

# Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini)

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Received 29 October 2002; accepted for publication 28 July 2003

Neotropical ants in the tribe Attini share the obligatory behaviour of cultivating fungi as an essential food source. Of this complex and well-studied mutualism, little is known about the biological traits of the phylogenetically basal attines, which may offer important clues to the origins and early evolution of this intricate symbiosis. In this paper we focus on the founding of new nests, a key to understanding evolutionary changes in many social insects, which has received comparatively little attention in attine ants. We present a comparative survey of nest-founding behaviour in Attini, based on 441 foundress nests of 20 species in eight attine genera. In general, attine queens are semiclaustral and haplometrotic when founding nests. We show that attine foundresses of most species use an inert platform (discarded forewings, roots, or rocks) on which the incipient fungal garden is physically isolated during nest foundation, and they conduct semiclaustral foundation. This behaviour is not shared with *Atta*, which places the incipient garden directly on the soil floor of the initial nest chamber, and conducts claustral foundation. Nest-founding maps congruently onto the major clades in attine phylogeny, suggesting that the behavioural mechanisms used to isolate the incipient garden may have been key innovations in the early evolution of attine fungiculture. The evolutionary sequence of gardening in Attini suggests a transformation series from retention and use of detached wings (suspended from the chamber ceiling or placed over the chamber floor) to root-suspension, and finally use of bare soil. We also discuss transitions from semiclaustral to claustral founding, as well as from haplometrosis to pleometrosis, from ecological and evolutionary perspectives. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 81, 39–48.

**ADDITIONAL KEYWORDS:** agriculture – behavioural flexibility – claustral – comparative study – foundress – haplometrosis – phylogeny – pleometrosis – semiclaustral.

## INTRODUCTION

Nest foundation in ants in general begins when the virgin queen leaves the natal nest, mates, finds a suitable place to establish the new nest, and initiates the first worker brood (Hölldobler & Wilson, 1990). Queens can found the nest on their own (haplometrosis), with the collaboration of two or more queens (pleometrosis), with the help of workers, or by fission of a mature colony (Hölldobler & Wilson, 1977, 1990). During the colony life-cycle the founding stage is the most

dangerous time because the nest is highly vulnerable to predation, competition, and opportunistic infections. This stage becomes even more precarious if the queen requires a symbiont to establish the new colony. In nest foundation of the fungus-growing ants (Attini), the queen needs to transport, nourish, and cultivate fungi with which she will nourish her brood; she uses nutrient reserves in her body, supplemented by eating trophic eggs, to nurture the fungal garden and brood (Huber, 1907; Wheeler, 1907; Weber, 1972; Montenegro, 1975; Mintzer, 1987; Rissing *et al.*, 1989; Fernández-Marín, Zimmerman & Wcislo, 2003). It is commonly assumed that all attine gynes carry the fungal hyphae from their natal nest to their new nests in the infrabuccal pocket (Weber, 1982; Mueller *et al.*, 2001), and that they expel them just prior to estab-

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lishing a new nest. Queen behaviour during the founding stage provides important information regarding the mechanisms of successful nest establishment (Hölldobler & Wilson, 1977, 1990). Moreover, mechanisms of foundation from basal, transitional, and derived attine queens could be important for understanding the origin or early evolution of the fungiculture behaviour.

Nest-founding behaviour has provided an important window for understanding the origin and evolution of traits in many social insects (e.g. collaboration among queen ants; Rissing *et al.*, 1989). The scantiness of information on attine nest foundation precludes generalizations about its importance in relation to the origin of fungiculture or evolutionary transitions in nest-founding mechanisms. Some information is now available for the highly derived genera *Atta* and *Acromyrmex*, and from a phylogenetically basal species, *Mycocepurus smithii*. An *Atta* foundress leaves the natal nest for a mating flight during the early wet season and establishes a colony without foraging (claustral foundation); she places the incipient fungal garden directly on the chamber floor and nourishes her brood and garden with trophic eggs and nutrient reserves in her body (Huber, 1907; Autouri, 1942; Mariconi, 1974; Weber, 1982; Mintzer, 1987). An *Acromyrmex* queen forages for substrate to grow the garden and uses her fungal garden to nourish her broods (semiclaustral foundation; see descriptions of *Ac. lundii* in Weber, 1972, *Ac. striatus* in Diehl-Fleig & Lucchese, 1992, *Ac. versicolor* in Rissing, Johnson & Pollack, 1986, *Ac. octospinosus* in Cordero, 1963 and Fernández-Marín *et al.*, 2003). In contrast to *Atta*, *Ac. octospinosus* queens employ roots as a platform where they attach the fungal garden (Fernández-Marín *et al.*, 2003). In a more basal attine ant, *M. smithii*, foundresses dig tunnels and chambers, and suspend one or both of their detached forewings from the ceiling of the chamber to be used as a platform for the garden (Fernández-Marín, 2000). For both *Ac. octospinosus* and *M. smithii* foundresses, the use of a platform may be a critical hygienic behaviour during establishment of the garden by physically isolating the fungal symbiont from soil microorganisms, which may be pathogens or competitors. These considerations raise the question as to whether nest-founding behaviours reflect historical relationships, or whether they are ecologically labile, without a strong phylogenetic bias.

