

Scaling of body weight and fat content in fungus-gardening ant queens: does this explain why leaf-cutting ants found claustrally?

J. N. Seal

Received: 25 February 2008 / Revised: 1 October 2008 / Accepted: 14 January 2009
© Birkhäuser Verlag, Basel/Switzerland 2009

Abstract Offspring traits are among the most important life history traits, yet we lack an adequate understanding of their role in social insect life history evolution. Colony founding in the fungus-gardening ants (Tribe Attini) is different from most other ant species because the queens forage during the founding phase. Queens of the most derived genus, *Atta*, are the only attines that exhibit the more typical claustral founding, where the queens seal themselves in a below-ground chamber and produce their first generation of workers with only body fat reserves. Here I report the dry weights, fat content and energetic value of newly mated queens of ten attine species. Published phylogenies were used to make inferences on the evolutionary transitions in this clade. It appears that the evolution of fungus-gardening was associated with the manufacture of smaller, leaner queens as basal taxa are characterized by small bodies that contain relatively less fat than derived taxa. Moreover, there appears to be an allometric function between fat, energetic content and dry weight, which means that for fatter and claustral queens to develop, they also must become larger.

Keywords *Atta* · *Trachymyrmex* · Claustral founding · Fat content · Life history evolution

Introduction

Social insects share many life-history characteristics with unrelated organisms such as plants or marine invertebrates as they have a motile pre-reproductive stage. This stage experiences high mortality where a few individuals out of thousands ever develop to maturity (Williams, 1975; Harper, 1977). Since the parents have little or no role in raising these offspring to adulthood, natural selection is thought to select for the production of offspring that are similar in size (quality). Parents can thus increase their fitness by producing large quantities of similar offspring (Smith and Fretwell, 1974; Lloyd, 1987). Under this scenario, an increase in the production of higher quality offspring would not result in higher fitness than allocating additional energy toward producing more, lower quality offspring.

Ecological conditions influence body size within populations such that, if conditions are typically poor for juvenile growth, larger, better-provisioned offspring and parents that produce offspring from stored food will be selected (a capital breeding strategy; Sibly et al., 1988; Stearns, 1992; Bourke and Franks, 1995; Jönsson, 1997; Turnbull et al., 1999; Jakobsson and Eriksson, 2000; Hendrickx et al., 2003; Johnson, 2006). On the other hand, if an environment has more predictable sources of mortality and plentiful food resources, it would be adaptive to produce offspring from food gathered during the reproductive period (an 'income-breeding' strategy; Stearns, 1992; Jönsson, 1997; Johnson, 2006).

Among the social insects, ants are well-suited for comparative studies on breeding strategy and body size (Peeters and Ito, 2001; Johnson, 2002; Howard, 2006; Johnson, 2006). Most ants exhibit so-called claustral founding, and this strategy is considered the derived reproductive strategy (Hölldobler and Wilson, 1990;

J. N. Seal (✉)
Department of Biological Science, Florida State University,
Tallahassee, FL, USA
e-mail: jon.seal@biologie.uni-regensburg.de

Present Address:

J. N. Seal
Universität Regensburg, Universitätsstraße 31,
Lehrstuhl Biologie I, 93040 Regensburg, Germany

Heinze and Tsuji, 1995; Peeters and Ito, 2001; Johnson, 2002). Claustrally founding queens initiate colonies by individually dispersing from the parent colony, sealing themselves in a subterranean chamber, and producing their first brood entirely from stored energy reserves that the queen obtained during the developmental (larval) or early adult stage in her parent colony (Hölldobler and Wilson, 1990; Peeters and Ito, 2001). These queens are relatively energetically expensive from the parent colony's perspective, because their bodies tend to be larger and they may be provisioned with larger amounts of lipids (>50% body fat), carbohydrates and storage protein than non-claustral queens (Haskins and Haskins, 1955; Boomsma and Isaaks, 1985; Keller and Passera, 1989; Hölldobler and Wilson, 1990; Boomsma et al., 1995; McInnes and Tschinkel, 1995; Wheeler and Buck, 1995; Tschinkel, 1996; Peeters and Ito, 2001; Hahn et al., 2004; Howard, 2006; Tschinkel, 2006). The production of such nutrient-laden, expensive foundresses has been proposed to be adaptive if mortality of semi-claustral queens (i.e., queens that forage) is high enough to offset the production of fully claustral queens (Bourke and Franks, 1995; Brown and Bonhoeffer, 2003).

