

Energetics of newly-mated queens and colony founding in the fungus-gardening ants *Cyphomyrmex rimosus* and *Trachymyrmex septentrionalis* (Hymenoptera: Formicidae)

JON N. SEAL and WALTER R. TSCHINKEL

Department of Biological Science, Florida State University, Tallahassee, Florida, U.S.A.

Abstract. The energetics of colony founding is investigated in the fungus gardening ants (Attini) *Trachymyrmex septentrionalis* and *Cyphomyrmex rimosus*. Similar to most ants, inseminated queens of these two species found nests independently unaccompanied by workers (haplometrosis). Whereas most ant founding queens seal themselves in a chamber and do not feed when producing a brood entirely from metabolic stores (claustral founding), the majority of fungus gardening ants must forage during the founding phase (semiclaustral founding). Laboratory-reared *T. septentrionalis* individuals comprise 84 dealate females collected after mating flights in June 2004. Twenty are immediately killed to obtain values for queen traits and another 20 after worker emergence for queen, fungus garden and worker traits. *Cyphomyrmex rimosus* comprise 22 dealate females collected in June 2005; ten of which are immediately killed and similarly prepared. Newly-mated *T. septentrionalis* queens have 25% of their dry weight as fat; whereas newly-mated *C. rimosus* queens contain 11% fat. These amounts are 50–75% less than most independently founding ant species. *Trachymyrmex septentrionalis* queens lose merely 5% of their energetic content during colony founding, whereas the total energetic content of their brood is more than three-fold the amount lost by the queen. Incipient *T. septentrionalis* colonies produce approximately half as much ant biomass per gram of fungus garden as do mature colonies. Similar to most ants, *T. septentrionalis* produces minim workers that are approximately 40% lighter than workers from mature colonies. Regardless of their size, *T. septentrionalis* workers contain much lower fat than do workers of claustral species. These data indicate that fungus gardening is adaptive because colonies can produce much cheaper offspring, making colony investment much lower.

Key words. Attini, claustral founding, *Cyphomyrmex rimosus*, Florida, fungus gardening ant, introduced species, queen fat content, semiclaustral founding, *Trachymyrmex septentrionalis*.

Introduction

Social insect colonies experience the highest mortality of their life cycle during the founding phase (Hölldobler & Wilson, 1990; Hunt, 1991), which is thought to be a major factor in the evolution of colony founding mode (Jeanne, 1991; Bourke & Franks, 1995; Heinze & Tsuji, 1995).

Similar to most social insects, ants reproduce by independent founding, where individual foundresses or small groups of foundresses start their colony without the help of workers (Hölldobler & Wilson, 1990; Bourke & Franks, 1995). The majority of ants have elaborated on this scheme by founding nests claustrally; the queen seals herself in a chamber and uses her stored body reserves to rear the first generation of workers (Hölldobler & Wilson, 1990). Alternatively, in semiclaustral founding, the queen supplements her stored body reserves by foraging for food that she uses to rear her first workers. This is most common in 'primitive' ants, especially ants in the subfamilies Ponerinae and Myrmeciinae

Correspondence: Jon N. Seal, Department of Biological Science, Florida State University, Tallahassee, Florida 32306-4370, U.S.A. Tel.: +1 850 644 9811; fax: +1 850 644 0481; e-mail: seal@bio.fsu.edu

(Haskins & Haskins, 1955), but it also occurs in some members of the more derived subfamily Myrmecinae (Brown, 1999; Johnson, 2002; Fernández-Marín *et al.*, 2004).

Claustral founding is thought to evolve when founding queen mortality is high enough to offset the increased cost of producing claustral queens (Brown & Bonhoeffer, 2003). Claustral queens are more costly for a parent colony than nonclaustral founders because, relative to nonclaustral founding queens, they require larger amounts of lipids, carbohydrates (Keller & Passera, 1989) and storage protein (McInnes & Tschinkel, 1995; Wheeler & Buck, 1995; Tschinkel, 1996; Johnson, 2002; Hahn *et al.*, 2004). The first generation workers of claustral founders are invariably smaller than workers in a mature colony (Hölldobler & Wilson, 1990). These small-bodied (minim) workers result from a size vs. number trade-off. The number of first generation of workers is much more important than their quality (size) in establishing a functional colony (Porter & Tschinkel, 1986). Therefore, these workers are as small as they can be, although still capable of performing the various behaviours necessary for colony persistence and growth (Haskins & Haskins, 1955; Porter & Tschinkel, 1986; Hölldobler & Wilson, 1990). This trade-off permits a queen with a fixed energy budget to produce more individual workers.

All of the fungus gardening ants (Myrmicinae: Attini) exhibit semiclaustral founding, except for those in the most derived genus *Atta* (Weber, 1972; Fernández-Marín *et al.*, 2004). Attines are obligately dependent on specific Basidiomycete fungi (Agaricales: Lepiotaceae) found only in association with these ants (Martin, 1987; Mueller *et al.*, 1998). Founding queens place a small piece of the parental colony's fungus garden in their infra-buccal pocket before mating flights (Quinlan & Cherrett, 1978; Mueller *et al.*, 2001). After the mating flights, the inseminated queen drops to the ground and excavates a small chamber where she regurgitates the pellet, which serves as the inoculum for the fungus garden (Quinlan & Cherrett, 1978; Fernández-Marín *et al.*, 2004). Most attines probably rear the first workers on this small fungus garden (Weber, 1972). Exceptions include the genus *Atta* and some species of *Acromyrmex* that appear to rear workers entirely on trophic eggs (von Ihering, 1898; Huber, 1905; Diehl-Fleig & de Araújo, 1996; Fernández-Marín & Wcislo, 2005).