To address this and other questions, we document the behaviour of attine queens during nest establishment and initiation of the fungal garden, and describe details of the architecture and placement of the garden, for species representing eight genera in central Panamá. We then map garden establishment mechanisms and mode of colony initiation onto the current

attine phylogenetic tree. This comparative survey allows us to discuss the significance of sanitation behaviour from both ecological and evolutionary perspectives. Also, we discuss concomitant transitions from semiclaustral to claustral foundation, and the occurrence and ecological importance of haplometrosis and pleometrosis in the attines.

## METHODS

### STUDY SITES AND COLLECTING SAMPLES

This study was conducted in central Panamá along Pipeline Road (PLR) in Soberanía National Park and Barro Colorado Island (BCI) during May–July 2000, February–May 2001, and June 2002. These periods include the second half of the dry season and early wet season. Additional collecting was done at the Smithsonian Canopy Crane site near Sherman (Colon Province) along the Caribbean coast. Both PLR and BCI are primary and older secondary forest (for details of the sites, see Croat, 1978; Leigh, Windsor & Rand, 1982; Condit *et al.*, 2001).

Nest-founding attines were located along edges of trails, on slopes near rivers and creeks, and on the forest floor, by searching between 08:00 and 18:00 h. Incipient attine nests were recognized by the presence of small mounds, turrets of excavated soil and auricles around the entrances; alternatively, foraging foundresses were tracked to their nests.

Each recently established nest was carefully excavated. We recorded the number of foundresses, the position of the fungal garden, its substrate (e.g. forewing, roots, or chamber floor), and the date of collection. Observations on the behaviour of queens that transported the garden from one nest to another were also recorded when possible. The following nest architecture characters were also noted: form of entrance, entrance diameter, tunnel diameter, size and depth of chamber. The nests were transported to the laboratory where the number of eggs, larvae, pupae, and substrate used were recorded using a stereomicroscope. In this study, we defined foundress nests as those containing the queen inside a primary chamber, along with fungal garden, eggs, larvae and pupae (nests containing callow or adult workers were not included). Generally, such nests would be less than 1 month post establishment. Voucher specimens are deposited in the Zoological Museum, University of Puerto Rico (Rio Piedra Campus), and the Dry Reference Collection, Smithsonian Tropical Research Institute (Panamá).

### ATTINE ANT PHYLOGENY

Phylogenetic analyses of Attini reported by Schultz & Meier (1995), Schultz (2000), Lattke (1999), and Wet-

terer, Schultz & Meier (1998) provide a well-resolved cladistic classification, which we used to map nest foundation behaviours. Attine phylogeny shows a basal monophyletic group that includes three genera: *Myrmicocrypta*, *Mycocepurus* and *Apterostigma*. A more derived group is formed by four genera *Mycetartotes*, *Mycetosoritis*, *Cyphomyrmex* and *Mycetophylax*. Finally, two clades form the last major branch: one as transitional–higher clade *Trachymyrmex* and *Sericomyrmex*, the other containing the highly derived attine genera, the leaf-cutting ants, *Acromyrmex* and *Atta*.

## RESULTS

In total, 441 foundress nests representing 20 species from eight genera were collected in central Panamá. Specific details concerning nest-founding behaviour and garden initiation are described below.

### MATING FLIGHTS AND SEASONAL PATTERNS OF NEST INITIATION

In general, the mating flights of attine queens occurred after the first substantial rains of the wet season (~May to June). An exception to this pattern is *Cyphomyrmex longiscapus*, for which foundress nests were sometimes found in the middle of the dry season (March). As inferred from the temporal distribution of foundress nests there is usually a single peak for mating flights each year, although additional, minor peaks (one or two) sometimes occur for particular species in the same location; the degree of synchronization varies as well (Table 2). For example, in central Panamá (Gamboa) a major mating flight of *Atta colombica* was recorded on 15 May 2001, with a minor peak in June. By comparison, mating flights of *Trachymyrmex cornetzi* logged in 2002 occurred from mid-May to late July.

