

Colony foundation, nest architecture and demography of a basal fungus-growing ant, *Mycocepurus smithii* (Hymenoptera, Formicidae)

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Abstract

The genus *Mycocepurus* is a phylogenetically basal attine ant, so studies of its biology may provide insight into the evolution of behaviours associated with fungus-growing that characterize the tribe Attini. *Mycocepurus smithii* from Puerto Rico produces sexual females from July to September, but no males were observed in 2 years of observations, confirming previous observations elsewhere. Colonies were founded between July and August and most nests were haplometrotic (85% of 74 nests). After excavating a tunnel and small chamber, a foundress queen inserted her fore wings into the ceiling and used the wing surfaces as a platform on which the incipient fungal garden was grown. Foundresses foraged for substrate to grow the fungus garden. Growth of incipient colonies was slow: the first workers emerged 2–5 months after colony founding and, after 8 months, colonies contained on average only a single worker.

Keywords: Attini, fungiculture, haplometrosis, hygiene, polygyny

Introduction

Studies of the natural history of the fungus-growing ants (Attini) have focused primarily on two genera, *Atta* and *Acromyrmex* (Weber 1972; Lofgren and Vander Meer 1986), which were christened as model systems for studying attine ant agriculture (Hölldobler and Wilson 1990). These two genera are highly derived and thus can provide little insight into the early stages of the evolution of fungiculture. In order to understand the origin and early evolutionary stages of fungiculture, data are needed on the natural history of basal genera, *Myrmicocrypta*, *Mycocepurus* and *Apterostigma* (Schultz and Meier 1995; Mueller et al. 2001), or on possible sister groups to Attini such as *Blepharidatta* (see Diniz et al. 1998).

Unfortunately, scant data are available detailing the biology and natural history of these basal taxa. The scarcity of observations likely stems from their inconspicuous nests and

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small colony size (Wheeler 1907; Weber 1945), even though a number of species that inhabit mesic Neotropical forests often have a high nest density in sites where they occur (e.g. *Myrmicocrypta buenzlii*, Weber 1972; *Apterostigma dentigerum*, Forsyth 1981).

Little is known about *Mycocepurus smithii* Forel, which is widely distributed from central Mexico to northern Argentina, and throughout the Caribbean (Kempf 1963). *M. smithii* is a tiny ant with monomorphic workers that live in small colonies of up to 100 adult workers with multiple queens (Kempf 1963). Their nests are excavated in soil and consist of a vertical series of oval chambers in which the fungus gardens are maintained on substrata of insect faeces and dead vegetable material (Wheeler 1907; Weber 1945). This study describes the life history traits of *M. smithii*, including information on colony founding and growth, architecture of incipient and established nests, and colony demography. Finally, we suggest character states that may be important for understanding the early evolution of fungiculture and colony foundation among attine ants.

Materials and methods

Foundress nests

From 19–26 July 1999, 109 new colonies were located and marked in a secondary forest in the Vega Alta State Forest, Puerto Rico. Based on collections of *M. smithii* colonies in July, we defined incipient or new nests as those with a soil mound <1 cm in diameter. Incipient nests discovered at the same time were considered a cohort. The course of colony growth was followed by excavating nests from cohorts at monthly intervals from July 1999 to March 2000, with the exception of November 1999 and February 2000. Queens with their gardens were transported to the laboratory, where we recorded the following data: the number of fore wings used as garden platforms, and the numbers of eggs, larvae, pupae, workers, and queens. The incipient fungus gardens were oven-dried at 50°C for 24 h and weighed.

Established nests

We studied five populations of *M. smithii* in the State Forests of Vega Alta, Maricao, Susúa, Guajataca and the Luquillo Experimental Forest at El Verde, Puerto Rico. Established colonies are easy to recognize because a mound of excavated soil from 1 to 9 cm in diameter usually surrounds the nest entrance (Torres 1989). Individual colonies were excavated, and the ants and their fungus gardens were placed in Petri dishes and transported to the laboratory. In the laboratory, all ants were separated from the fungal garden and preserved in ethanol. We defined non-reproductive colonies as those without alates, while reproductive colonies were those that contained alates. The number of workers and dealate or alate individuals per colony was recorded. The reproductive status of supernumerary females found in each colony was not determined, and thus it was not possible to infer the social structure in colonies with more than one dealate female. During colony excavations we recorded, when possible, details of nest architecture including the diameter, length and orientation of tunnels, size of the tunnel entrance, and the number, size and depth of the chambers. Voucher specimens are deposited in the Dry Reference Collection, Smithsonian Tropical Research Institute, Ancón, Panamá City, Republic of Panama.