In the present study, the traits of foundresses are described in two species of fungus-gardening ant: *Cyphomyrmex rimosus* Spinola and *Trachymyrmex septentrionalis* McCook. *Cyphomyrmex* is a 'lower' (basal) attine genus whereas the genus *Trachymyrmex* is a 'higher' attine, basal to the lineage containing the highly derived leaf-cutting ants (Mueller *et al.*, 1998; Wetterer *et al.*, 1998). The study has two objectives. First, founding queen traits of these two species are compared and interpreted in the context of the phylogeny of the Attini. Second, data are presented on the relationships among foundresses, fungus gardens and workers of incipient colonies of *T. septentrionalis*. Finally, the transition that this higher attine undergoes during colony ontogeny is explored by comparing the size of workers and the productivity of fungus gardens of incipient and mature colonies of *T. septentrionalis*.

Materials and methods

Trachymyrmex septentrionalis is a common ant in sandy soils of eastern North America, occupying a region that extends to 40°N (Weber, 1970). This species is among the most abundant ants in longleaf pine-turkey oak sandhills of the Apalachicola National Forest of northern Florida, which may contain over 1000 nests per hectare (Seal & Tschinkel, 2006). The soil is characterized by deep, almost pure quartz sand entisols that contain very little organic matter (Brown *et al.*, 1990). *Cyphomyrmex rimosus* is a widespread introduced species found throughout the south-eastern coastal plain (Deyrup, 2000) in a variety of urban and woodland habitats. To a certain extent, it is sympatric with *T. septentrionalis* in the Apalachicola National Forest, although it appears to be more abundant in the wetter 'flatwoods' where *T. septentrionalis* is largely absent (Lubertazzi, 2003). Similar to most ants in this area, both species produce sexuals during the spring (March to May) and release their sexuals for mating flights by the end of May or early June. Mating flights appear to be highly synchronized events that largely take place over the course of a few days; dealate females become difficult to find by July. The weeks immediately preceding mating flights are characterized by an almost rainless period that marks the transition from rainfall derived from frontal boundaries to convective monsoon-like afternoon rains (Chen & Gerber, 1990). Thus, by the first heavy rain, the sexuals have matured and are ready to fly.

Eighty-four dealate *T. septentrionalis* queens were collected 3–5 June 2004 between 10.00 and 13.00 h from a single site within the Apalachicola National Forest (30°22.56'N, 84°22.01'W). Mating flights were observed approximately 1 h before the first dealate was collected. Twenty-two dealate *C. rimosus* queens were collected from the same site, at approximately the same time of day, on 13–14 June 2005. All of these queens were collected along the edge of a mixed pine-oak secondary growth stand and a relatively barren and mowed power and gas pipeline easement that is approximately 45 m wide at this location. The queens were collected within 2 m of one edge in a strip 75 m long (150 m²). This same area harboured 37 adult colonies of *T. septentrionalis* and at least three *C. rimosus* colonies.

Within hours of their collection, the queens were taken to the laboratory in a cooler, chilled briefly (4 °C) and weighed live on a microbalance. Twenty *T. septentrionalis* and ten *C. rimosus* queens were killed, oven dried and their body fat extracted for 24 h in a Soxhlet extractor column (Soxhlet, 1879) using diethyl ether. This procedure estimates queen condition (fresh, dry, lean and fat weights) immediately after their mating flights. Energetic contents of ants were obtained by multiplying lean weights by 18.87 J mg⁻¹ and fat weights by 39.33 J mg⁻¹ (Peakin, 1972).

The remaining live queens were placed in individual test tubes partly filled with water behind a cotton plug and placed in a room kept at 24.5–25 °C, the optimal temperature for the growth of their fungus (Powell & Stradling, 1986). The queens were offered substrates *ad libitum* at least twice weekly. The substrates consisted of frass from eastern

tent caterpillars [*Malacosoma americanum* Fabricius (Lasiocampidae)] and tussock moth caterpillars [*Orgyia detrita* Guérin-Méneville (Lymantriidae)] reared on black cherry (*Prunus serotina* Ehrh.) and oak (*Quercus virginiana* Mill. and *Q. nigra* L.) leaves, respectively. Queens were also supplied with catkins (staminate flowers) from oak trees (*Quercus laevis* Walter and *Q. laurifolia* Michx.). When given a choice, mature colonies prefer caterpillar frass and oak catkins (Seal, 2006). At weekly intervals, unused substrates and fungus garden refuse were removed from each tube. The ants were supplied with a new test tube when mould appeared on the glass or unused substrates. Workers emerged by mid-July, after which colonies were not fed. At the end of July 2004, 20 incipient *T. septentrionalis* colonies were randomly selected and killed by freezing. The contents of each tube were sorted (queen, workers, brood, fungus garden), dried, weighed and their fat extracted. The energetic value of each incipient colony was calculated from these data, as described above. Of the 12 *C. rimosus* queens not immediately killed, only three were observed to cultivate yeast on supplied frass pieces, and the remaining nine were dead by 10 July 2005. For this reason, the remainder of the data presented here concern only *T. septentrionalis*.