### SOCIAL ORGANIZATION OF FOUNDRESS NESTS: HAPLOMETROSIS AND PLEOMETROSIS

Nests of attines are generally established by a single queen (haplometrosis; Table 1). A low percentage of the nests in nearly half of the species in our study appear to be occasionally founded cooperatively by 2–4 queens (pleometrosis). However, whether these pleometrotic nests remain polygynous is unknown.

### NEST ARCHITECTURE AND LOCATION

Incipient foundress nests consist of an entrance with a small mound or circle of excavated soil around it, or with an auricle (see fig. 1 of Mueller & Weislo, 1998). The entrance leads to a short vertical tunnel that opens into a wall or ceiling of the oval to circular gar-

**Table 1.** Haplometrosis and pleometrosis in attine ants. The table includes only species for which eight or more nests were collected in Panamá. <sup>1</sup>specimens from Puerto Rico: Fernández-Marín (2000); <sup>2</sup>Fernández-Marín *et al.* (2003); <sup>3</sup>Rissing *et al.* (1986); <sup>4</sup>Mariconi (1974), <sup>5</sup>Echols (1966)

Species	No. nests	% with 1 foundress	% with > 1 foundresses
<i>Mycocepurus smithii</i> <sup>1</sup>	63	88	12
<i>Mycocepurus smithii</i>	12	91	9
<i>Mycocepurus tardus</i>	11	100	0
<i>Cyphomyrmex longiscapus</i>	12	100	0
<i>Trachymyrmex bugnioni</i>	13	100	0
<i>Trachymyrmex cornetzi</i>	22	100	0
<i>Trachymyrmex zeteki</i>	171	99	1
<i>Trachymyrmex</i> sp. 3	11	91	9
<i>Trachymyrmex</i> sp. 8	8	100	0
<i>Sericomyrmex amabilis</i>	44	91	9
<i>Acromyrmex echinator</i>	12	100	0
<i>Acromyrmex octospinosus</i>	86	96.5	3.5
<i>Acromyrmex octospinosus</i> <sup>2</sup>	245	99	1
<i>Acromyrmex versicolor</i> <sup>3</sup>	64	44	56
<i>Atta colombica</i>	25	100	0
<i>Atta capiguara</i> <sup>4</sup>	26	96	4
<i>Atta texana</i> <sup>5</sup>	97	97	3

den chamber that is usually 1–4 cm below the soil surface, except for *Mycocepurus* queens, which dig their chambers at a depth of 5–12 cm (Table 2). The chamber generally has a smooth surface, except in nests of *C. longiscapus*, *Acromyrmex* spp. and *Atta colombica*.

External architectural traits of some attine nests make it easy to find incipient ones. *C. longiscapus* and *T. zeteki* nests have conspicuous auricles and are often located on slopes along creeks (*C. longiscapus*) or roads (*T. zeteki*). *Mycocepurus* spp., *Sericomyrmex amabilis*, *T. bugnioni*, *T. cornetzi* and *Trachymyrmex* spp. 3–7 have a small mound of soil around the entrance and are located on the forest floor, although never near the slopes along creeks. *Myrmicocrypta* nests are also located on the forest floor, but have only a small mound of soil, while those of *Apterostigma* are found in cavities within the forest floor. *Acromyrmex* nests are found along the slopes of creeks and roads; the entrances are always covered with compacted, excavated soil grains. Nests of *Atta colombica* have a ring of excavated soil surrounding the entrance and are typically located in flat, open places within or in clearings outside the forest. With the exception of *Apterostigma* and *Myrmicocrypta*, the remaining genera included here are easily found on the forest floor.

**Table 2.** Architectural features and dimensions of attine foundress nests. A hole is the entrance to an underground cavity

