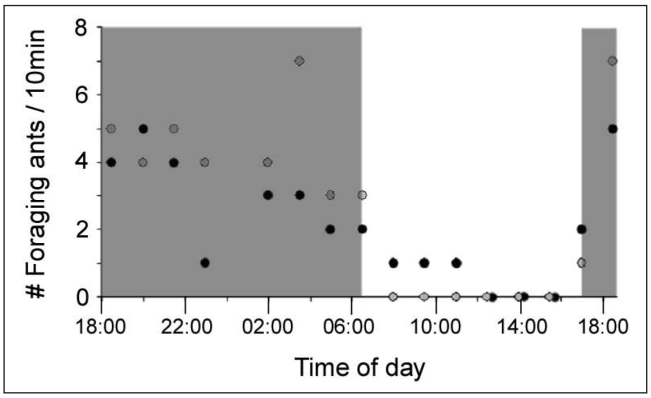

Figure 2. Activity pattern of two *B. brasiliensis* colonies during a 24-hour observation period. The two colonies are represented by white or black dots, respectively. Nighttime hours are indicated by grey background shading. The daylight hours do not correlate exactly with sunrise and sunset because the nests were observed in a closed-canopy rainforest habitat on a cloudy day (i.e., the onset of daylight is delayed by approximately 30 min after sunrise, and the beginning of darkness occurred approximately 30 min before sunset).

but at noon this activity ceased as well and no further workers were observed until the late afternoon hours (17.00 h). At dusk (18.30 h), foraging resumed and continued until the next morning (8.00 h).

B. brasiliensis appeared to be omnivorous and scavenged or preyed on insects and other small arthropods, especially ants. In one instance, foragers of *B. brasiliensis* were observed to prey on a worker of the ant genus *Cephalotes*. When this predation event was discovered, a few *Blepharidatta* workers were already attacking the *Cephalotes* worker. The victim's legs and antennae were cut off and then it was dragged to the nest. There it was stored in front of the nest beside the entrance. The next day the immobilized *Cephalotes* prey was lying at the same position and head movements showed that the individual was still alive.

Besides preying on insects, *B. brasiliensis* scavenged arthropod corpses. A wide variety of exoskeleton parts from different arthropods were found in nest chambers, which reflect a general carnivorous foraging behavior. These exoskeleton parts could be assigned to beetles, bees, cicadas, crickets, termites, spiders and ants of the genera *Cephalotes*, *Pheidole*, *Camponotus* and *Pachycondyla*. The fact that foragers could be baited with cream-of-rice suggests that *B. brasiliensis* may also collect nutrient-rich plant structures, such as seeds or elaiosomes, in addition to the arthropod diet. Moreover, feeding on plant juices (e.g., extrafloral nectaries, fruits) can also not be ruled out.

Behavior towards fungi

In order to test the hypothesis that a close relative of fungus-growing ants is a facultative fungivore and therefore may exemplify a transitional state between hunter-scavengers and fungus-growers, feeding preferences of eleven *B. brasiliensis* colonies were observed in artificial nest chambers. Only the fungus garden of *Apterostigma auriculatum* attracted the attention of *B. brasiliensis* foragers. On March 8th, during a 24-hour observation, three *B. brasiliensis* workers of colony TRS000224-09 were observed on a garden fragment of *A. auriculatum* where they cropped mycelium tufts with rapid mandibular motions. Other ants were licking the mycelium. One worker cut out a piece of mycelium, which had roughly the size of its head (approximately 1 mm), and carried it to the colony. There it presented it to other workers, which licked the garden and possibly fed on it. On March 9th five workers of the same colony were observed on the fungus fragment. Again, they cut out a piece of garden with mycelium on it and brought it to their colony. There, the cultivar piece was presented to other workers, which licked the mycelium and fed on it.

Workers of no other laboratory colony were observed on experimental garden fragments. Garden fragments of *Cyphomyrmex faunulus, Mycetarotes acutus* and *Myrmicocrypta sp*. colonies were neither licked nor brought to the nest. Instead, *Blepharidatta* workers removed these garden fragments from the colony. Of the eleven colonies tested, one was a facultative fungivore with respect to the cultivars used, the other colonies showed no evidence of facultative fungivory. We never observed *B. brasiliensis* workers to forage on mushrooms growing among the leaf litter on the forest floor, but more carefully planned observations would be necessary to rule out cryptic foraging on mycelium growing in the leaf litter.