In nature, incipient queens start their fungus garden by re-gurgitating an inoculum contained in their infrabuccal pocket (von Ihering, 1898; Quinlan & Cherrett, 1978). To prevent founding failure due to inoculum loss, which commonly occurs under laboratory conditions (J. N. Seal, unpublished data), each *T. septentrionalis* queen was provided with 0.1 mg healthy fungus garden from a laboratory colony. Because fungus gardens of incipient queens weigh on average 35 mg (see results), this inoculum amounts to only 1 : 350 of the garden that the queen eventually produces.

Data analysis followed a pathway of inferred causation. First, traits (dry, fat and lean weight, and energetic value) of newly-mated *T. septentrionalis* queens were compared with those of queens that had produced workers. Then, whether initial (postmating flight) fresh weight predicted the weight of the fungus garden and the traits of the workers (worker mean dry, lean and fat weight, and worker energetic value) was tested. At the next step, whether the weight of the fungus garden predicted worker traits was examined. Finally, the traits of workers from these incipient colonies were compared with those from field and laboratory colonies. Nine *T. septentrionalis* laboratory colonies had been fed a mixture of the above substrates *ad libitum* in the spring of 2003 and 19 unmanipulated field colonies were collected in June 2003. Nineteen of these (laboratory and field) colonies were considered mature because they produced sexuals, and provided production and energetic values for sexuals in addition to new workers. Nine colonies of these colonies were designated as immature because they produced only worker brood (J. N. Seal unpublished data). Ten dark (old) workers (Tschinkel, 1999) were randomly selected from each of these colonies, weighed individually, denuded of their fat and reweighed.

Data were \log_{10} -transformed and analysed with analyses of variance and linear regression using Statistica version 6.1 (Statsoft, 2003). To examine allometric patterns, simple

t-tests were used to test whether slopes differed from 1.0. Analysis of covariance was used to test for heterogeneity of slopes. When parametric assumptions were not met, nonparametric tests were employed. Percentages were arcsine transformed (Sokal & Rohlf, 1995). Power analyses were also conducted using G-Power (Faul & Erdfelder, 1992) on tests that yielded low *P*-values, but were otherwise > 0.05 .

Results

Newly-mated queen traits

Newly-mated *T. septentrionalis* queens were more than three-fold larger than *C. rimosus* queens in several measures (Table 1). The live weight of a newly-mated queen was an accurate indicator of her condition because it was positively correlated with dry weight, lean weight, fat and energetic content (Table 2). Larger queens of both species were disproportionately energetically more expensive to produce than smaller queens because the linear relationship between dry weight and energetic content was positively allometric on a log-log scale (*T. septentrionalis*: $r^2 = 0.95$, $\log_{10} J = 1.341 + 1.181 (\log_{10} \text{dry weight})$, $H_0: B1 = 1$, $t_{18} = 3.15$, $P < 0.01$; *C. rimosus*: $r^2 = 0.95$, $\log_{10} J = 1.4301 + 1.2784 (\log_{10} \text{dry weight})$, $H_0: B1 = 1$, $t_8 = 2.572$, $P < 0.05$). Larger queens of both species were also disproportionately more energetically expensive than smaller queens because queen dry weight and queen fat were positively allometric (*T. septentrionalis*: $r^2 = 0.72$, $B1 = 1.87 \pm 0.27$; $H_0: B1 = 1$, $t_{18} = 3.19$, $P < 0.001$; *C. rimosus*: $r^2 = 0.67$, $B1 = 3.055 \pm 0.804$; $H_0: B1 = 1$, $t_{18} = 2.56$, $P < 0.05$; Fig. 1). From a different perspective, total dry weight and lean weight exhibit negative allometry (*T. septentrionalis*: $r^2 = 0.78$, $B1 = 0.72 \pm 0.09$; $H_0: B1 = 1$, $t_{18} = 3.12$, $P < 0.001$; *C. rimosus*: $r^2 = 0.78$, $B1 = 0.671 \pm 0.125$; $H_0: B1 = 1$, $t_{18} = 5.37$, $P < 0.01$). As queen dry weight of both species increases, lean weight increases by only approximately one half as much.

Queen traits after worker-emergence

Of the 64 *T. septentrionalis* queens that were not immediately killed, 34 survived to produce workers, one survived

Table 1. Weights (mg), percent fat (dry weight) and energetic content (J) of newly-mated queens of *Cyphomyrmex rimosus* and *Trachymyrmex septentrionalis*.

	<i>Cyphomyrmex rimosus</i>	<i>Trachymyrmex septentrionalis</i>
Fresh (live) weight (mg)	1.03 ± 0.04	3.34 ± 0.04
Dry weight (mg)	0.41 ± 0.01	1.79 ± 0.03
Lean weight (mg)	0.363 ± 0.011	1.33 ± 0.016
Fat content (mg)	0.045 ± 0.01	0.456 ± 0.164
Percent Fat (mg)	10.8 ± 1.5	25.4 ± 0.63
Energetic content (J)	8.58 ± 0.40	43.02 ± 0.87

Data are mean ± SE.