Species	No. nests	Time collected	External appearance	Entrance diam. (mm)	Tunnel diam. (mm)	Chamber depth (mm)	Chamber volume (cm <sup>3</sup> )	Garden dry weight (mg)
<i>Myr. ednaella</i>	2	Feb, June	Mound	2.5	2.5	34	1.08	1.22
<i>Myc. smithii</i>	12	May–June	Mound	1.2 ± 0.4	1.6 ± 0.2	125 ± 37	1.2 ± 0.6	0.73
<i>Myc. tardus</i>	11	May–June	Mound	2 ± 0.4	1.3 ± 0.1	118 ± 45.1	1.4 ± 1.2	0.3 ± 0.1
<i>Mycocephurus</i> sp. 2	2	May	Mound	1.8 ± 0.4	1.3 ± 0.1	118 ± 45.1	1.4 ± 1.2	0.21
<i>Mycocephurus</i> sp. 4	1	May	Mound	1.8	1.3	3	1.1	–
<i>Apterostigma</i> sp. 1	2	May	Hole	1.4	–	–	–	0.15
<i>C. longiscapus</i>	11	March	Auricle	1.4 ± 0.4	<0.2	11 ± 5	1.1 ± 1	0.23 ± 0.02
<i>T. zeteki</i>	74	April–July	Auricle	4.4 ± 1.2	2.9 ± 0.5	31.6 ± 24.6	4.6 ± 5.2	4.8 ± 2.1
<i>T. bugnioni</i>	13	May–July	Mound	2 ± 0.4	1.7 ± 0.3	27 ± 14	2 ± 1.4	3.16 ± 2.4
<i>T. cornetzi</i>	22	May–July	Mound	0.25 ± 0.4	2.5 ± 0.3	52 ± 19	3.8 ± 2.4	1.4 ± 2.8
<i>Trachymyrmex</i> sp. 3	11	May–June	Mound	2.8 ± 0.6	2.5 ± 0.4	52 ± 14	2.9 ± 1.6	3.47 ± 4.9
<i>Trachymyrmex</i> sp. 4	1	June	Mound	2.5	2.5	36	1.4	0.1
<i>Trachymyrmex</i> sp. 5	1	June	Mound	–	–	38	2.0	1.2
<i>Trachymyrmex</i> sp. 6	1	June	Crater	2.8	–	48	3.5	0.3
<i>Trachymyrmex</i> sp. 7	8	May–June	Crater	1.8 ± 0.4	1.6	35	2.9	0.4
<i>S. amabilis</i>	37	May–July	Mound	2.6 ± 0.3	2.3 ± 0.2	49 ± 12	3.5 ± 2.1	8.3 ± 5.5
<i>Ac. octospinosus</i>	56	April–July	Mound	17.14	14.5 3.3	35 ± 17	68 ± 13	24 ± 26
<i>Ac. cf. volcanus</i>	3	June	Mound	18 ± 0.5	10.1 ± 3	24 ± 10	71 ± 10.2	15 ± 12
<i>Ac. echinator</i>	4	June	Mound	12.8	12.8	11.4	56.4	–
<i>A. colombica</i>	24	May–June	Crater	14.2	20.1 ± 0.2	39 ± 21	53.5 ± 11	–

#### MODE OF GARDEN FOUNDATION: SEMICLAUSTRAL AND CLAUSTRAL

Apparently, with the exception of *Atta*, all attine foundresses forage for garden substrata (semiclaustal foundation). Foundresses of *Ac. octospinosus*, *Ac. echinator*, *Ac. cf. volcanus*, *Apterostigma* sp.1, *T. bugnioni*, *T. zeteki*, and *T. cornetzi*, *Trachymyrmex* spp. 3 and 7 were observed foraging. Queens of *Mycocephurus smithii*, *M. tardus* and *Mycocephurus* sp. 2, foraged close (<25 cm) to the nest entrance. For the remaining foundresses in this study, the fungal gardens contained substrata from outside the nests and the entrances were open, which suggests that queens of these species also exhibit semiclaustal foundation. Some nests of *Atta colombica*, *Ac. octospinosus*, *Ac. cf. volcanus*, *Myc. smithii*, *Myc. tardus*, *T. zeteki*, and *T. cornetzi* contained living queens in the primary chamber either without, or with a dead, fungal garden. Presumably the former had yet to initiate their gardens. *Ac. octospinosus* foundresses were observed trying to enter other conspecific nests, but were usually rejected aggressively by the resident queens. Foundresses of *T. zeteki*, *T. cornetzi*, *Apterostigma* sp. 1 and *Ac. octospinosus* were observed moving the incipient fungal garden from one nest to another, new nest.

#### FUNGAL GARDEN PLATFORMS

Garden placement in recently established attine nests is strongly associated with the phylogenetic position of the genera (Table 3, Fig. 1). Queens from basal attines and one species of a more derived genus utilize detached wings as a garden platform. Transitional–higher genera and the highly derived genus *Acromyrmex* utilize roots for the platform, while the most highly derived *Atta* place the garden on the chamber floor. All the foundresses that use a platform place their eggs and juveniles in the garden; in contrast, *Atta* foundresses place their eggs directly in the soil. Additional specific details follow about garden placement.