Discussion

Nest distribution

The nest distribution of *B. brasiliensis* within the mapping plots C and D showed a random distribution pattern. Random patterns are expected when nest sites and food sources are abundant and evenly distributed in microhabitats (Levings and Traniello, 1982; Levings, 1983), intra- or inter-specific competition does not drive communities to overdispersion (Levings and Traniello, 1982; Levings and Franks, 1982; Ryti and Case, 1992), and microclimatic conditions are constant (Doncaster, 1981). For this terra firme rainforest habitat near Manaus we observed a homogeneously structured leaf litter stratum (Rabeling and Verhaagh, unpubl. data), which is concordant with the random nest patterns in leaf litter nesting ants.

We found *B. brasiliensis* in only 62.5 % of our survey plots, which suggests a patchy distribution of populations in this Amazonian rainforest (Table 1). One explanation for this patchy distribution may be that *B. brasiliensis* colonies have functionally flightless queens like other *Blepharidatta* species (Diniz et al., 1998). Wing buds are undeveloped in *B. brasiliensis*, the queens' dispersal potential is therefore limited and new nests are founded probably by colony fission in the vicinity of a maternal nest. This dispersal behavior is likely to result in locally dense but sporadically distributed colony aggregations.

B. brasiliensis colonies were found in the leaf litter where they inhabited hollow branches and cavities between leaves, which represented abundant nest opportunities in this terra firme habitat. Preying and scavenging on highly abundant prey (e.g., arthropods), feeding on extrafloral nectaries, and possibly tending coccids (as in *B. conops* [Diniz et al., 1998]), makes it unnecessary to specialize on resource bonanzas, which are patchy in both space and time. Nest distribution patterns of *B. brasiliensis* therefore appear to reflect more the abundant distribution of adequate nests sites on the forest floor and the short dispersal distances of ergatoid queens, rather than any patchily distributed food sources.

The ground ant communities in the terra firme forest in this study were not mapped entirely, but focused on *Blepharidatta* and attine ants. The nest distribution pattern of this partial community, including attine species, was also found to have a random pattern (Rabeling and Verhaagh, unpubl. data). The biology of other carnivorous and leaf-litter nesting ant species therefore would need to be studied to determine if interspecific interactions are an important factor in the spatial structuring of this ant community.

The study of distribution patterns of *B. conops* in a Brazilian savannah (cerrado) revealed that nests in this population are overdispersed instead of random, statistically supported by a high z-value ($Z = 8.19$) (Diniz et al., 1998). The regularly spaced nesting pattern is caused by intraspecific competition for food items between established colonies and results in the exclusion of new colonies from already established territories (Diniz et al., 1998). The comparison of spatial patterns between *B. brasiliensis* and *B. conops* suggests that food resources for *Blepharidatta* ants may be more limited in the cerrado than in the terra firme rainforest in Amazonia.

Behavioral ecology and origins of fungiculture

The observation that workers of one *B. brasiliensis* colony brought pieces of an *Apterostigma auriculatum* garden to nest mates who fed on it is a case of facultative mycophagy. Even though it was a rare observation it demonstrates that the food preferences in *Blepharidatta brasiliensis* were variable to the extent that some specimens accepted the *Apterostigma* garden as a possible food source.

Mueller et al. (2001) reviewed seven possible hypotheses, which could explain the origin of fungus-growing behavior in attine ants. These hypotheses group into two basic models: the traditional "Consumption First" and the alternative "Transmission First" model. According to the "Consumption First" model, fungi were a natural part of the ants' diet prior to the fungiculture origin. Subsequently, the ants evolved the ability to cultivate fungus and convert it into a reliable and abundant food source and finally developed means to transmit their fungal garden between parent and offspring nest. According to this model, the ants controlled the domestication process and fungi underwent a cultivation event like human crops. The alternative "Transmission First" model suggests that specialized fungi used ants as vectors for dispersal prior to fungiculture. Subsequently, the ants incorporated fungi in their diet and finally evolved the ability to cultivate them by adding substrate. Mueller et al. (2001) concluded that one hypothesis of the traditional model and one of the alternative model most likely explain the origin of fungus-growing behavior.

Emery's (1899), Santschi's (1910) and Farquharson's (1914) hypothesis conform to the traditional "Consumption First" model and suggest that a hypothetical Attini ancestor took advantage of adventitious fungi growing on walls of their leaf litter nests. Our observation of the nesting behavior of *B. brasiliensis* in an Amazonian rainforest habitat is consistent with this model, and describes the possible ecological circumstances under which the attine ancestor could have existed. All nests found were located either in twigs, cavities of rotting branches or between leaves. Similar nesting habits are reported from an undescribed *Blepharidatta* species inhabiting the Atlantic rainforest of Bahia, Brazil, which construct their nests in rolled-up leaves (Diniz et al., 1998). In contrast, the savannah dwelling species *B. conops* lives in subterranean nests dug into the soil (Diniz et al., 1998) and this species is therefore less informative with respect to the ancestral ecological conditions, because the ant-fungus mutualism supposedly arose in the tropical rainforests of South America (Weber, 1972).