Results

Architecture of foundress nests

The general structure of 74 foundress nests of *M. smithii* consisted of an entrance 2.1 ± 0.2 mm (mean \pm SD) in diameter, leading to a vertical tunnel of 1.5 ± 0.35 mm and 3–5 cm long (46 of 74 observations), which then opened into an oval-shaped expansion of the tunnel that was ~ 3.5 mm diameter and ~ 10 mm long. From there the tunnel continued until it opened into the ceiling of the primary chamber. Primary chambers were located 10.3 ± 2.23 cm below the surface and were irregularly shaped ovals with smooth walls, and had a mean volume of 1.9 ± 1.0 cm³ ($n=74$).

The majority of recently founded colonies (63/74=85.1%) had their incipient fungal gardens attached to one ($n=60$) or rarely two ($n=3$) discarded fore wings that were suspended from the ceiling of the primary chamber. In 11 colonies (14.9%) there were no fore wings present, including four nests that lacked a garden (all collected in July), while in the other seven (one in July 1999, one in December 1999 and five in March 2000) the gardens were attached directly to the earthen ceiling.

Architecture of established nests

Established nests of *M. smithii* were subterranean and had a single tunnel entrance surrounded by a mound of excavated soil. The entrance opening was 1.6 ± 0.35 mm in diameter ($n=138$). The tunnel often contained two or three small pseudo-chambers approximately 3–5 mm in height, and 3–5 mm in width, in which garden substrata were stored. The tunnel consisted of a narrow section that was approximately 1.27 ± 0.37 mm wide and 30.0 mm long, followed by a wider section that was 3.6 ± 0.25 mm wide and 10.0 ± 0.15 mm long, with alternating narrow and expanded sections running the length of the tunnel. The expanded sections were used to store soil and garden substrate. Horizontal lateral tunnels, 1.4 ± 0.2 mm ($n=4$) in diameter, branched at right angles to the main tunnel and extended 6.2 ± 4.1 cm ($n=11$) to lateral chambers. Lateral tunnels entered the lateral chambers near the base of the chambers. Chambers ranged from 3.5 to 38.0 cm³ ($n=87$) and were located primarily at depths between 20.0 and 85.0 cm below the soil surface ($n=42$). Among the non-reproductive nests, 53.6% ($n=41$) had one or two chambers and 42.7% had more than two chambers (range=1–4). Of the reproductive nests, 94% ($n=18$) had more than three chambers (range=2–7). In established colonies the fungus gardens were suspended from the chamber ceiling and attached directly to the soil, and remnants of fore wings were never observed in these mature nests. Fully developed fungus gardens were organized in vertically oriented, curtain-like folds. The garden filled nearly the entire chamber, except for a narrow gap between the chamber walls and the garden surface.

Demography of foundress nests

Of 74 new nests collected, 85% had a single foundress (haplometrosis). One nest lacked a foundress but the queen may have been overlooked during excavation of the nest. Among the remaining pleometrotic nests, seven contained two foundresses, one contained three and one contained four at the time of collection.

Of 109 incipient colonies excavated in July, 67% were alive at the time of their collection, and the remaining 33% were devoid of live ants or fungal gardens (Table I). In July, the

Table I. Status of young nests of *Mycocrepus smithii* collected in Vega Alta State Forest, Puerto Rico.