##### Forewing platforms

Alate females from seven attine species of four basal genera retained their detached forewings, which were used as platforms for initiating the gardens (Table 3). Queens of *Myrmicocrypta*, *Mycocephurus* and *Apterostigma* – the most basal clade of the Attini – usually insert one or two wings into the ceiling of the subterranean chamber. *Myrmicocrypta ednaella* foundresses used their two forewings as a platform for the incipient garden, and either inserted them into the ceiling of the excavated chamber ( $n = 1$ ) or attached

**Table 3.** Position and type of platform used by attine queens to establish the fungal garden. *N* = no. total nests; F = forewing; R = rock; S = soil; IN = incomplete nest – signifies that no garden was present in the nest chamber. <sup>1</sup>specimens from Puerto Rico (Fernández-Marín, 2000); <sup>2</sup>*Atta sexdens rubrupilosa*\* attaches the fungal hyphae to the chamber floor (Autuori 1942)

Attine group	Species	<i>N</i>	Ceiling				Bottom		
			F	R	S	Roots	F	S	IN
Basal	<i>Myrmicocrypta ednaella</i>	2	2	0	0	0	0	0	0
	<i>Mycocepurus smithii</i> <sup>1</sup>	74	63	0	10	0	0	0	1
	<i>Mycocepurus smithii</i>	12	8	0	0	0	0	0	4
	<i>Mycocepurus tardus</i>	11	10	0	0	1	0	0	0
	<i>Mycocepurus</i> sp. 2	2	2	0	0	0	0	0	0
	<i>Mycocepurus</i> sp. 4	1	1	0	0	0	0	0	0
	<i>Apterostigma</i> sp. 1	2	2	0	0	0	0	0	0
Lower	<i>Cyphomyrmex longiscapus</i>	12	0	0	0	0	8	4	0
Transitional, Higher	<i>Trachymyrmex bugnioni</i>	13	0	0	4	9	0	0	0
	<i>Trachymyrmex cornetzi</i>	22	0	1	0	18	0	0	3
	<i>Trachymyrmex zeteki</i>	171	0	4	1	163	0	0	3
	<i>Trachymyrmex</i> sp. 3	11	0	0	0	11	0	0	0
	<i>Trachymyrmex</i> sp. 4	1	0	0	0	1	0	0	0
	<i>Trachymyrmex</i> sp. 5	1	0	0	0	1	0	0	0
	<i>Trachymyrmex</i> sp. 6	1	0	0	0	1	0	0	0
	<i>Trachymyrmex</i> sp. 7	8	0	0	0	8	0	0	0
	<i>Sericomyrmex amabilis</i>	44	0	3	0	38	0	0	3
Advanced, Higher	<i>Acromyrmex echinatio</i>	12	0	0	0	12	0	0	0
	<i>Acromyrmex octospinosus</i>	86	0	0	0	83	0	0	3
	<i>Acromyrmex</i> cf. <i>volcanus</i>	4	0	0	0	3	0	0	1
	<i>Atta colombica</i>	25	0	0	0	0	0	25	0
	<i>Atta sexdens rubrupilosa</i> <sup>2</sup>							*	

them to rootlets entering the chamber ( $n = 1$ ). In both cases the forewings were positioned in the form of a cross with the intersection near the apical regions. In *Mycocepurus* nests the garden was suspended from one forewing attached to the ceiling or surrounded an inserted wing (Fig. 2). In one *M. tardus* nest it was suspended from a root, and no forewing was found in the chamber. One active nest of *Apterostigma* sp. 1 contained a foundress; the garden was built on two forewings and attached to roots in a cavity. One abandoned nest contained a garden built on a forewing attached to the ceiling chamber. A more derived species, *Cyphomyrmex longiscapus*, facultatively places the wings horizontally on soil pillars on the chamber floor. In some nests of this species the gardens were supported by 3–4 short pillars; in 8 out of 12, however, detached forewings were found, indicating that either pillars or wings could be employed as platforms.

#### Root platforms

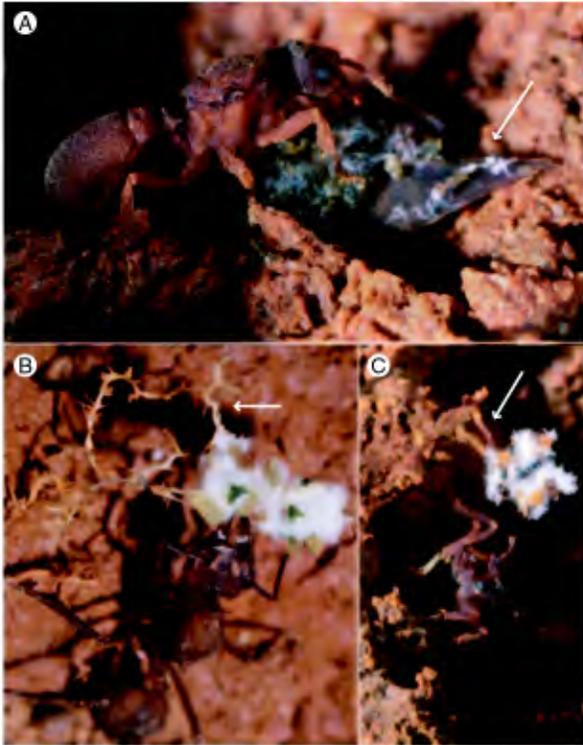
Queens usually discard wings after mating in 12 attine species representing genera of the derived clade [*Trachymyrmex* + *Sericomyrmex*] + [*Acromyrmex* +