Table 2. Correlation coefficients between fresh weights obtained immediately after collection and traits (dry and lean weights, fat and energetic contents) of queens and brood of both collection times.

Dependent measure	Postmating flight <i>C. rimosus</i> queens	Postmating flight <i>T. septentrionalis</i> queens	Postworker emergence <i>T. septentrionalis</i> queens
Dry weight	0.73**	0.86***	0.23, ns
Lean weight	0.79**	0.91***	0.67**
Fat content	0.41, ns	0.56*	-0.47*
Energetic content	0.64*	0.77***	0.01, ns
Brood weight	NA	NA	0.26, ns
Total ant production	NA	NA	0.35, ns
Mean worker weight	NA	NA	-0.16, ns
lean weight.	NA	NA	-0.26, ns
Fat content	NA	NA	0.26, ns
Energetic content	NA	NA	0.08, ns
Energetic content	NA	NA	0.37, ns
Fungus garden weight	NA	NA	-0.07, ns

The data were log transformed.

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns, $P > 0.05$.

but did not produce any workers and three died after producing workers. The fresh weights of postmating flight queens did not appear to be a factor in founding success. Survival to worker production was not related to initial queen live weight: successful queens weighed 3.37 ± 0.152 mg, whereas those dying prior to reproduction weighed 3.47 ± 0.30 mg (Mann-Whitney $U = 375$, $P > 0.10$). Live weights taken after mating flights were not strong predictors of postworker-emergence queen traits (Table 2). The single exception was lean weight and perhaps queen fat content. Queens that were heavier before the production of brood tended to have higher lean weights after ($r = 0.67$, $P < 0.01$) but their fat content was lower ($r = -0.47$, $P < 0.05$; Table 2).

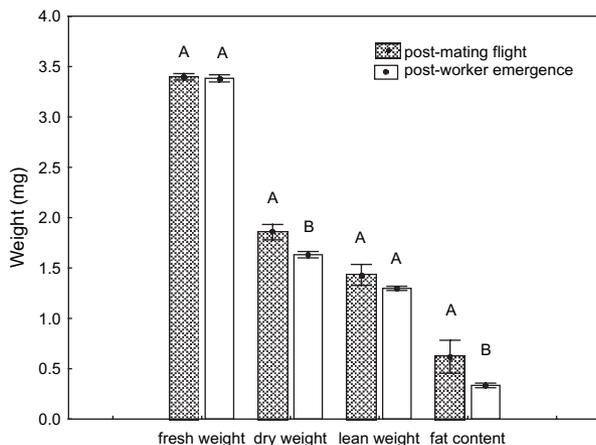


Fig. 1. Fresh, dry and lean weights, and fat content of *Trachymyrmex septentrionalis* queens. Error bars indicate standard errors. Shaded bars indicate postmating flight queens and open bars indicate post-emergence queens. Significant differences are denoted by different letters ($P < 0.05$, t -tests).

Initial queen live weight was not correlated with traits of offspring or worker biomass (Table 2). However correlations with total ant production ($r = 0.35$, $P > 0.14$) and total energetic content of workers ($r = 0.37$, $P > 0.11$) had low power and therefore a high probability ($\alpha > 0.5$) of making a type II error (concluding that there is no trend when one in fact exists).

The linear relationship between final queen dry weight and final fat content was not different from the slope for newly-mated queens ($F_{1,36} = 0.966$, $P > 0.33$; Fig. 2). Specifically, it was positively allometric ($r^2 = 0.61$, $B1 = 2.41 \pm 0.46$; $H_0: B1 = 1$, $t_{18} = 3.09$, $P < 0.01$) and not significantly different from 2 ($H_0: B1 = 2$, $t_{18} = 0.899$, $P > 0.38$; Fig. 2). Therefore, queens lost an amount of fat proportional to their

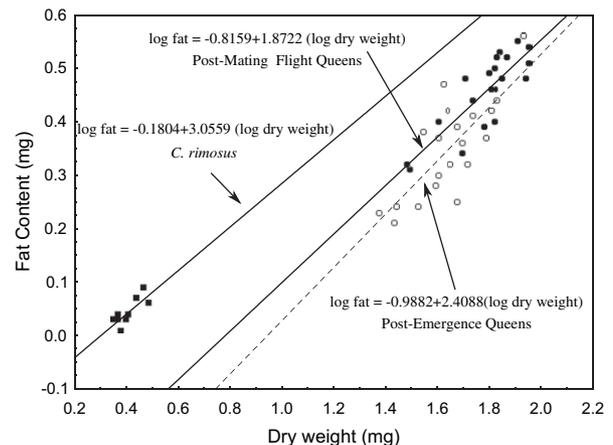


Fig. 2. Linear plot of the fat content and dry weight of postmating-flight queens and postemergence queens. Data were log-transformed. ■, *Cyphomyrmex rimosus* queens; ●, postmating flight *Trachymyrmex septentrionalis* queens (solid line); ○, postworker emergence *Trachymyrmex septentrionalis* queens (dashed line).

body weight by the time that they grew a garden and produced brood.