There are several lineages of derived ants that have reverted to semi-claustral founding, in which the founding queen's offspring are nourished almost exclusively from food that the queen gathers while foraging. Notably, semi-claustral founding is common among harvester ants (*Messor* spp. and *Pogonomyrmex* spp.) (Brown, 1999; Johnson, 2002, 2006) and is the norm in most fungus-gardening ants (tribe Attini), except for the most-derived genus, *Atta* (Fernández-Marín et al., 2004; Seal and Tschinkel, 2007).

By mapping traits of queens onto a phylogeny, it is possible to observe transitions in life-history strategies that are coupled with queen characteristics and founding mode. Elucidating such evolutionary links between stages in the life cycle and the life-history strategies of different lineages of ants has previously been difficult as such comparative studies are limited by the quality of the phylogeny used to make inferences (Harvey and Pagel, 1991). However, the fungus-gardening ants are an exception as a recent phylogeny has benefited from molecular, morphological, physiological and behavioral data and thorough sampling at lower taxonomic levels (Schultz and Brady, 2008).

Among the Attini, variation in founding mode is associated with pronounced variation in queen and worker body size and colony size. The basal members of this tribe, the 'paleoattini,' are characterized by small workers and small colonies (e.g., *Myocepurus*, *Myrmecocrypta* and *Apterostigma*). In contrast, the more derived genera, the 'neo-attini,' tend to be larger-bodied and have larger colonies, especially species in the 'higher attine' genera, such *Sericomyrmex*, *Trachymyrmex*, *Acromyrmex* and *Atta*

(Weber, 1972; Wirth et al., 2003; Schultz and Brady, 2008). The most derived members in the 'higher attine' group are the leaf cutting ants, *Acromyrmex* and *Atta*, which are among the largest ants in the world in terms of body and colony size (5,000–50,000 workers in *Acromyrmex* to more than 1 million workers in *Atta*).

Here I report data on queen size and quality (fat content) from several species of 'neoattine' species (Schultz and Brady, 2008) collected in the Republic of Panamá and also published data (Seal and Tschinkel, 2007) to document variation in size and quality among queens of different genera. Previously, Seal and Tschinkel (2007) found that two attini species had queen fat content well below the threshold necessary for claustral founding (Keller and Passera, 1989; McInnes and Tschinkel, 1995). Seal and Tschinkel (2007) speculated that body fat might exhibit a scaling relationship with regard to body size across the entire tribe. Specifically, they found that queen fat content was positively allometric with body size, and by extrapolating the data, they predicted that *Atta* queens would be claustral because they were large and fat enough to be so. It seemed that while the evolution of fungus gardening was associated with producing lean queens, subsequent radiations saw the increase in body size and fat content of founding queens. However the support for this interpretation was not very robust because their study had data from only two attine species that occupied intermediate positions in the phylogeny.

Materials and methods

The Attini occur in warm temperate and tropical latitudes of the western hemisphere in a zone extending from 40°N (Illinois and New York) to 45°S (Patagonia) (Weber, 1972; Farji Brener and Ruggiero, 1994). Most of the data reported here is drawn from specimens collected in Panamá during April and May 2006. Young queens collected in Florida (30°22.56 N, 84°22.01 W) in May 2006 and those reported by Seal and Tschinkel (2007) were also included.

Mating flights of attines generally take place one day after a heavy rain. In Panamá, this often corresponds with the first significant rainfall of the wet season (late April to early May). In Florida, attine flights typically occur after the start of the first afternoon, convective thunderstorm of the early summer (late May to early June; Seal and Tschinkel, 2007). While this appears to be the case for *Cyphomyrmex rimosus* and other attines (Seal and Tschinkel, 2007), *C. longiscapus* and *Apterostigma* spp. in Panama produce sexuals throughout the year (Fernández-Marín et al., 2004; Seal and Tschinkel, 2007).

All specimens of *Atta* spp. were collected during or immediately following flights, which was greatly

facilitated by the attraction of alates to lights and the fact that many source colonies were located near buildings. Five *Atta sexdens* female alates (gynes) were collected at approximately 21:00 h during a flight that took place near a *Rey* Supermarket along the Corredor Norte in Panama City on 27 April 2006. Four additional *Atta sexdens* gynes were collected at the nest during a flight 20:00–22:00 h on 6 May 2006 near Edificio 183 in Gamboa (9°7'N, 79°42'W). At the same location, forty *A. cephalotes* gynes were collected approximately at 09:00 h on 6 May 2006 after a mating flight.

Six *Acromyrmex octospinosus* and seven *A. echinator* queens were collected 16:00–18:00 h 12–13 May 2006 along the embankment across from the old school house in Gamboa. At this time, queens could be easily spotted as they foraged. These queens were probably products of a mating flight within the past few days since some queens were followed to their fungus gardens, which were small and contained no larvae.