*Atta*]. In foundress nests of *Trachymyrmex*, *Sericomyrmex* and *Acromyrmex* rootlets are part of the nest-founding repertoire and used as platforms (Table 3). Each foundress excavates a primary chamber in the soil, after which she cleans the soil from the roots (generally less than 2 mm in diameter) that she encounters. She then attaches a fungus pellet, suspending it above the chamber floor. Foundresses apparently do not select specific plant species: for example, in four nests of *Ac. octospinosus* roots were identified as *Cordia alliodora* (Boraginaceae), *Paullinia* sp. (Sapindaceae), *Geonoma* sp. (Palmae) and *Desmopsis* sp. (Annonaceae). Moreover, in some cases foundresses appear to have an alternative if they do not encounter roots in the primary chamber, attaching the pellet to a rock or to the chamber ceiling (13% of  $n = 374$  nests). In one case roots were present in the primary nest chamber, but the garden was attached to the ceiling (Table 3).

#### No platforms

*Atta colombica* foundresses discard their wings after mating, seal their nest entrances and tunnels with soil





**Figure 2.** Foundress queens with incipient gardens in three species of Attini. (A) *Mycocepurus smithii* foundress carrying a small fungal garden on a forewing (arrow); (B) recently established nest of *Acromyrmex octospinosus* with garden suspended from rootlet (arrow); (C) young nest of *Trachymyrmex bugnioni* with garden suspended from rootlets (arrow).

fungus and care for the brood, although co-founding (pleometrosis) commonly occurs (Table 1). In our study, *Mycocepurus smithii* appears to be pleometrotic, as does *Acromyrmex versicolor* (Rissing & Pollack, 1987). Studies of co-founding in *Acromyrmex* (Rissing *et al.*, 1986, 1989; Diehl-Fleig & de Aráujo, 1996) and *Atta* (Echols, 1966; Mintzer, 1987) suggest that it is associated with a high spatial density of nests and with the advantages conferred by cooperative defence against predators. It may also be advantageous if it increases the survival and growth of fungi during this stage. An additional advantage is that co-founding may accelerate the production of workers, which is known to enhance the survival of nests of the harvester ant *Messor perdandei* (= *Veromessor pergandei*; Rissing & Pollack, 1987; Hölldobler & Wilson, 1990). In Attini, foundresses typically leave their natal nests carrying a small pellet of fungal hyphae to provision their gardens; those leaving without a pellet have to acquire one elsewhere. The loss of a pellet prior to or during nest foundation is potentially catastrophic, as is the loss of the forewings in some basal

attine taxa. Co-founding may serve as insurance against such a catastrophe, although Mintzer (1987) suggested that co-founding as a strategy to prevent the loss of fungi is unlikely, because the frequency of *Atta* foundresses without a pellet is low in nature, as is true for *Acromyrmex striatus* (Diehl-Fleig & de Aráujo, 1996). *Acromyrmex* accepts new gardens and survives (Bot, Rhener & Boomsma, 2001), suggesting that attines naturally lose them on occasion (see Mueller, Rehner & Schultz, 1998). Our study also shows that attine foundresses do lose the pellet (or leave the natal nest without it) on occasion; in addition, the garden may fail during nest foundation. In either case, queens can remedy the situation by (1) joining a colony with an established garden; (2) recruiting other foundresses with pellets to co-found; (3) stealing fungi from another nest; or (4) finding an external source of suitable fungi. Detailed behavioural studies are unavailable for most species, and therefore we lack data to discriminate among these alternatives.

#### NEST ARCHITECTURE

The initial nest chambers of the attine ants we studied are usually located 1–8 cm below the surface, where fine rootlets are generally most extensive in tropical forests (Sanford, 1989; Cavalier, Estevez & Arjona, 1996). At these depths queens may maintain homeostatic conditions conducive to the establishment and growth of a new colony (fungal garden and brood). Moreover, as the size of the primary chamber is relatively small in all attines, it may be more easily maintained in a hygienic state relative to a larger area. In proportion to its body size, the primary chamber of an *Atta colombica* foundress nest is generally smaller than that of other attines, even though *Atta* foundresses are larger (e.g. ~7–10 times larger than *Acromyrmex*; H. Fernández-Marín, pers. observ.). The former are the only attines that regularly place both their gardens and brood directly on the chamber floor; a smaller chamber presumably minimizes exposure to soil-borne pathogens.