By the time that queens produced their first workers, their amount of body fat fell from $25.4 \pm 3\%$ (percent dry weight \pm SE) to $21 \pm 4\%$ or 80% of the original content (Mann-Whitney $U = 83$, $P < 0.001$). This fat loss accounted for the 5% loss of total dry weight ($t_{38} = 3.17$, $P < 0.01$) because lean weights did not change significantly ($t_{38} = 1.24$, $P > 0.22$; Fig. 1). There was also a significant decrease in the queens' energetic value (Fig. 1). By the time that she had produced brood, the average queen contained 38.2 ± 0.96 J, which is a net loss of 5.2 J ($t_{38} = 3.68$, $P < 0.001$). This loss was much less than the average energetic value of brood (19.69 ± 2.43 J) (Mann-Whitney $U = 24$, $P < 0.001$). Therefore, the energetic content of brood is almost four-fold greater than the amount of energy lost by the queen. It should be noted that this probably represents a conservative estimate because only fully eclosed workers were used to estimate energetics and many nests contained larvae and pupae.

Comparisons with older *T. septentrionalis* colonies

Trachymyrmex septentrionalis colonies increase substantially in size during the transition from the incipient stage to sexual maturity. The colony size appears to increase from an average of three workers and 35 mg of fungus garden in incipient colonies to more than 200 workers and > 4 g of fungus garden in mature colonies (Table 3). Workers from incipient colonies were significantly smaller (0.29 ± 0.09 mg) and weighed significantly less than those from mature (0.50 ± 0.08 mg) and immature colonies (0.50 ± 0.06 mg; $F_{2,44} = 38.93$, $P < 0.001$; Fig. 3). Incipient workers had significantly lower lean weights (incipient: 0.25 ± 0.07 mg vs. 0.43 ± 0.06 mg (mature) and 0.44 ± 0.06 mg (immature); $F_{2,44} = 49.4$, $P < 0.001$) but similar amounts of fat (0.06 ± 0.05 mg; $F_{2,44} = 0.586$, $P > 0.55$) compared with workers from immature and mature colonies (0.06 ± 0.02 mg and 0.07 ± 0.03 mg, respectively) (Fig. 3). Therefore, incipient workers had the highest percent fat (19 vs. 12% for immature and mature colonies combined) because their bodies were smaller ($F_{2,43} = 3.91$, $P < 0.05$). Nonetheless, workers from incipient colonies had the lowest energetic value and were cheaper to produce (6.87 ± 0.609 J vs. 10.89 ± 0.394 J and 10.53 ± 0.495 J for mature and immature colonies, respectively, $F_{2,45} = 18.98$, $P < 0.001$).

The productivity of incipient colonies appeared to be lower than to those of larger colonies. Immature and mature colonies (henceforth 'older' colonies) were pooled because the

Table 3. Fungus garden weights and worker number of incipient and older colonies of *Trachymyrmex septentrionalis*.

	Incipient	Immature	Mature
Fungus garden weight (g)	0.034 ± 0.02	2.54 ± 1.7	4.81 ± 2.4
Worker number	3 ± 2	160 ± 106	223 ± 160

Data are mean \pm SE.

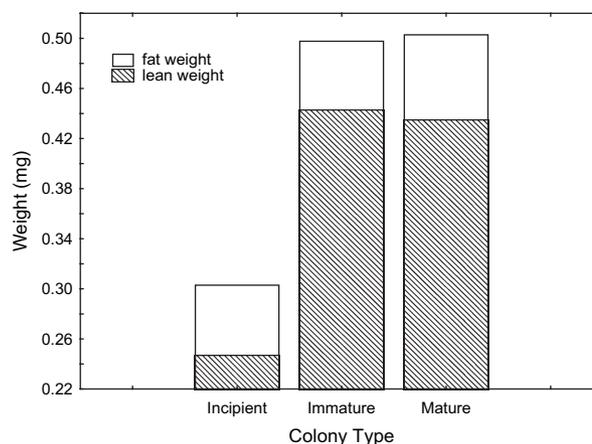


Fig. 3. Means of lean weights and fat content of *Trachymyrmex septentrionalis* workers by colony type. The open area indicates fat content and the hatched area indicates lean weights.

small number of immature colonies ruled out the use of analyses of covariance (Sokal & Rohlf, 1995). The rates of ant production relative to fungus garden weight for older colonies appeared different than for incipient colonies ($F_{1,41} = 4.33$, $P < 0.05$, Fig. 4). Clearly, there is a positive slope among the older colonies (1.095 ± 0.162 , $r^2 = 0.65$, $P < 0.001$) that was not significantly different from 1.0 ($H_0: B1 = 1$, $t_{25} = 0.585$, two-tailed $P > 0.55$). The slope among the incipient colonies (0.511 ± 0.237 , $r^2 = 0.22$, $P < 0.05$, Fig. 4) was significantly less than 1.0 ($H_0: B1 = 1$, $t_{16} = 2.06$, one-tailed $P < 0.05$). This suggests that, even though a proportional change in fungus garden weight in an older colony results in a proportional change in the amount of ant production, a similar increase in fungus garden size of an incipient colony may result in a less-than-proportional increase in ant production.