Date	No. of colonies collected		No. of eggs	No. of larvae	No. of pupae	No. of workers	Garden dry weight (g)
	Total	Alive					
July 1999	28	27	0.4 ± 1.0 (0–4)	0.1 ± 0.5 (0–2)	–	–	0.003 ± 0.003 (0.000–0.011)
August 1999	23	19	2.0 ± 1.0 (0–4)	0.4 ± 0.5 (0–4)	–	–	0.005 ± 0.004 (0.000–0.013)
September 1999	15	8	2.0 ± 0.6 (1–3)	1.1 ± 0.7 (0–2)	1.0 ± 0.6 (0–2)	0.6 ± 1.1 (0–3)	0.008 ± 0.004 (0.005–0.015)
October 1999	11	8	2.3 ± 0.8 (2–4)	0.8 ± 0.9 (0–2)	0.8 ± 0.8 (0–2)	0.5 ± 0.8 (0–2)	0.006 ± 0.002 (0.004–0.008)
December 1999	8	2	4.0 ± 1.4 (3–5)	2.0 ± 2.8 (0–4)	0.0 ± 0.0 (0–0)	2.5 ± 0.7 (2–3)	0.018 ± 0.005 (0.013–0.023)
January 2000	7	5	2.4 ± 1.8 (0–4)	0.6 ± 0.6 (0–1)	0.4 ± 0.6 (0–1)	2.6 ± 1.5 (0–4)	0.009 ± 0.005 (0.004–0.019)
March 2000	17	5	1.6 ± 1.1 (0–3)	0.4 ± 0.6 (0–1)	0.2 ± 0.4 (0–1)	1.0 ± 0.7 (0–2)	0.013 ± 0.004 (0.010–0.020)

Data are based on active nests and those dead at the time of excavation; ontogenetic data are averaged among nests within a cohort. Data are given as mean ± SD with ranges presented below in parentheses.

wings in all founding nests were overgrown with white hyphae of the fungal symbiont. By August, the gardens in all nests were attached directly to the chamber ceiling, and fore wings were completely surrounded by the garden. Eggs were observed on the surface and embedded in the garden, but were never on the chamber soil. In July and August 1999, the nest entrances were open and foundresses were observed outside the nest foraging for caterpillar faeces ($n=5$). The first appearance of workers occurred in the second month (September). The numbers of eggs and brood increased from September to January. In March, the numbers of workers and juveniles were significantly less than in colonies from December 1999 and January 2000 (Table I).

Demography of established nests

A total of 154 established nests from *M. smithii* were excavated between May 1998 and March 2000, including 135 non-reproductive (87.7%), 18 reproductive (11.7%), and one senescent (0.6%) nest (Table II). Reproductive nests contained more workers than in non-reproductive nests (77.2 ± 41.0 versus 41.6 ± 36.9 , respectively), as well as more dealate females (5.7 ± 4.3 versus 1.4 ± 1.6 , respectively). Twenty-four non-reproductive nests were without dealate females, while 76, 34 and one non-reproductive nests contained one, two to five, and more than five dealate females, respectively. Of the reproductive nests, 12 contained two to five dealate ants and six had more than five dealate ants. Fifteen reproductive colonies were collected from July to September 1999 (Table II), when colony founding occurred. These nests contained between one and 23 alate females (4.7 ± 6.1). In seven reproductive nests there were one to five alate females that had their fore wings partially cut off, presumably by nest-mates. One senescent nest collected in February 1999 contained nine living dealate females and a single worker, and the fungal gardens in all three chambers were dead. No males were observed at any time during this study (also Fernández-Marín 2000).

Table II. Chronology of nest excavations in five different localities in Puerto Rico (data from 154 colonies include the number of colonies collected by site, and the percentage of colonies containing sexual individuals).

Year	Month	Site	No. of nests	Percentage of nests containing alates
1998	May	El Verde	5	–
	June	El Verde	4	–
	September	Vega Alta	7	–
	October	Vega Alta	7	–
1999	February	Vega Alta	6	–
	April	Guajataca	5	–
	July	Vega Alta	28	21.4
	August	El Verde	19	31.6
	August	Guajataca	20	5.0
	September	Maricao	7	28.0
	October	Maricao	7	–
	November	Susúa	16	6.2 ^a
	November	Vega Alta	6	16.6 ^b
	2000	February	Vega Alta	5
March		Vega Alta	12	8.3 ^b

^aIn one nest, one alate female pupated 12 days after collection.

^bIn one nest, one alate female had partially cut fore wings.

Morphological features of the fore wings

The fore wings of *M. smithii* alate females have a morphological trait that may be associated with their use as platforms. The wings have an area lacking veins and pigmentation in the apical field. This spot is crescent-shaped and measures 0.36 ± 0.015 mm in length and 0.093 ± 0.009 mm in width ($n=36$). Representative material was examined by J. Billen (personal communication) and he found no evidence of a glandular structure on the wings.

