

Research article

Nest-founding in *Acromyrmex octospinosus* (Hymenoptera, Formicidae, Attini): demography and putative prophylactic behaviors

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Summary. Foundresses of the leaf-cutting ant *Acromyrmex octospinosus* in central Panamá forage for leaves as garden substrate (semi-claustral foundation). The fungal pellet and substrate usually are attached to rootlets, which are used as a platform for the garden. This arrangement keeps the garden suspended away from the earthen chamber of the underground nest during early colony growth, and we hypothesize that it serves to minimize contact between the garden and contaminants. *A. octospinosus* foundresses produce from 3 to 7 workers in 2.7 months after founding the nest, but workers do not forage for substrate at this time. Incipient nests died or were abandoned at a monthly rate of ca. 50%. We show that ants routinely clean their legs before manipulating the garden substrate. We also describe how foundresses use their forelegs to rub the surface of the metapleural gland (MPG), and they then use typical grooming behaviors to pass the forelegs through the mouthparts, after which the ant then licks the garden substrate. Similarly, ants apparently use their mouths to transfer fecal droplets to their legs. We briefly discuss the functional significance of these grooming behaviors, and hypothesize that they are prophylactic behaviors that may help the foundress maintain a hygienic garden.

Key words: Attini, metapleural gland, leg grooming.

Introduction

The nest founding behavior of the fungus-growing ants (Attini) has been postulated as the most elaborate among ants (Mintzer, 1987; Hölldobler and Wilson, 1990; Wirth et al., 2003). This is largely because attine queens must complete many activities associated with fungiculture behavior, including the collection of garden substrate, and the establishment, growth, and care of the young gardens. Apart from

details on foundress number or whether nests are semi-claustral or claustral, fungal gardening behavior in foundress nests is not well known (e.g., *Atta sexdens*, Huber, 1907; Autouri, 1942; *Atta texana*, Mintzer, 1987; *Acromyrmex versicolor*, Rissing et al., 1986; 1989; *Acromyrmex striatus*, Diehl-Fleig and Araújo, 1996; *Mycocepurus smithii*, Fernández-Marín, 2000). Recently it has been suggested that attine ants can do little to prevent microbial infection of the fungal garden (Wirth et al., 2003). A variety of studies, however, indicate that attines possess a number of mechanisms to control and inhibit infections by alien fungi and other microorganisms. These countermeasures against microbes include: an acidic pH in the fungus garden (Powell and Stradling, 1986), antibiotic secretions from the metapleural gland (Bot et al., 2002) and the symbiotic association with *Streptomyces* bacteria, which control the specialized, co-evolved and highly devastating fungal parasites in the genus *Escovopsis* (Currie et al., 1999; 2003). From this perspective, it appears that fungiculture in the attines is a system of disease management (Mueller and Gerardo, 2002). This perspective, however, ignores the importance of prophylaxis, or hygiene and disease prevention, in fungal garden management by the attines.

In this study we describe the nest-founding behavior of *A. octospinosus* queens, and present quantitative information on the rates of growth and mortality or abandonment of incipient nests. We describe leg-grooming behaviors associated with glandular and fecal secretions, and briefly discuss whether such grooming represents prophylactic behaviors that help the ants maintain a hygienic fungal garden.

Materials and methods

Nest founding of *A. octospinosus* was studied in central Panamá (near Gamboa, Colon Province) from May to August 2001. The identification of *A. octospinosus* foundresses was determined following Schultz et al. (1998). On May 18 and 20, 245 incipient nests of *A. octospinosus* were

located on a creek bank near Gamboa, and on a road bank of Pipeline Road (4.9 km) in Parque Nacional Soberanía (PNS), and marked to record both growth and mortality rates. Of the 245 nests marked, 22 were collected on May 20, 21 on June 19, 15 on July 19, and 8 on August 4 to record the number of eggs, larvae, pupae, workers, and foundresses, and the dry mass of the fungal garden. For each nest the garden position (where it was attached), and queen number were recorded. Each month after nests were marked they were censused to determine survival rates. Incipient nests were 'alive' if they contained a foundress and/or a fungus garden. Nests without gardens and foundresses were 'missing nests;' since foundresses sometimes relocate nests, we do not know if these 'missing nests' represent foundress mortality or nest relocation. Each month, surviving nests were collected randomly to determine the growth rates. Foraging trips of the queens were recorded when possible. No predators, parasites or pathogens of the foundresses and fungus garden were observed.