Except for the published data set and two additional *Cyphomyrmex rimosus* queens collected in Florida on 27 May 2006 along the same edge of a mixed pine-oak secondary growth (30°22.6'N, 84°22'W) (Seal and Tschinkel, 2007), all the remaining Attini were collected in Panamá along several creek embankments that intersect the first 2–5 km of Pipeline Road in the Parque Nacional Soberanía 3 May to 7 May (9°9.2'N, 79°44.2'W). Incipient nests were easily spotted after carefully inspecting the leaf litter and stream embankments (Fernández-Marín et al., 2004). Most of these queens were collected at or near their incipient nests 10:00–15:00 h. Queens collected inside nests with incipient fungus gardens that contained brood, primarily *C. longiscapus* and *Apterostigma* sp., were excluded from the analysis, since their condition (fat content) would likely be lower since they had already begun the brood rearing process (Seal and Tschinkel, 2007). As a rough indicator of time since mating flights, only queens were used whose fungus gardens had platforms (the dealate's discarded wings) that were visibly larger than the amount of fungal mycelia (Fernández-Marín et al., 2004). This yielded values that approximate the condition of post-mating flight queens.

After collection the queens were killed by freezing and then oven-dried. Their body fat was then extracted in a Soxhlet extractor column using diethyl ether until constant weight was reached (Soxhlet, 1879). Extracting time depended on the size of the ant and therefore, the species. *Cyphomyrmex* spp. generally required at least 12 h, *Trachymyrmex* spp. and *Sericomyrmex amabilis*, 24 h; *Acromyrmex octospinosus*, 72 h; and *Atta* spp., at least 168 h (7 days). Several specimens of these species were extracted for an additional 5 days with little or no change in weight to verify full extraction. After extraction, individual queens were then dried and then re-weighed.

Energetic contents of ants were obtained by multiplying lean weights by 18.87 J/mg and fat weights by 39.33 J/mg (Peakin, 1972). These constants have been used for estimating the energetic content of ants (Boomsma and Isaaks, 1985; Keller and Passera, 1989; Tschinkel, 1993; McInnes and Tschinkel, 1995) and other insects (Sartori et al., 1992). Data were \log_{10} transformed and analyzed with ANOVA and linear regression (Statistica, version 6.1; Statsoft, 2003).

Species identifications of the Panamanian attines were aided by an unpublished key made available by Koos Boomsma (Dijkstra and de Fine Licht, 2007) and help in the field by Hermogenes Fernández-Marín. To avoid taxonomic confusion, terminology was kept consistent with recent work on Attini founding biology (Fernández-Marín et al., 2004). Two specimens of a species of *Trachymyrmex* were not pictured in Dijkstra and de Fine Licht (2007) and probably represents an undescribed species within the *T. cornetzi* complex since it was very similar in appearance to *T. cornetzi* (Fernández-Marín et al., 2004; Dijkstra and de Fine Licht 2007). This morphospecies is indicated as *Trachymyrmex* cf. *cornetzi* sp. 3. Identifications of the *Acromyrmex* species were also aided by comparing the dry weights in this study with unpublished data from Koos Boomsma and Else Fjerdingstad. The presence of the social parasite *Acromyrmex insinator* was also ruled out by using the descriptions in Schultz et al. (1998).

Results

The most striking observation in these attines is a transition from small, lean and energetically poor queens to extremely large, fat and energetically endowed queens; variation that spans at least four orders of magnitude (Table 1). The leanest queens were in the intermediate genus, *Cyphomyrmex*, which contained 12–15% fat. The fattest young queens were in the most derived genus, *Atta*, which contained approximately 40% body fat (Table 1). The size distributions of nearly all species are non-overlapping; so that each species appears to have a unique dry weight range. This pattern is clearly seen across the dry weight ranges of *Sericomyrmex amabilis*, the three species of *Trachymyrmex* that have sample sizes >2 , the two species of *Acromyrmex* ($F_{5,77} = 1263.1$, $P < 0.0001$, Table 1, Fig. 1a), and between the two species of *Atta* ($F_{1,47} = 212.6$, $P < 0.001$) (Table 1). However, the sample size of *C. longiscapus* is too small ($n = 3$) to draw a similar conclusion about the two *Cyphomyrmex* species, since they appear to have similar weights ($t_{13} = 0.068$, $P = 0.95$) and percents fat ($t_{13} = 0.84$, $P = 0.41$) (Table 1).