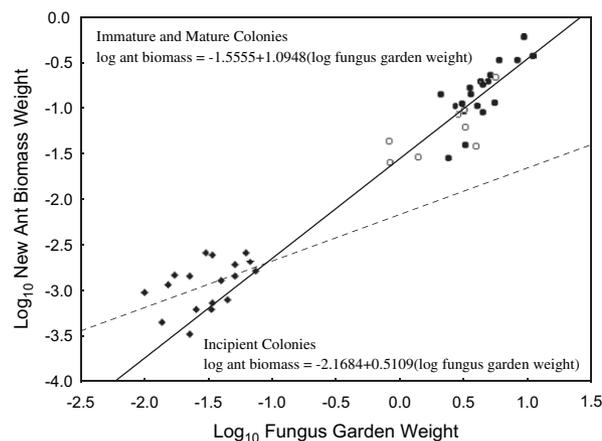


Fig. 4. Fungus garden weight in relation to total ant production for the three *Trachymyrmex septentrionalis* colony types. Data were \log_{10} -transformed. \blacklozenge , Incipient colonies; \circ , immature colonies; \bullet , mature colonies.

Discussion

The present study indicates that the principle adaptation in the early evolution of fungus-gardening is the ability to produce small and energetically cheap offspring. The use of fungus garden during colony founding allows the fat contents of *C. rimosus* (11%) and *T. septentrionalis* queens (25%) to be much lower than those of other semiclaustral-founding ant species. Keller & Passera (1989) report a fat content of 43% and 46% for *Manica rubida* and *Leptothorax nylanderi*, respectively, and Johnson (2002) reports 30–35% for *Pogonomyrmex californicus*. Similarly, founding queens of the socially parasitic form of the fire ant (*Solenopsis invicta* and *Solenopsis geminata*) contain 35–40% fat compared with fully claustral queens that contain 45–50% fat (McInnes & Tschinkel, 1995; Tschinkel, 1996). Therefore, brood production in the two species reported in the present study appears to be heavily dependant on the performance of their fungus garden, and not simply augmented by queen foraging activity as in other semiclaustral founders (Johnson, 2002). Indeed, the energetic content of brood is much higher than the amount of energy lost by the queens. The origin of this additional energy is the fungus garden. Additionally, *T. septentrionalis* workers do not appear to serve as food reservoirs to be used by fellow nest-mates, as often occurs in other social insects (Tschinkel, 1987; Cassill & Tschinkel, 1999). *Trachymyrmex septentrionalis* workers from mature colonies contain less than 15% body fat, which is considerably lower than the 40–60% reported by Tschinkel (1993) for *Solenopsis invicta*. It appears then that the *T. septentrionalis* fungus garden nourishes the ants and brood during all stages of colony ontogeny.

The low productivity of incipient *T. septentrionalis* colonies reported in the present study might be explained by the limits of a single ant (the queen) maintaining a healthy fungus garden. The queen must perform all the tasks of mature colonies, such as foraging and preparing substrates (Quinlan & Cherrett, 1977), as well as tending to the garden (Bass & Cherrett, 1994, 1996). Ant fungus gardens are susceptible to an exceptionally virulent fungal pathogen (*Escovopsis* spp.), which kills and consumes ant fungi and leads to colony failure (Currie *et al.*, 1999, 2003). This pathogen only becomes detectable in incipient *Atta colombica* colonies once workers emerge, apparently because they bring in spores when foraging (Currie *et al.*, 1999). In the case of semiclaustral attines, such as *T. septentrionalis*, the queen foraging period may be minimized to lower the chance of *Escovopsis* exposure and other sources of mortality but still allow construction of a garden large enough to produce some workers. In other words, founding queens might sacrifice garden productivity if it increases the chances of producing workers without an *Escovopsis* outbreak. This is supported by the observation that none of the incipient colonies lose their gardens to this pathogen, whereas five of the remaining 11 *T. septentrionalis* colonies (from queens collected in 2004 but otherwise not part of the study) lose their gardens to *Escovopsis* in the period July 2005 to January 2006, more than 1 year after workers first emerge from this cohort.

Although the evolution of fungus gardening probably releases colonies from the constraint of producing fat females necessary for claustral founding, the tribe is characterized by a pronounced increase in body size and a return to claustral founding in the most derived genera (Hölldobler & Wilson, 1990; Mueller *et al.*, 1998). The most derived attines are the leaf-cutting ants, among which are the largest queens on earth; *Atta* queens typically weigh 400–800 mg (Mintzer, 1987, 1990), more than two-fold greater than *T. septentrionalis* or *C. rimosus* queens. The apparent similarity of the slopes of queen fat content and dry weight across these two species suggests a common allometric relationship between queen fat and body size. Allometric growth patterns are characteristic of holometabolous insects, and they are thought to arise from trade-offs among imaginal disks for the fixed amount of food reserves obtained during the larval stage (Wheeler, 1986; Nijhout & Wheeler, 1996).