Discussion

The foundresses of a phylogenetically basal attine, *Mycocepurus smithii*, use their fore wings as platforms for their incipient fungal gardens, and nest tunnel architecture enables the efficient storage and transportation of substrata and nesting materials within a tunnel system while allowing workers to pass by. Foundress nests sometimes contain multiple dealate females, and there is an evident lack of males.

Nest architecture

Nest architecture of some basal attine ants was described by Wheeler (1907) and Weber (1972), but did not include *Mycocepurus*. In Puerto Rico individual colonies of *M. smithii* had single nest entrances, although the high density of nests and close proximity of entrances could lead to the mistaken conclusion that there are multiple nest entrances. Kempf (1963) reviewed but doubted earlier reports that *M. smithii* has multiple nest entrances, although detailed studies on intra-specific variation in nest architecture are lacking, so conflicting reports may be correct. *Mycocepurus tardus* and *M. smithii* from Costa Rica and Trinidad (and independent samples from Panama) also have single entrances, as does *M. goeldii* in Guyana and Brazil (U. G. Mueller, personal communication).

Several architectural traits of attine nests have been proposed as adaptive features, such as tunnel systems that aid drainage and ventilate nests (Weber 1972; Mueller and Wcislo 1998), or features that increase the flow of traffic into nests (Howard 2001). The head width of workers and queens of *M. smithii* were approximately 0.7 mm and 0.9 mm, respectively, and the tunnel diameter was 1.3 mm, which was too narrow for two ants to pass one another. The tunnels of all nests had expanded sections ~3.6 mm in diameter, interspersed along the tunnel system, which provided sufficient room for individuals to readily pass one another, but still narrow enough to ensure contact among ants to facilitate information exchange. Maintaining a narrow tunnel presumably has lower construction costs and may serve other purposes such as preventing intrusions by larger predatory insects or stabilizing the temperature and humidity of the nest environment (Hölldobler and Wilson 1990).

Colony demography

In Puerto Rico, *M. smithii* annual production of alate females and nest founding peaked from July to September (Table II). These activities coincided with the onset of the rainy season, as is characteristic of the majority of Neotropical attine ants (Weber 1972; Fernández-Marín et al. 2004; but see Mueller and Wcislo 1998). Colony foundation seems to be associated with a time of high soil humidity when excavation is relatively easy, the desiccation risk to incipient gardens is minimized, and garden substrata are abundant (Weber 1972).

This is the first study to describe the fore wing spot that lacks pigmentation in Attini, though its functional significance, if any, is unknown. In addition to *Mycocepurus smithii*, this unusual feature is also present in alate females in all three basal genera (*Mycocepurus*, *Apterostigma* and *Myrmicocrypta*; T. Schultz, personal communication; H. Fernández-Marín, personal observation). This trait helps support the monophyly of the basal attine genera (Kusnevov 1963; Lattke 1999; Schultz 2000; see Schultz and Meier 1995). Transparent fore wing spots were not noted by Kempf (1963) in the revision of the genus *Mycocepurus*, nor do they appear in the drawing of the *Myrmicocrypta* wing by Weber (1972), indicating that both authors apparently overlooked this feature. The use of wings as platforms suggests that further studies are warranted.

Although we have not observed *M. smithii* queens shedding their wing(s), attaching the discarded wings to the chamber ceiling, nor the initial cultivation of the fungal garden, our observations suggest the following sequence of events during colony foundation. Prior to nest excavation founding queens either shed their wings at the excavation sites or retain them until a short tunnel is excavated. During some nest excavations we encountered abandoned fore wings in these places ($n=3$), and we observed foundresses moving through the tunnels with the fore wings in their mandibles ($n=2$). The dealate foundress then continues excavating to a depth of approximately 10 cm and constructs the primary chamber where she then places the fore wings in the ceiling of the chamber. The use of fore wings and other objects as a platform may be a specialized behaviour that serves to enhance garden hygiene during the initial stage of garden establishment (Fernández-Marín et al. 2004). The fore wings-as-platform is likely to have a temporary function, because fungal hyphae quickly grow out from the wing and attach themselves to the chamber ceiling.