Twenty-five artificial nests were built to record the nest-founding behavior of queens. Artificial nests were made of transparent plastic boxes ($7.4 \times 7.4 \times 3.1$ cm), and were partially filled with soil to maintain an empty area (chamber, of approximately 35.0 cm^3) in the box core. Part of a root was attached so that it entered the chamber. Artificial nests were placed on slopes of small creeks near Gamboa. Twenty-five incipient nests of *A. octospinosus* were collected from Pipeline Road-PNS (between 0 to 4.9 km) and the queen and the small garden were placed in the chamber of artificial nests. After putting the queen and garden in the artificial nests, the entrance was closed with soil for 24 h, allowing the queens to attach their garden to the root and acclimate to their new nests. We recorded ant activity in the artificial nests from May 26 to August 4, in sessions of 30 min to 7 h, between 08:00 and 18:00 h, including a total of 145 h observations, focusing on behaviors associated with tending their gardens. *A. octospinosus* queen are relatively big, and relatively easy to observe using the naked eye, so we did not use video recording. Reproduction and details of brood tending behaviors of the foundresses were not recorded. We recorded distance and times of the foraging trips, and determined the frequency of the behavior acts of the queen. During the study, the first workers emerged and we also recorded their behaviors.

Results and discussion

A. octospinosus exhibited haplometrotic colony foundation. Ninety-nine percent of 245 nests were founded by a single queen, and 3 nests (1%) contained three queens. Other *Acromyrmex* species are haplometrotic (*A. niger* and *A. balzani*; Fowler, 1992), but *A. versicolor* is pleometrotic (Rissing et al., 1986). Monthly survivorship of *A. octospinosus* incipient nests was 52% in the first month ($n = 223$), 60% in the second month ($n = 94$), and 50% after 2.7 months ($n = 51$), which is higher than survival rates for incipient nests of other *Acromyrmex* species (e.g., mortality rate in two-month old nests of *A. niger* was 88%, and 83% for *A. balzani*; Fowler, 1992). Our results may overestimate mortality rates of foundresses; an empty chamber or dead garden does not show unequivocally that the foundress died, because she could relocate the nest.

Ninety-seven percent of the incipient nests ($n = 245$) had roots as a garden platform, and 3% used the shoots of *Selaginella* sp. (Lycopodiales, Selaginellaceae) as a platform, around which the nest chambers were constructed. In incipient nests (from May–July) the garden was always attached to rootlets that entered the underground chamber, and thus the fungal garden was suspended within the chamber so that it did not touch the earthen walls, floor or ceiling of the cham-

ber. Furthermore, foundresses also stripped the soil from rootlets before attaching the garden. In slightly older nests, gardens were usually suspended from a platform, but some nests were attached to the earthen ceiling of the chamber (8 of 26 nests in August). Eggs and juveniles always were attached to, or embedded in, the garden, and were never found on the earthen floor. The use of objects as platforms by foundresses apparently is widespread among attines, except for foundresses of the highly derived *Atta* (Fernández-Marín et al., in press). The functional significance of platform use is presently not known. Hypothetically, foundress ants may use platforms as a prophylactic behavior to physically isolate the fungal garden from soil-borne pathogens before the workers eclose and begin tending the gardens. Suspending the garden from rootlets may also give the foundress more room to work and manipulate the fungus garden.