The fat content of attine queen bodies presents a slightly different picture. While the two species of *Acromyrmex* are

Table 1 Mean dry weights, percents fat and energetic value of 12 attine species

Species	<i>N</i>	Dry weight (mg) ± SD	Percent fat ± SD	Joule ± SD
<i>Cyphomyrmex longiscapus</i>	3	0.44 ± 0.03	15 ± 4	10 ± 1
<i>Cyphomyrmex rimosus</i> ^a	12	0.44 ± 0.08	12 ± 6	9 ± 2
<i>Sericomyrmex amabilis</i>	10	2.11 ± 0.26	34 ± 3	55 ± 8
<i>Trachymyrmex cornetzi</i>	11	1.31 ± 0.09	29 ± 1	33 ± 2
<i>Trachymyrmex septentrionalis</i> ^b	21	1.79 ± 0.13	25 ± 2	43 ± 4
<i>Trachymyrmex zeteki</i>	27	2.42 ± 0.2	24 ± 3	58 ± 6
<i>Trachymyrmex</i> cf. <i>cornetzi</i> sp. 3	2	2.26 ± 0.58	20 ± 6	52 ± 16
<i>Trachymyrmex</i> cf. <i>zeteki</i> sp. 3	1	2.2 ± 0	33 ± 0	56 ± 0
<i>Acromyrmex echinator</i>	7	13.5 ± 1.2	28 ± 6	333 ± 43
<i>Acromyrmex octospinosus</i>	6	19.22 ± 0.91	34 ± 2	495 ± 24
<i>Atta sexdens</i>	9	317.3 ± 20.2	39 ± 2	8,251 ± 660
<i>Atta cephalotes</i>	37	417.1 ± 19.1	40 ± 1	11,273 ± 510

^a Most data (*N* = 10) are from Seal and Tschinkel (2007)

^b All data are from Seal and Tschinkel (2007)

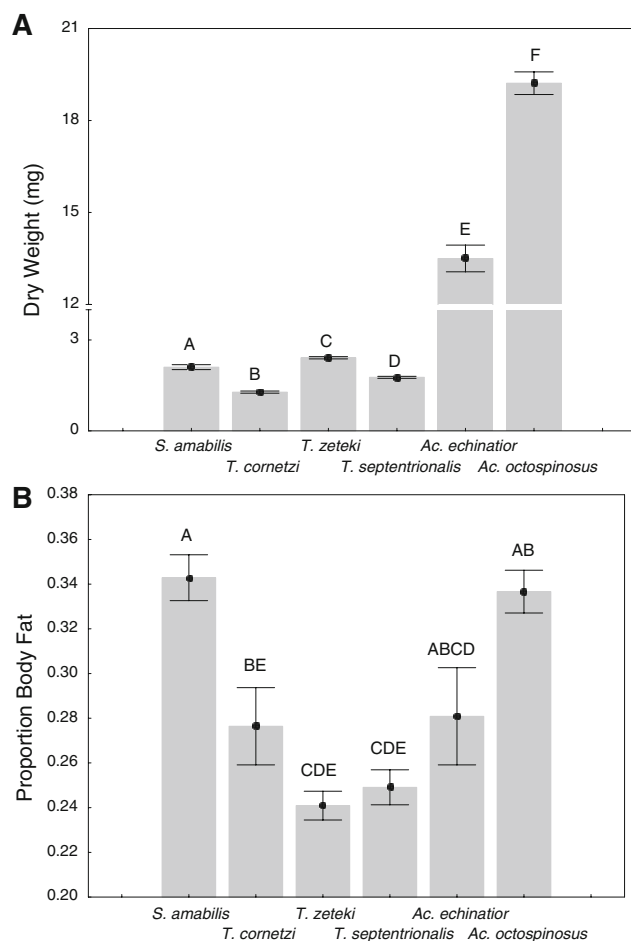


Fig. 1 **a** Mean dry weight of six higher Attini (±SE). All species had significantly different dry weights. **b** Mean percent fat (±SE) of these four species and the two *Acromyrmex* species. To use the approximate scale for comparison purposes, the data from the two species of *Atta* were omitted from these figures. Species sharing similar letters are not significantly different ($P < 0.05$, Scheffé's test)

clearly intermediate in size between the *Trachymyrmex* + *Sericomyrmex* group and *Atta* (Table 1, Fig. 1a), and variation in dry weight spans at least one order of magnitude, these increases were not matched by increases in percent body fat in these five species, although significant variation was found ($F_{5,77} = 13.8$, $P > 0.0001$, Fig. 1b). *Sericomyrmex amabilis* and *Ac. octospinosus* had similar body fat percentages (~30%) whereas the three *Trachymyrmex* species generally contained less fat (Fig. 1b).