#### MODE OF GARDEN FOUNDATION: EVOLUTIONARY TRANSITION FROM SEMI-CLAUSTRAL TO CLAUSTRAL

Most attine foundresses conduct foraging trips, which suggests that they cannot grow the fungus pellet solely with stored nutrients. For 19 of the species included here, we either directly observed extranidal foraging, or inferred its occurrence because the garden substrata included material from outside the nest. Semi-claustal founding is also known from *Ac. lundii*, *T. septentrionalis*, *C. costatus* (Weber, 1972), *Ac. striatus* (Diehl-Fleig & Lucchese, 1992), *Ac. versicolor* (Rissing *et al.*, 1986), *Ac. octospinosus*

(Cordero, 1963; Fernández-Marín *et al.*, 2003) and *Mycocepurus smithii* (Weber, 1945; Fernández-Marín, 2000). After foraging for substrata and establishing a garden, foundresses confine themselves to the nest, tending the garden and rearing the brood. This behaviour reduces their exposure to predation and augments their ability to control the garden and brood (Diehl-Fleig & Lucchese, 1992). However, this confinement is not always absolute, as foundresses of *M. smithii*, *T. zeteki*, *T. bugnioni*, *Apterostigma* sp. 1, *Ac. octospinosus* and *Ac. echinator* continued to forage for a time after the eclosion of the first workers. Cordero (1963) and Fernández-Marín *et al.* (2003) observed similar behaviour in a nest of *Ac. octospinosus*, in which foundresses ceased foraging when there were approximately nine workers.

Evolutionary transitions from semiclaustral to claustral nest foundation are probably shaped in part by trade-offs between predation risk to the adult and nutrient availability for the garden and brood rearing (Montenegro, 1975; Rissing *et al.*, 1989; Diehl-Fleig & Lucchese, 1992; Thompson, 2002). *Atta* gynes are large by comparison with those of other genera, and the evolution of claustral foundation may be associated with an increase in body size and capacity to store larger reserves of food. The general assumption is that claustral ants produce fewer, smaller workers because they have less fat and energy to nourish the brood than those which forage (Keller & Passera, 1989; Thompson, 2002). The small body size of most attine gynes (range 4.5–10.5 mm in length compared with *Atta* foundresses, 20–27 mm) may preclude claustral foundation because they may not be able to store enough nutrients to nourish the garden and rear the brood. Comparing the number of broods that are produced by *Atta* vs. *Myc. smithii* and *Ac. octospinosus*, Fernández-Marín *et al.* (2003) reported that *Atta* foundresses produced ~100 workers in 3 months, while semiclaustral attine foundresses produce from 3 to 7 workers in the same time. Weber (1972) suggested that the former use their metabolized muscles to nourish their brood. Additionally, this could increase worker production if larger queens (such as those of *Atta*) have a lower specific respiratory rate than smaller ones, and if claustral ant foundresses produce smaller workers (Keller & Passera, 1989).

#### ORIGIN OF USING FOREWINGS AS A GARDEN PLATFORM

The origin and evolution of wings in insects enabled aerial dispersal and colonization of new habitats (Wigglesworth, 1973; Harrison, 1980; Trueman, 1990). In general, alate female ants (gynes) disperse from their natal nests, drop the wings after mating but before excavating a nest (Hölldobler & Wilson, 1990), as do

the derived attines, *Atta*. Gynes of basal attine genera, in contrast, retain and transport detached forewings using their mandibles (see also Fernández-Marín, 2000). Their use as a platform can be considered a novel adaptation. Each wing appears to be a firm sheet, consisting of two chemically inert cuticular layers, protected with waxes (Locke, 1974; Brudsky, 1994). We hypothesize that they are used as a clean, hygienic surface on which the small pellet of hyphae can grow.