Two hypotheses by von Ihering (1894) and Forel (1891) proposed that attine ancestors fed on fungi growing on insect corpses accumulating in middens in nest chambers and that ants might have cropped fungal hyphae growing inside their wooden nests. These ideas have been rejected because lepiotaceous fungi are saprophytic and specialized on leaf litter decomposition. Chitinolytic enzymes, which are needed to decompose arthropod exoskeletons, are produced only in low concentration, hyphae do not grow into insect parts (Weber, 1972; Martin, 1974), and free-living Lepiotaceae rarely grow on decomposing wood in a natural environment (Dennis, 1952; Singer, 1986; Johnson, 1999). However, *B. brasiliensis* workers constructed nests in rotting wood and sealed off the functional nest chamber from natural cavities in branches and between leaves, with walls built from debris and insect carcasses. Fungal mycelium was observed on both nest walls within branches and chitin particles, but *B. brasiliensis* workers were never observed to crop these fungi. Diniz et al. (1998) reported that *B. conops* forms rings of insect carcasses around their nest entrances and also found fungus-overgrown exoskeleton parts within the nest chambers.

Given these observations, von Ihering's (1894) and Forel's (1891) scenarios appear plausible under the assumption that the attine ancestor grew a variety of different fungi in addition to lepiotaceous fungi. Today, cultivation of non-lepiotaceous fungi is only found in populations of the *Apterostigma pilosum* species group, which grow pterulaceous fungi (Basidiomycota: Pterulaceae) instead of lepiotaceous fungi (Munkacsi et al., 2004; Villesen et al., 2004). Munkacsi et al. (2004) propose that ants in the "*A. pilosum* group inadvertently domesticated the pterulaceous cultivar, which then displaced the cultivar originally grown by these ants". This scenario assumes that lepiotaceous fungi were cultivated by the attine ancestor from the beginning of the symbiosis 45–65 million years ago.

The alternative hypothesis would be that a hypothetical ancestor may have been associated with a variety of different fungi to which they were frequently exposed, either growing on walls of wooden nest, on insect carcasses, incorporated in nest construction (e.g., walls delimiting chambers) or on internal refuse dumps. Over time, the saprophytic Lepiotaceae were able to cope most successfully with the different substrates offered to them by the ants, including insect feces, leaf litter fragments, chitin pieces, and wood fragments. Finally, lepiotaceous fungi out-competed rival cultivars and, to speculate further, may have swept through the ancestral attine population, which lead to the co-evolutionary reciprocity between the two monophyletic groups of ants and fungi. However, such a hypothesized selective sweep has not been demonstrated for any attine cultivar, yet.

The observations on *B. brasiliensis* natural history and behavioral ecology did not yield additional information bearing on the three hypotheses of the traditional model. The "stored seed" (von Ihering, 1894), "mycorrhizae" (Garling, 1979) and "ant feces" hypotheses (Forel, 1902) remain unlikely, because (a) *B. brasiliensis* was neither observed to forage for seeds nor were granaries found in its nests (although, workers could be baited with cream-of-rice); (b) they did not construct soil nests and therefore, have limited contact to mycorrhizal fungi (although mycorrhizal fungi can be found occasionally in rotting wood); and c) neither lepiotaceous nor pterulaceous fungi are coprophilous (specialized on feces).

Recently, Sánchez-Peña (2005) proposed the novel hypothesis that attine ancestors foraged on fungus-growing beetle larvae and started cultivating the ambrosia beetle cultivar. This hypothesis is unlikely because ambrosia-beetle fungiculture arose probably after the origin of fungiculture in the ancestor of attine ants (Mueller et al., 2005).

The single hypothesis of the alternative "Transmission First" model, the "infrabuccal pellet" hypothesis (Bailey, 1920), is neither strengthened nor invalidated by our observations on *B. brasiliensis*. To our knowledge, *Blepharidatta* ants have never been observed to feed on mushrooms and thus, no conclusions about possible spore dispersion through infrabuccal pellets can be made. It should be noted at this point, that litter dwelling ants are most likely to feed on mycelium within the leaf litter layer, a feeding behavior that is unlikely to be observed. However, Mikheyev (pers. comm.) observed a case of facultative mycophagy in *Wasmannia* cf. *rochai*; *Wasmannia* and *Blepharidatta* are sister taxa in the tribe Blepharidattini (Wheeler and Wheeler, 1991; Bolton, 1995; Bolton, 2003) and are closely related to the Attini. Workers of *W.* cf. *rochai* foraged on a fruiting body of *Oudemansiella canarii* (Basidiomycota, Tricholomataceae) and fungal tissue has been detected in the ants' digestive system via molecular methods (Mikheyev, pers. comm.). Whether the ants were feeding on the fruiting body and ingested spores, or whether they foraged on insect larvae living within the mushroom and accidentally ingested fungal tissue, was not shown. To gauge the likelihood of Bailey's "infrabuccal pellet hypothesis" for the origin of attine fungiculture, future research on both *Blepharidatta* and *Wasmannia* should include molecular and microscopic examination of the infrabuccal pocket and the stomach contents.