Interspecific comparisons among three recognized clades of fungus gardening ants (*Atta* and *Acromyrmex*), non leaf-cutting higher attines (*Trachymyrmex* and *Sericomyrmex*), and the intermediate attine genus (*Cyphomyrmex*) indicate that this sample of the tribe is characterized by a positive allometry between queen fat content and dry weight (slope ± SE = 1.12 ± 0.034 , two tailed t test: $t_8 = 3.59$, $P < 0.01$). This means that a tenfold increase in dry weight corresponds to a thirteen fold increase in fat content (Fig. 2a). This means that as body size increases in these queens, lean weight (fatless weight) increases at a lower rate than the fat content. There also appears to be a tribe-wide positive allometry between dry weight and energetic content (slope ± SE = 1.026 ± 0.006 , two tailed t test: $t_8 = 4.24$, $P < 0.0001$), such that energetic content increases at a faster rate than dry weight in these attine taxa.

Discussion

The major finding of this paper is that attine queens appear to be characterized by a positive allometry between dry weight and fat and energetic content. On average, larger

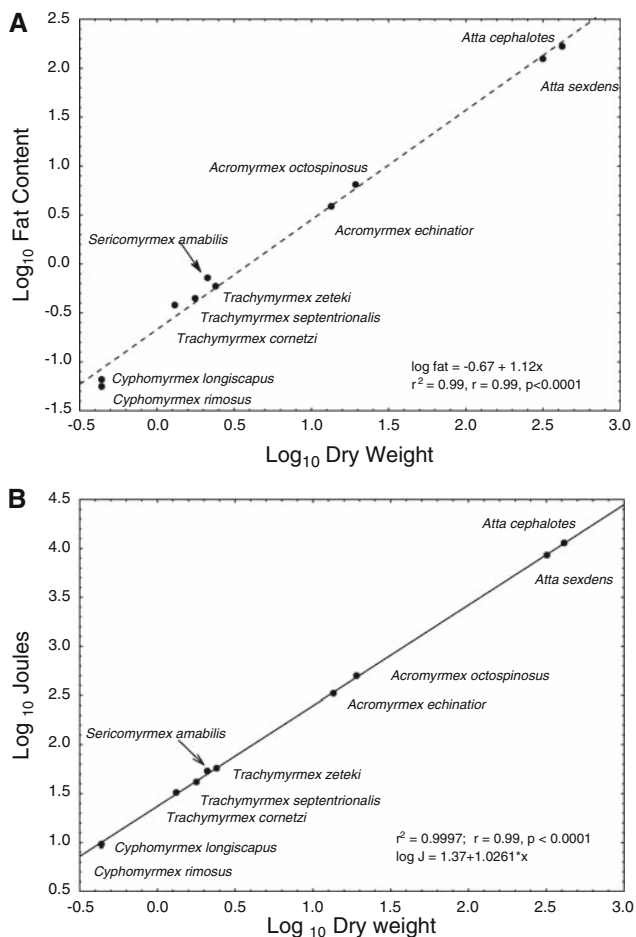


Fig. 2 **a** Log–log plot of fat content as a function of total dry weight of ten attine species. This slope has a positive allometry. **b** Log–log plot of energetic content (J) as a function of total dry weight of ten attine species. This slope has a positive allometry. The three specimens of the two species of *Trachymyrmex* were excluded from this analysis for brevity (the fit does not change)

bodies are disproportionately fatter and energy richer than smaller bodies. In other words, fat and energetic content increase at a faster rate than does overall body weight. This indicates that fat deposition became important during the adaptive radiation of the Attini. Additionally, this relationship appears to limit the amount of fat that a queen accumulates, which therefore influences colony founding mode. Small queens are less able to found nests claustrally and raise both brood and a garden on body resources alone.

Although the evolution of fungus gardening and its consequences on founding mode will remain unknown until analyses are performed on the paleoattini and the yet unknown extant sister taxa to the attines (Schultz and Brady, 2008), it seems probable that early fungus-gardening species likely produced small and lean queens. Fungus-gardening may have removed the selective pressure to produce fat-laden queens capable of rearing brood without

foraging. This interpretation is supported by the finding that the attine queens in this study are leaner than other semi-claustral myrmecines (Keller and Passera, 1989; McInnes and Tschinkel, 1995; Tschinkel, 1996; Howard, 2006). Even though *Atta* queens are the fattest attines with 40% body fat, they still contain less fat than other claustral species, which typically contain $\pm 50\%$ body fat (Keller and Passera, 1989; McInnes and Tschinkel, 1995; Tschinkel, 1996; Howard, 2006). In fact, 40% body fat is consistent with semi-claustral founding or parasitic queens in other ant genera (Keller and Passera, 1989; Tschinkel, 1996), making *Atta* queens the leanest ants capable of claustral founding. Perhaps this underscores the importance of fungiculture as an important evolutionary change that unifies this lineage of ant species.