#### EVOLUTIONARY TRANSFORMATIONS OF NEST FOUNDING BEHAVIOUR

Based on current phylogenetic hypotheses (Schultz & Meier, 1995; Wetterer *et al.*, 1998; Lattke, 1999; Schultz, 2000) we suggest that the use of wings as a platform is part of the groundplan for the tribe Attini, and that the physical isolation of the garden on a relatively inert surface was likely to have been an important early advance in the evolution of fungiculture. *Cyphomyrmex longiscapus* queens differ in the placement and orientation of the wing, although it still separates the garden from the chamber floor. Intraspecific variation in availability of supports may help explain variation in placement (Table 3) and also the evolutionary transition from the use of detached forewings to rootlets as a platform. In *M. smithii*, some queens have partially chewed wings in the natal nest (Fernández-Marín, 2000), which might make them unsuitable as platforms. In the absence of forewings, some *Mycocepurus* queens (8.7% of 92 nests; Fernández-Marín, 2000) initiate gardens directly on the soil ceiling substrate. Alternatively, they also can use roots, as in the case of *M. tardus*. Variability in platform type can be found among rootlet-using species of *Trachymyrmex* and *Sericomyrmex*. If rootlets are not present but a rock protrudes from the ceiling, foundresses suspend gardens from the rock surfaces; if rocks are not present the garden is planted directly on the chamber ceiling.

A second transition occurred in *Atta* as queens evolved claustral founding and lost the use of platforms. In all other known attine genera the foundresses forage for organic matter as garden compost (Table 3), which may contaminate the soil chamber. A claustral strategy is likely to enhance the survival of *Atta* foundresses, which are a conspicuous and nutritionally rich food item for numerous predators, including indigenous humans (Ramos-Elorduy *et al.*, 1997). A transition to claustral foundation would also reduce the introduction of contaminants relative to the more open nests of the less derived attines (Martin, 1987; Currie, Mueller & Malloch, 1999). Currie *et al.* (1999) found that garden contamination of *Atta colombica* nests increases once workers

begin to forage for garden substrate, while claustral nests were without *Escovopsis* infection.

Attine foundresses evolved novel behaviours to utilize existing structures (wings), or incorporate environmental objects (rootlets, rocks) into their behavioural repertoire during nest foundation, in order to isolate gardens from the soil. Additionally, suspending the garden from a platform enables queens to manipulate it without contaminating it. Ongoing studies are being undertaken to assess whether use of platforms (1) reduces susceptibility of the fungal symbionts to competition or attack by microbes in the soil or those introduced into the fungal chambers by ants, and (2) allows queens to easily manipulate their gardens. Further, there is a need to identify the source of damage to wings of basal attine gynes. Observations so far suggest that nestmates may mutilate the wings of gynes to limit their reproductive options (Fernández-Marín, 2000), as is known for a ponerine ant (Peeters & Higashi, 1989). Further research is needed. If substantiated, then the transition from the use of forewing as a garden platform to roots and rocks may have evolved as an escape from enforced slavery by nestmates (Peeters & Higashi, 1989; Rosengren, Sundström & Fortelius, 1993). Finally, the fact that *Atta* foundresses do not use a platform suggests that the infection of the incipient fungal garden is a result of foraging activity (see Currie *et al.*, 1999). Preliminary data show some contamination of the fungal garden in *Ac. octospinosus*, consistent with the hypothesis that foundresses accidentally bring in contaminants. Moreover, *Atta* foundresses could possibly use antibiotic secretions from exocrine glands to sterilize the earthen chamber (North *et al.*, 1997); however, no data currently exist to refute or support this hypothesis.

### CONCLUSIONS

This comparative study of the natural history of the Attini offers new perspectives on colony foundation behaviour, including that associated with the origin of fungiculture. Detailed behavioural studies of the strategies employed by foundresses that have lost their fungal symbionts may provide clues relating fungiculture to attine social organization. Further study of the natural history of basal attines, *Myrmicocrypta*, *Mycocephurus* and *Apterostigma*, as well as the more derived genera such as *Cyphomyrmex*, is necessary to establish attine groundplan behaviours. Our evidence suggests that fungus-growing ants have evolved behaviours to reduce competition between their fungal symbionts and a multitude of potential microbial competitors that are present in the soil or transported into the nest (Martin, 1987).

### ACKNOWLEDGEMENTS

Thanks are due to the following people and organizations. Stephen Rehner made suggestions and reviewed an early version of the manuscript. Koos Boomsma, Catherine Duckett, David Roubik, Ted Schultz, Sergio Sanchez-Peña and an anonymous reviewer commented on the manuscript. Adam Hart made suggestions during the development of this study. Ana Portugal assisted with the search for nests. Marcos Guerra took the photographs. The Autoridad Nacional del Ambiente de Panamá generously provided collecting and export permits. Ulrich Mueller identified some of the queens. This work was carried out with the support of the Short-Term Fellowship and A. F. Mellon Award from the Smithsonian Tropical Research Institute (STRI) to H. F. M., the Luquillo Long-Term Ecological Research Program funded by the USA National Science Foundation (DEB-9705814), the University of Puerto Rico and USDA Forest Service; and general research funds from STRI to W. T. W.

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