This allometry suggests that the deposition of fat in larval queens is constrained by body size. A ten-fold increase in dry weight results in a 100-fold increase in fat content, but the variation in dry weights for these species is quite low in magnitude (0.35–0.49 mg in *C. rimosus* and 1.48–1.79 mg in *T. septentrionalis*) and in biological relevance. Queen condition is virtually uncorrelated with founding success, fungus garden weight and offspring traits. Therefore, if there is direct selection for increased fat necessary for claustral founding (> 50% body fat; (Keller & Passera, 1989; McInnes & Tschinkel, 1995; Tschinkel, 1996; Johnson, 2002), there is an upper limit imposed by body size in these ants. The only attine queens known to be capable of producing offspring from metabolic stores, *Atta* and, to a limited extent, *Acromyrmex* (Diehl-Fleig & de Araújo, 1996), have the largest and perhaps the fattest bodies. Interpolating a 430 mg (dry weight) *Atta cephalotes* queen collected in Trinidad in July 2003 during a mating flight (J. N. Seal, unpublished data) onto the allometric curve in the present study results in a prediction of 180 mg of fat, or approximately 45% body fat, close to values expected for claustral founding species. That overall size constrains the deposition of body fat is supported by two additional accounts. Mintzer (1987) show that a small-queened species, *Atta texana*, exhibits higher success when founding nests in groups rather than independently. Additionally, Diehl-Fleig & de Araújo (1996) show that groups of *Acromyrmex striatus* foundresses do not forage but are essentially able to produce brood claustrally whereas single foundresses have to forage to produce brood. It would appear that small foundresses effectively pool their resources and produce brood from trophic eggs that they otherwise would not be able to do independently. This calls for a more comprehensive comparative analysis on founding biology across the entire tribe of fungus gardening ants.

Acknowledgements

We thank Joshua King and Kevin Haight who read earlier versions of this manuscript. Comments by two anonymous reviewers helped improve the manuscript. The USDA Forest

Service graciously allowed us to study in the Apalachicola National Forest. The project was carried out during the tenure of a Florida State University PEG (Program Enhancement Fund) Grant and NSF (0212085) Grant to W.R.T.

References

- Bass, M. & Cherrett, J.M. (1994) The role of leaf-cutting ant workers (Hymenoptera: Formicidae) in fungus garden maintenance. *Ecological Entomology*, **19**, 215–220.
- Bass, M. & Cherrett, J.M. (1996) Leaf-cutting ants (Formicidae, Attini) prune their fungus to increase and direct its productivity. *Functional Ecology*, **10**, 55–61.
- Bourke, A.F.G. & Franks, N.R. (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey.
- Brown, M.J.F. (1999) Semi-claustral founding and worker behaviour in gynes of *Messor andrei*. *Insectes Sociaux*, **46**, 194–195.
- Brown, M.J.F. & Bonhoeffer, S. (2003) On the evolution of claustral colony founding in ants. *Evolutionary Ecology Research*, **5**, 305–313.
- Brown, R.B., Stone, E.L. & Carlisle, V.W. (1990) Soils. *Ecosystems of Florida* (ed. by R. L. Myers and J. J. Ewel), pp. 35–69. University of Central Florida Press, Orlando, Florida.
- Cassill, D.L. & Tschinkel, W.R. (1999) Regulation of diet in the fire ant, *Solenopsis invicta*. *Journal of Insect Behavior*, **12**, 307–328.
- Chen, E. & Gerber, J.F. (1990) *Climate. Ecosystems of Florida* (ed. by R. L. Myers and J. J. Ewel), pp. 11–34. University of Central Florida Press, Orlando, Florida.
- Currie, C.R., Mueller, U.G. & Malloch, D. (1999) The agricultural pathology of ant fungus gardens. *Proceedings of the National Academy of Science of the U.S.A.*, **96**, 7998–8002.
- Currie, C.R., Wong, B., Stuart, A.E. *et al.* (2003) Ancient tripartite coevolution in the attine ant-microbe symbiosis. *Science*, **299**, 386–388.
- Deyrup, M.A. (2000) Exotic ants in Florida. *Transactions of the American Entomological Society*, **126**, 293–326.
- Diehl-Fleig, E. & de Araújo, A.M. (1996) Haplometrosis and pleometrosis in the ant *Acromyrmex striatus* (Hymenoptera: Formicidae). *Insectes Sociaux*, **43**, 47–51.
- Faul, F. & Erdfelder, E. (1992) *GPOWER: A Priori, Post-Hoc, and Compromise Power Analyses for MS-DOS*. Psychologisches Institut der Universität Bonn, Germany.
- Fernández-Marín, H. & Wcislo, W.T. (2005) Production of minima workers by gynes of *Atta colombica* Guérin-Ménéville (Hymenoptera: Formicidae: Attini) that lack a fungal pellet. *Journal of the Kansas Entomological Society*, **78**, 290–292.
- Fernández-Marín, H., Zimmerman, J.K. & Wcislo, W.T. (2004) Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). *Biological Journal of the Linnean Society*, **81**, 39–48.
- Hahn, D.A., Johnson, R.A., Buck, N.A. & Wheeler, D.E. (2004) Storage protein content as a functional marker for colony-founding strategies: a comparative study within the harvester ant genus *Pogonomyrmex*. *Physiological and Biochemical Zoology*, **77**, 100–108.
- Haskins, C.P. & Haskins, E.F. (1955) The pattern of colony foundation in the archaic ant *Myrmecia regularis*. *Insectes Sociaux*, **2**, 115–125.
- Heinze, J. & Tsuji, K. (1995) Ant reproductive strategies. *Researches on Population Ecology*, **37**, 135–149.
- Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Harvard University Press, Cambridge, Massachusetts.
- Huber, J. (1905) Über die Koloniengründung bei *Atta sexdens*. *Biologisches Centralblatt*, **25**, 606–635.
- Hunt, J.H. (1991) Nourishment and the evolution of the social Vespidae. *The Social Biology of Wasps* (ed. by K. G. Ross and R. W. Matthews), pp. 426–450. Cornell University Press, Ithaca, New York.
- von Ihering, H. (1898) Die Anlange neuer Kolonien und Pilzgärten bei *Atta sexdens*. *Zoologischer Anzeiger*, **21**, 238–245.
- Jeanne, R.L. (1991) The swarm-founding Polistinae. *The Social Biology of Wasps* (ed. by K. G. Ross and R. W. Matthews), pp. 191–231. Cornell University Press, Ithaca, New York.
- 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.
- Keller, L. & 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.
- Lubertazzi, D. (2003) Ant community change across a ground vegetation gradient in north Florida's longleaf pine flatwoods. *Journal of Insect Science*, **3**, 17.
- Martin, M.M. (1987) *Invertebrate-Microbial Interactions: Ingested Fungal Enzymes in Arthropod Biology* pp. 91–126. Cornell University Press, Ithaca, New York.
- McInnes, D.A. & Tschinkel, W.R. (1995) Queen dimorphism and reproductive strategies in the fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Behavioural Ecology and Sociobiology*, **36**, 367–375.
- Mintzer, A.C. (1987) Primary polygyny in the ant *Atta texana*: number and weight of females and colony foundation success in the laboratory. *Insectes Sociaux*, **34**, 108–117.
- Mintzer, A. (1990) Foundress female weight and cooperative foundation in *Atta* leaf-cutting ants. *Applied Myrmecology: A World Perspective* (ed. by R. K. Vander Meer, K. Jaffe and A. Cedeno), pp. 180–183. Westview Press, Boulder, Colorado.
- Mueller, U.G., Rehner, S.A. & Schulz, T.R. (1998) The evolution of agriculture in ants. *Science*, **281**, 2034–38.
- Mueller, U.G., Schultz, T.R., Currie, C.R. *et al.* (2001) The origin of the attine ant-fungus mutualism. *Quarterly Review of Biology*, **76**, 169–197.
- Nijhout, H.F. & Wheeler, D.E. (1996) Growth models of complex allometries in holometabolous insects. *The American Naturalist*, **148**, 40–56.
- Peakin, G.J. (1972) Aspects of productivity in *Tetramorium caespitum* L. *Ekologia Polska*, **20**, 55–63.
- Porter, S.D. & Tschinkel, W.R. (1986) Adaptive value of nanitic workers in newly founded red imported fire ant colonies (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, **79**, 723–726.
- Powell, R.J. & Stradling, D.J. (1986) Factors influencing the growth of *Attamyces bromatificus*, a symbiont of attine ants. *Transactions of the British Mycological Society*, **87**, 205–213.
- Quinlan, R.J. & Cherrett, J.M. (1977) The role of substrate preparation in the symbiosis between the leaf cutting ant *Acromyrmex octospinosus* (Reich) and its food fungus. *Ecological Entomology*, **2**, 161–170.
- Quinlan, R.J. & Cherrett, J.M. (1978) Studies on the role of the infrabuccal pocket of the leaf-cutting ant *Acromyrmex octospinosus* (Reich) (Hymenoptera: Formicidae). *Insectes Sociaux*, **25**, 237–245.
- Seal, J.N. (2006) *The functional ecology of the obligate mutualism between the higher attine Trachymyrmex septentrionalis and a symbiotic fungus*. PhD Thesis, Florida State University, Tallahassee, Florida.