The evolution of larger and fatter queens was probably made possible by increasing the size of the colony and its ability to acquire resources, which, in turn, allows the production of larger foundresses. It would be impossible for a species with small colonies and small queens to produce an ant with the fat content of an *Atta* capable of claustral founding. For example, the average mature colony of *T. septentrionalis* in north Florida produces an average of 24 female sexual offspring (Seal and Tschinkel, 2008) or a total of 1,023 J, whereas a single *Atta* queen may contain about ten times this value (Table 1) and a single *Atta* colony may produce thousands of gynes per year (Weber, 1972). Furthermore, comparative studies (Shik, 2008) and focused work on fire ants and harvester ants (Tschinkel, 1999; Smith and Tschinkel, 2006; Tschinkel, 2006) suggest that colonies achieve higher fitness by increasing colony size. This may be true across the higher attini because colony size and queen size both appear to span several orders of magnitude. For example, colony sizes in *T. septentrionalis* are on the order of 10^2 workers (Seal and Tschinkel, 2006, 2008), *Acromyrmex landolti*, 10^4 workers, (LaPointe et al., 1998; S.L. LaPointe, pers. comm.) and *Atta colombica* $> 10^6$ workers (Weber, 1972; Wirth et al., 2003). Dry weights and energetic value of queens also increase roughly by an order of magnitude between these three genera (Table 1). Therefore, queen size in the Attini would appear to increase at least an order of magnitude for a similar increase in colony size. However, except for Seal and Tschinkel (2006, 2008) no studies have documented relationships between aspects of sexual output and colony size for most Attini.

The rough similarity of percent fat of the three *Trachymyrmex* species suggests that regardless of their overall size, around 25–29% body fat is adequate to meet the metabolic demands of the founding period. The finding that *S. amabilis* contains about the same amount of body fat as the two species of *Acromyrmex* also indicates that around 34% body fat is adequate for these species, even

though there is about a ninefold difference in dry weight—*S. amabilis* has similarly sized bodies to the five species of *Trachymyrmex*. Therefore similar fat contents would appear to be adequate for the production of brood in bodies of vastly different size. It might be adaptive for *Acromyrmex* to have a greater lean mass because their colonies are generally large, queens mate multiply (Murakami et al., 2000) and store more sperm to do so (Tschinkel, 1987). On the other hand, *Sericomyrmex* ants do not have cuticular actinomycetes that produce antibiotics as defenses and could possibly have higher energy expenditure during the founding period as a consequence (Currie et al., 2006; H. Fernandez-Marin, pers. comm.).

Why is there variation in fat content that is below the threshold for founding claustrally? Fatter bodies might be able to forage less during the founding phase. Attine fungus gardens are susceptible to fungal pathogens that invariably leads to colony failure (Currie et al., 2003), spores of which appear to be brought in to the nest by foragers (Currie et al., 1999). *Trachymyrmex septentrionalis* foundresses generally cease foraging after they build up a garden, which may be 4 weeks or more before the first minors emerge and the first pathogen attacks took place several weeks and months later (Seal and Tschinkel, 2007; J.N. Seal, unpubl. data). Reducing the time spent of the nest could be important in less derived lineages because of the susceptibility to pathogen attacks, since brood production during founding in *T. septentrionalis* comes primarily from the fungus garden (Seal and Tschinkel, 2007). In claustral species, time spent out of the nest is reduced entirely. *Atta colombica* foundresses can even rear brood in the absence of a fungus garden (Fernández-Marín and Wcislo, 2005). Therefore, an advantage of having a fatter body is to reduce time spent out of the nest and in creating an independence from the fungus garden during colony founding.

Notable absences in the data include several ‘paleoattine’ genera (e.g., *Mycocepurus*, *Myrmecocrypta*, and *Apterostigma*; Schultz and Brady, 2008). Inclusion of samples from these species would be particularly enlightening for our understanding of the origin and early evolution of semi-claustral founding in these ants. The data for only two *Cyphomyrmex* species, both of which belong to clades differing in their type of fungal cultivar and associated pathogens (Schultz and Brady, 2008) also weakens the conclusions. Nevertheless this study reveals that Schultz and Brady’s (2008) phylogeny provides a framework that can be used to link aspects of one life-history variable (e.g., queen size) with aspects of colony size, energetics, among other ‘sociometric’ traits (Tschinkel, 1991) in the quest of understanding the ‘as yet almost unattempted enterprise’ (Bourke and Franks, 1995) of social insect life history evolution.