The growth of *M. smithii* colonies is very slow, in comparison to that of *Atta* species. In *M. smithii*, the first workers emerge approximately 2–5 months after colony founding, but up to 9 months after nest founding the colony may still have only one worker (Table I).

In contrast, *Atta* colonies that are 2–3 months old typically have 60 to >100 workers (Autuori 1942; Fowler et al. 1986). *M. smithii* is a basal attine, and may possess some primitive fungiculture traits (e.g. they cultivate more than one type of fungal symbiont, which are closely related to free-living species of *Leucocoprinus*, Mueller et al. 1998; and employ as fungal substrata dead vegetable matter and pieces of insects, Weber 1945). Such comparisons suggest that in the evolution of Attini the ants may have domesticated their fungi to improve the nutritive properties, which may explain the faster growth rates of more derived attines. Alternatively, faster growth rates in higher Attini might be associated with increased egg production by the larger gynes.

We hypothesized that *M. smithii* is potentially secondarily polygynous (Kempf 1963 suggests that it is a polygynous species), which is based on: (1) reproductive nests have a large number of dealate ants in comparison with non-reproductive nests; (2) queens use fore wings as platforms, and alate females with cut fore wings presumably would be socially handicapped for independent nest founding; and (3) pleometrosis occurs in some nests of this species. Dissections of dealate females from *M. smithii* colonies with multiple dealate females showed that females of the same nests have yellow bodies and developed ovaries, which suggests that multiple females have active reproductive roles (H. Fernández-Marín, unpublished data).

If females use wings as platforms in incipient nests, what is the fate of dealate females that had their fore wings damaged or removed in the maternal nest? We offer three possible answers. First, dealate females may remain in the maternal nest (secondary polygyny), and thus act as a social insurance. Rosengren et al. (1993) proposed that *Formica* nests accept extra queens, or exploit daughter females that are prisoners of the resident queen and workers. Peeters and Higashi (1989) reported that *Diacamma australe* gamergates mutilate sister gamergates in a manner that limits their ability to reproduce. Second, dealate females may escape or leave the maternal colony on foot to found their own nests by attaching the incipient fungal gardens to rocks or rootlets which they clean as a substitute for wing platforms. Third, dealate females may enter incipient or established colonies and labour alongside the original foundresses in co-operatively founding the colony or in worker production. These possibilities all assume that dealate females are able to mate near or in the natal nest, or that females can reproduce parthenogenetically.

The possibility that females reproduce parthenogenetically is suggested by the conspicuous lack of males in reproductive colonies (Fernández-Marín 2000). In an early survey, Kempf (1963) reported on reproductive females, and he noted “three males taken by Dr. W. E. Kerr at Rio Claro, São Paulo, Brazil, seem to represent this caste of *smithii*”. Thus there is an element of uncertainty in Kempf’s report. The curious lack of males in *M. smithii* is not limited to the Puerto Rican populations. In Panama, among 48 reproductive nests collected from May to July 2000, a total of 512 alate and 183 dealates females were counted but not a single male was found (H. Fernández-Marín, unpublished data). The lack of males suggests either that males leave the nest much earlier than females and are extremely rare, or absent in this species, or the species is infected by *Wolbachia*, which is known to alter reproduction in its insect hosts in several ways, including male killing (Werren 1997). A definitive test that males are lacking necessitates the dissection of dealate females from functioning colonies to determine if the spermathecae contain sperm.

In conclusion, we report the first study of the demography of a basal attine, and we also describe the nest architecture of young and established colonies, the use of wings as a platform for incipient fungal gardens, the presence of multiple dealate females (potential secondary polygyny), and an evident lack of males. However, it is inappropriate to consider

all the behavioural traits of *M. smithii* as ancestral for attines. Recently, comparative studies of nest founding in attines show that the use of fore wings and semi-claustral behaviour are ancestral (Fernández-Marín et al. 2004). Other traits, however, such as the lack of males or multiple dealate females could be derived traits (autapomorphies), because males are known in other *Mycocepurus* species and all remaining attine genera (Kempf 1963; Weber 1972; Villesen et al. 2002). We require additional information on the other basal attines to help improve our understanding of the origin and evolution of fungiculture in these ants.

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