Conclusions and future research directions

The novel information on the natural history and behavioral ecology of *Blepharidatta brasiliensis* support Emery's (1899) "adventitious fungi on nest walls" hypothesis for the origin of the ancestral attine-fungus association. The new information also suggests to reconsider von Ihering's "arthropod corpses" (1894) and Forel's "rotting wood" (1891) hypotheses as a likely origin of fungiculture within the attines, because *Blepharidatta* ants are in close contact to fungi growing on the walls of their wooden nest and also on insect carcasses, which they use for nest wall construction. These observations and the fact that ants in the *Apterostigma pilosum* group experienced a convergent coevolution with coral mushrooms (Pterulaceae) (Munkacsi et al., 2004; Villesen et al., 2004) lend support to the hypothesis that the attine ancestor possibly cultivated a variety of different fungi, beside lepiotaceous fungi. Because the majority of attine species are, like *B. brasiliensis*, inhabitants of moist tropical rainforest, the most likely evolutionary reconstructions would postulate a rainforest habitat also for the ancestral attine ant. The ancestral attine was therefore probably a leaf litter dweller, building small to medium sized nests (e.g., 20–200 workers) either directly in the leaf litter or in decaying wood. This attine ancestor may have been a partial predator or scavenger, accumulating exoskeleton refuse and other debris within or near the nest chamber. Such refuse provides a rich substrate for saprophytic fungi that may grow from spores within the refuse, or grow into the refuse from the surrounding wooden or leafy nest walls. At a subsequent stage, mycelium was facultatively cropped by ants and became a part of their diet, leading eventually to true fungal cultivation (i.e., adaptive substrate addition) and vertical transmission of the cultivated fungi by dispersing females.

This hypothetical scenario should be explored more with detailed observations of the foraging behavior and ecology of the three rainforest dwelling *Blepharidatta* species, *B. brasiliensis*, *B. sp. nov*. (Diniz et al., 1998), and *B. sp. nov*. (www.antweb.org). Comparative observations of these *Blepharidatta* species, in conjunction with observations on ants in the sister genus *Wasmannia,* on non-leafcutting Attini and other outgroup taxa from the subfamily Myrmicinae, will most likely allow reconstruction of the behavioral transitions and feeding adaptations that preceded the suite of derived fungi-culturing behavior exhibited by extant attine ants. Special attention should be given to the identification of the diversity of fungi, which are sampled and manipulated by these ants. In addition, preference tests could systematically explore the feeding preferences and tolerance of these ants towards fungi in and outside the family Lepiotaceae.

Acknowledgements

Research at the experimental field site of the Centro de Pesquisa Agroflorestal da Amazônia Ocidental da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) was conducted under SHIFT Project ENV 52-2 (collaboration between EMBRAPA Manaus and the State Museum of Natural History Karlsruhe) and funded by the Bundesministerium für Bildung und Forschung BMBF, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq (BMBF 01LT0014 / CNPq 690018/00-2). CR and MV would like to thank the CNPq and the Instituto Brasileiro do Meio Ambiente dos Recursos Naturais Renováveis (IBAMA) for permission to conduct fieldwork in Brazil; BMBF and CNPq for funding; Marcos Garcia from EMBRAPA Amazônia Ocidental for excellent collaboration and logistic support in Manaus. UGM would like to thank the Instituto Nacional de Pesquisas Amazônicas (INPA), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Instituto Brasileiro do Meio Ambiente dos Recursos Naturais Renováveis (IBAMA) (Licença 009/00-DIFAS, 02001.004380/99-90AC) for permission to conduct field work in Brazil; NSF for funding field research (NSF-IRCEB award DEB-0110073; NSF-CAREER award DEB-9983879). C. Roberto F. Brandão, Heraldo Vasconcelos and Ted Schultz provided invaluable logistical support for research in Brazil. We thank Mike Ryan, Matthew Leibold, Pupating Eufinger and two anonymous reviewers for helpful comments on this manuscript.

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