Acknowledgments I thank Hermogenes Fernández-Marín for his generosity in taking the time to show how and where to find and identify the ants. Koos Boomsma, Joshua King, Ulrich Mueller and an anonymous reviewer provided helpful advice and criticisms that greatly improved this manuscript. Koos Boomsma also provided help in identifying species of *Acromyrmex* and *Trachymyrmex* and access to unpublished data. Adrian A. Smith gave up some of his future vision acuity by looking for dark brown ants on a dark brown clay rainforest floor. Kevin Haight made sure the extracting apparatus did not explode and Betty Gaffney donated ether. Walter Tschinkel provided space and advice. Marc Seid and Bill Wcislo helped with logistics. Jim Hunt and Fran James provided useful comments during the planning phase of this project. Orelis E. Arosemena and the Autoridad Nacional del Ambiente de Panamá facilitated and granted the necessary collecting and export permits. Financially, this project was supported by Dr. Horace Loftin and his endowment for tropical biology at Florida State University, a Short Term Fellowship from the Smithsonian Tropical Research Institute and a grant from the Theodore Roosevelt Memorial Fund/The American Museum of Natural History.

References

- Boomsma J.J. and Isaaks J.A. 1985. Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **18**: 19–27
- Boomsma J.J., Keller L. and Nielsen M.G. 1995. A comparative analysis of sex ratio investment parameters in ants. *Funct. Ecol.* **9**: 743–753
- Bourke A.F.G. and Franks N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, NJ. 529 pp
- Brown M.J.F. 1999. Semi-claustral founding and worker behaviour in gynes of *Messor andrei*. *Insect. Soc.* **46**: 194–195
- Brown M.J.F. and Bonhoeffer S. 2003. On the evolution of claustral colony founding in ants. *Evol. Ecol. Res.* **5**: 305–313
- Currie C.R., Bot A.N.M. and Boomsma J.J. 2003. Experimental evidence of a tripartite mutualism: bacteria protect ant fungus gardens from specialized parasites. *Oikos* **101**: 91–102
- Currie C.R., Mueller U.G. and Malloch D. 1999. The agricultural pathology of ant fungus gardens. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 7998–8002
- Currie C.R., Poulsen M., Mendenhall J., Boomsma J.J. and Billen J. 2006. Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Science* **311**: 81–83
- Dijkstra M.B. and de Fine Licht H.H. 2007. Non leaf-cutting attine ants in Gamboa (Panama). Unpublished Key, Copenhagen, Denmark
- Farji Brener A.G. and Ruggiero A. 1994. Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range size. *J. Biogeogr.* **21**: 391–399
- Fernández-Marín H. and Wcislo W.T. 2005. Production of minima workers by gynes of *Atta colombica* Guérin-Ménéville (Hymenoptera: Formicidae: Attini) that lack a fungal pellet. *J. Kansas Entomol. Soc.* **78**: 290–292
- Fernández-Marín H., Zimmerman J.K. and Wcislo W.T. 2004. Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). *Biol. J. Linn. Soc.* **81**: 39–48
- Hahn D.A., Johnson R.A., Buck N.A. and Wheeler D.E. 2004. Storage protein content as a functional marker for colony-founding strategies: a comparative study within the harvester ant genus *Pogonomyrmex*. *Physiol. Biochem. Zool.* **77**: 100–108
- Harper J.L. 1977. *Population Biology of Plants*. Academic Press, San Diego, California. 892 pp