- Seal, J.N. & Tschinkel, W.R. (2006) Colony productivity of the fungus-gardening ant, *Trachymyrmex septentrionalis* McCook, in a Florida pine forest (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, **99**, 673–682.
- Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*. WH Freeman, New York, New York.
- Statsoft (2003) *STATISTICA (Data Analysis Software System)*. Statsoft: Tulsa, Oklahoma.
- Soxhlet, F. (1879). Die Gewichtsanalytische Bestimmung des Milchfettes. *Polytechnisches J*, **232**, 461–465
- Tschinkel, W.R. (1987) Seasonal life history and nest architecture of a winter active ant, *Prenolepis imparis*. *Insectes Sociaux*, **34**, 143–164.
- Tschinkel, W.R. (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecological Monographs*, **63**, 425–457.
- Tschinkel, W.R. (1996) A newly-discovered mode of colony founding among fire ants. *Insectes Sociaux*, **43**, 267–276.
- Tschinkel, W.R. (1999) Sociometry and sociogenesis of colony-level attributes of the Florida harvester ant (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, **92**, 80–89.
- Weber, N.A. (1970) Northern extent of attine ants (Hymenoptera: Formicidae). *Proceedings of the Entomological Society of Washington*, **72**, 414–415.
- Weber, N.A. (1972) *Gardening Ants: The Attines*. American Philosophical Society, Philadelphia, Pennsylvania.
- Wetterer, J.K., Schultz, T.R. & Meier, R. (1998) Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. *Molecular Phylogenetics and Evolution*, **9**, 42–47.
- Wheeler, D.E. (1986) Developmental and physiological determinants of caste in social hymenoptera: evolutionary implications. *The American Naturalist*, **128**, 13–34.
- Wheeler, D.E. & Buck, N.A. (1995) Storage proteins in ants during development and colony founding. *Journal of Insect Physiology*, **41**, 885–894.

Accepted 24 May 2006

First published online 8 August 2006

Copyright of *Physiological Entomology* is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.