As with natural nests, in 19 of 25 artificial nests the foundresses attached the fungus garden to roots placed in the chamber. The remaining nests were empty 24 h later, and in 2 cases we observed the foundress moving the garden to a new site. Of the 19 nests we studied, foundresses displayed 12 behaviors associated with gardening (Table 2). Workers displayed the same behaviors as foundresses, except for those involved with initiating a garden or defending a young nest (Table 2). In very young nests workers did not forage for substrate, but, when the colony is slightly larger, both workers and queen foraged (Cordero, 1963; Fernández-Marín et al., in press), and in older nests, only the workers foraged.

A. octospinosus queens foraged for fresh vegetable matter to grow the fungus garden from 5 cm to 4 m ($n = 56$, 1.34 ± 1.68 m) away from the nest, on trips that lasted from 25 s to 182 min ($n = 70$, 16.1 ± 30.5 min). During our observations foundresses made 0–3 trips per day, usually between 9:00–15:00 h, and they collected pieces of fresh leaves ($n = 88$), roots ($n = 2$), blades of cut grass ($n = 3$), or flowers ($n = 12$). In 77 foraging trips, 64% of foundresses returned with garden substrate.

A. octospinosus foundresses produced from 3 to 7 workers in approximately 3 months (Table 1). In a similar period, another semi-claustral founding species, *Mycocepurus smithi*, produced from 0 and 3 workers (Fernández-Marín, Zimmerman, Wcislo and Rehner, in review), while the claustral *Atta* produced 60 or more workers (Autouri, 1942; Fowler et al., 1986). Additional data are needed to ascertain whether limited worker production is widespread in Attini, and if so, how low growth rates relate to mode of nest founding and attendant dangers (e.g., risk of infection of the garden or adult predation).

Foundresses displayed two putative hygienic behaviors. First, they cleaned their legs before contacting the fungus garden, employing leg and mouth grooming behavior, and using drops of fecal secretion. Before tending the garden, ants consistently cleaned their legs (foundresses: 96% of 436 observations; workers: 80% of $n = 333$ observations). Grooming the legs immediately prior to touching, tending, or walking over the garden also occurs in other attine foundresses, *Acromyrmex echinator*, *Trachymyrmex zeteki*, and two species of *Apterostigma*, and in workers of *A. echinator* and

Table 1. Ontogeny and dry mass of fungus gardens of a cohort of incipient *A. octospinosus* colonies during the first two and a half months (mean \pm standard deviation)

Date	No. Colonies Collected	Content of the colonies collected				
		No.eggs	No. larvae	No. pupae	No. workers	Dry mass garden (g)
May, 20	22	7.9 \pm 5.2	0	0	0	0.007 \pm 0.009
June, 19	21	5.3 \pm 3.6	4.4 \pm 3.4	0.9 \pm 1.3	0	0.031 \pm 0.025
July, 19	15	5.1 \pm 2.6	2.9 \pm 1.9	1.1 \pm 1.2	2.7 \pm 3.2	0.022 \pm 0.018
August, 4	8	4.8 \pm 3.9	5.2 \pm 3.9	1.7 \pm 2.1	8.3 \pm 3.6	0.054 \pm 0.032

Table 2. Behavioral activity of *A. octospinosus* foundresses and workers observed during nest founding and in early growth stages. Cleaning the chamber area means the ants were employing mouth-grooming behavior on the bottom and walls of the chamber, and over the roots. (+) Observed, (-) not observed

Behavior acts	Foundresses	Workers
Cleaning the chamber area	+	+
Attaching the fungus garden	+	-
Cleaning legs	+	+
Foraging for substrate	+	-
Preparing substrate	+	+
Drawing the foreleg over the meatus of metapleural gland	+	+
Removing pieces of the garden	+	+
Inoculating the substrate mass with fungus mycelium	+	+
Attending garden and broods	+	+
Storing garbage	+	+
Maintaining garbage in the chamber	+	+
Aggressively defending the nest and fungus garden against con-specific foundresses	+	-

T. jamaicensis (HFM, pers. obs.); grooming behavior was also reported in *Atta sexdens* queens (Autouri, 1942). We hypothesize that grooming behavior is widespread in attine foundresses and has a prophylactic function to minimize contaminants in the young fungal gardens.