- Harvey P.H. and Pagel M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford. 239 pp
- Haskins C.P. and Haskins E.F. 1955. The pattern of colony foundation in the archaic ant *Myrmecia regularis*. *Insect. Soc.* **2**: 115–125
- Heinze J. and Tsuji K. 1995. Ant reproductive strategies. *Res. Pop. Ecol.* **37**: 135–149
- Hendrickx F., Maelfait J.P., Speelmans M. and van Straalen N.M. 2003. Adaptive reproductive variation along a pollution gradient in a wolf spider. *Oecologia* **134**: 189–194
- Hölldobler B. and Wilson E.O. 1990. *The Ants*. Harvard University Press, Cambridge, MA. 746 pp
- Howard K.J. 2006. Three queen morphs with alternative nest-founding behaviors in the ant, *Temnothorax longispinosus*. *Insect. Soc.* **53**: 480–488
- Jakobsson A. and Eriksson O. 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos* **88**: 494–502
- Johnson R.A. 2002. Semi-claustral colony founding in the seed-harvester ant *Pogonomyrmex californicus*: a comparative analysis of colony founding strategies. *Oecologia* **132**: 60–67
- Johnson R.A. 2006. Capital and income breeding and the evolution of colony founding strategies in ants. *Insect. Soc.* **53**: 316–322
- Jönsson K.I. 1997. Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos* **78**: 57–66
- Keller L. and Passera L. 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). *Oecologia* **80**: 236–240
- LaPointe S.L., Serrano M.S. and Jones P.G. 1998. Microgeographic and vertical distribution of *Acromyrmex landolti* (Hymenoptera: Formicidae) nests in a neotropical savanna. *Environ. Entomol.* **27**: 636–641
- Lloyd D.G. 1987. Selection of offspring size at independence and other size- versus-number strategies. *Am. Nat.* **129**: 800–817
- McInnes D.A. and Tschinkel W.R. 1995. Queen dimorphism and reproductive strategies in the fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **36**: 367–375
- Murakami T., Higashi S. and Windsor D. 2000. Mating frequency, colony size, polyethism and sex ratio in fungus-growing ants (Attini). *Behav. Ecol. Sociobiol.* **48**: 276–284
- Peakin G.J. 1972. Aspects of productivity in *Tetramorium caespitum* L. *Ekol. Pol.* **20**: 55–63
- Peeters C. and Ito F. 2001. Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annu. Rev. Entomol.* **46**: 601–630
- Sartori M., Keller L., Thomas A.G.B. and Passera L. 1992. Flight energetics in relation to sexual differences in the mating behaviour of a mayfly, *Siphonurus aestivalis*. *Oecologia* **92**: 172–176
- Schultz T.R., Bekkevold D. and Boomsma J.J. 1998. *Acromyrmex insinuator* new species: an incipient social parasite of fungus-growing ants. *Insect. Soc.* **45**: 457–471
- Schultz T.R. and Brady S.G. 2008. Major evolutionary transitions in ant agriculture. *Proc. Natl. Acad. Sci. U.S.A.* **105**: 5435–5440
- Seal J.N. and Tschinkel W.R. 2006. Colony productivity of the fungus-gardening ant, *Trachymyrmex septentrionalis* McCook, in a Florida pine forest (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **99**: 673–682
- Seal J.N. and Tschinkel W.R. 2007. Energetics of newly mated queens and colony founding in the fungus-gardening ants *Cyphomyrmex rimosus* and *Trachymyrmex septentrionalis* (Hymenoptera: Formicidae). *Physiol. Entomol.* **32**: 8–15
- Seal J.N. and Tschinkel W.R. 2008. Food limitation in the fungus-gardening ant, *Trachymyrmex septentrionalis*. *Ecol. Entomol.* **33**: 597–607
- Shik J.Z. 2008. Ant colony size and the scaling of reproductive effort. *Funct. Ecol.* **22**: 674–681
- Sibly R., Calow P. and Smith R.H. 1988. Optimal size of seasonal breeders. *J. Theor. Biol.* **133**: 13–21
- Smith C.C. and Fretwell S.D. 1974. The optimal balance between size and number of offspring. *Am. Nat.* **108**: 499–506
- Smith C.R. and Tschinkel W.R. 2006. The sociometry and sociogenesis of reproduction in the Florida harvester ant, *Pogonomyrmex badius*. *J. Insect Sci.* **6**: 11
- Soxhlet F. 1879. Die Gewichtsanalytische Bestimmung des Milchfettes. *Polytech. J.* **232**: 461–465
- Statsoft 2003. STATISTICA (data analysis software system) Version 6.1. Tulsa, OK
- Stearns S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford. 262 pp
- Tschinkel W.R. 1987. Relationship between ovariole number and spermathecal sperm count in ant queens: a new allometry. *Ann. Entomol. Soc. Am.* **80**: 208–211
- Tschinkel W.R. 1991. Insect sociometry, a field in search of data. *Insect. Soc.* **38**: 77–82
- Tschinkel W.R. 1993. Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecol. Monogr.* **63**: 425–457
- Tschinkel W.R. 1996. A newly-discovered mode of colony founding among fire ants. *Insect. Soc.* **43**: 267–276
- Tschinkel W.R. 1999. Sociometry and sociogenesis of colony-level attributes of the Florida Harvester Ant (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **92**: 80–89
- Tschinkel W.R. 2006. *The Fire Ants*. Harvard University Press, Cambridge, MA. 723 pp
- Turnbull L.A., Rees M. and Crawley M.J. 1999. Seed mass and the competition/colonization trade-off: a sowing experiment. *J. Ecol.* **87**: 899–912
- Weber N.A. 1972. *Gardening Ants: the Attines*. American Philosophical Society, Philadelphia, PA. 146 pp
- Wheeler D.E. and Buck N.A. 1995. Storage proteins in ants during development and colony founding. *J. Insect. Physiol.* **41**: 885–894
- Williams G.C. 1975. *Sex and Evolution*. Princeton University Press, Princeton, New Jersey. 200 pp
- Wirth R., Herz H., Ryel R.J., Beyschlag W. and Hölldobler B. 2003. *Herbivory of Leaf-Cutting Ants: A Case Study on Atta colombica in the Tropical Rainforest of Panama*. Springer Verlag, Berlin, Germany. 230 pp