If the foundresses exited the nest chamber (e.g., to forage for substrate), upon returning they cleaned their legs and also used their mouth to collect fecal secretions, which were then transferred to the legs using regular grooming behavior (foundresses: 96% of 152 observations; workers: 80% of 15). To spread these secretions, the foundress curled her gaster and moved her head to take a drop of feces in her mouth, and then groomed her legs as described above, presumably transferring fecal secretions from the mouth to the legs. During our observations, foundresses apparently showed more attention and care in cleaning and using fecal secretions than workers, and this difference may be associated with low levels of worker foraging in the early stages of colony growth.

A second putative hygienic behavior is observed when foundresses convert pieces of leaves to garden substrate, which involves three steps: licking (rasping the surface of the leaf), maceration (biting and transforming the leaf into a macerated mass), and inoculation (placing drops of fecal secretion on the macerated mass, and placing the mass on the fungus garden; Quinlan and Cherrett, 1977; Weber, 1982). When a foundress prepared the substrate she used her

forelegs and mouthparts (n = 49) to hold the mass off the ground, minimizing contact between the earthen chamber and the substrate. While licking leaves, the foundress frequently paused to draw the foreleg in a manner such that the tarsi and metatarsi were rubbed on the meatus of the metapleural gland; then, she passed the foreleg through the mouthparts to lick the tarsi and metatarsi; finally, she again licked the substrate in the same position where she previously stopped (142 observations of foundresses, and 26 in workers). This behavior was observed from 1 to 26 times on a single piece of leaf. We hypothesize that this behavior enables the transfer of metapleural gland compounds to the substrate. After licking the leaf, ants thoroughly macerated it and curled the thorax to place the gaster in contact with it, placing from 1 to 7 drops of fecal secretion on the mass, and then she placed the mass on the fungus garden. Workers regularly apply fecal secretions to the substrate in *Acromyrmex* (Quinlan and Cherrett, 1977), which serves to transfer fungal enzymes via the ant to the garden, which increases fungal growth (Boyd and Martin, 1975). The use of these secretions by foundresses may have a similar function. We hypothesize that they also may facilitate the removal or decontamination of particles (spores, microbes, etc.) remaining on the ant's body, substrate or in the garden.

The use of forelegs associated with the metapleural gland described above suggests that metapleural secretions (MPS) are actively spread by the ants. Metapleural gland com-

pounds are known to inhibit bacterial and fungal growth in isolation, and many studies have hypothesized that they have such hygienic functions for gardens in vivo (e.g., Schildknecht and Koob, 1971; Beattie et al., 1985; 1986; Schoeters and Billen, 1993; Ortius-Lechner et al., 2000; Poulsen et al., 2002a, b; Bot et al., 2002), and in the survival of individual ants (Poulsen, 2002b), but in most cases experimental evidence is lacking (Currie, 2001). When the metapleural gland is experimentally closed, a fungal pathogen (*Metarhizium*) is often lethal to individual ants, while it is relatively harmless when the gland is open (Poulsen et al., 2002b). Schoeters and Billen (1993) proposed that the MPS are passively spread from the gland and then are distributed over the body by grooming behavior. Earlier Brown (1968) hypothesized that ants actively transfer metapleural compounds, based on his observations that some ant species draw the foreleg over the meatus of the metapleural gland, and spread the secretion over her body using grooming behavior. Brown postulated that ants have total control of where and when they supply MPG secretions, and our results support his hypothesis. In addition to antibiotic properties that safeguard individual worker ants, we hypothesize that MPS are spread by foundresses during the substrate preparation to suppress microbes before it is used to grow a fungal crop. Additional studies are needed to assess this hypothesis.

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