Food limitation in the fungus-gardening ant, *Trachymyrmex septentrionalis*

JON N. SEAL and WALTER R. TSCHINKEL   Department of Biological Science, Florida State University, Tallahassee, Florida, U.S.A.

Abstract. 1. Most ants are more like plants and marine invertebrates than other insects because the adults are sessile and the immature stages are motile.

2. This paper reported the results from a field experiment that increased food levels on the fungus gardening ant, *Trachymyrmex septentrionalis*, which has served as a model system for understanding the complexities of these obligate mutualisms.

3. Food supplementation increased the growth of the symbiotic fungus and this led to an increase in the amount of fungal biomass. The amount of fungal biomass was generally more important in determining the amount of ant offspring than colony size.

4. The results indicate that the population is food limited and colonies may compete for relatively rare fungal substrate. However, competition in this species is not obvious.

Key words. Attini, chitin, colony growth, competition, complex societies, leaf-cutting ant.

Introduction

Most social insect populations are unlike those of other insects because adult colonies are generally sessile entities and depend on motile forms to complete the sexual life cycle (Wilson, 1971; Hölldobler & Wilson, 1990). More like marine invertebrates, social insects exhibit indeterminate growth (Bourke & Franks, 1995) and thus grow and reproduce by making quantities of slightly different versions of a basic *body* plan. For example, colonies produce large and fat females that will become reproductives (queens) or smaller and lean females that will become workers (Wheeler, 1986; Wheeler, 1991; Hunt & Amdam, 2005). As colonies grow from a single foundress to a mature colony, they typically undergo a process known as sociogenesis (Wilson, 1983, 1985), the patterns of which are thought to reflect the outcome of natural selection operating at the level of the colony (Oster & Wilson, 1978; Tschinkel, 1993, 1999; Korb & Heinze, 2004; Tschinkel, 2006).

The performance of social insect colonies is often dependent on the quality of their immediate environment because their ability to move to a new one is relatively limited. Within ecological contexts, food supplementation experiments have been used to illustrate the proximate role of food in colony performance in taxa ranging from annual social wasps (Hunt & Dove,

2002; Seal & Hunt, 2004) to ants, which are all perennial (Deslippe, 1994; Herbers & Banschbach, 1998; Morales & Heithaus, 1998; Sorvari & Hakkarainen, 2007). In nearly all cases, added food causes colonies to produce more sexual biomass, but also because sexual and worker investment are traded off with each other over time in perennial species (Brian & Elmes, 1974; Brian *et al.*, 1981; Elmes & Wardlaw, 1982a; Cassill, 2002), the outcome may not be straightforward. Understanding how colonies partition food has implications in ecosystems where ants in particular are dominant or keystone members of their community (Hölldobler & Wilson, 1990; Tobin, 1994; Agosti *et al.*, 2000; Sorvari & Hakkarainen, 2005, 2007).

Integral to the success of many species of social insects are their mutualisms with microorganisms (Davidson *et al.*, 2003). The best studied of the social insect mutualisms are those between fungi and fungus gardening ants (tribe Attini)(Mueller & Gerardo, 2002). These ants have evolved an obligate symbiosis with specific strains of basidiomycete fungi, which they cultivate on items they collect while foraging (called *substrates*)(Weber, 1972). The fungus in turn breaks down these items using a wide spectrum of enzymes (Martin, 1987; D'Ettorre *et al.*, 2002; Richard *et al.*, 2005). These ants and their fungi have been the subject of phylogenetic analysis (Mueller *et al.*, 1998), behaviour (Roces, 2002; Seal & Tschinkel, 2007b), comparative studies on colony foundation (Fernández-Marín *et al.*, 2004), and ecology (Wirth *et al.*, 2003; Seal & Tschinkel, 2006). These ants are a remarkably successful group and inhabit a large zone

Correspondence: Jon N. Seal, Biologie I, Universität Regensburg, Universitätsstraße 31, D-93040 Regensburg, Germany. E-mail: jon.seal@biologie.uni-regensburg.de

in the Americas spanning from 40°N (Illinois and New York) to 45°S (Patagonia) (Weber, 1972; Farji Brener & Ruggiero, 1994). Yet they have been the focus of very few ecological studies that investigate the roles of food availability, fungal, and ant production on the life history of these ants, even although these ants can be quite abundant and presumably ecologically important (Wirth *et al.*, 2003; Seal & Tschinkel, 2006).

The following paper reports the results from a food supplementation experiment on a fungus gardening ant, *Trachymyrmex septentrionalis* McCook. Ants in the genera *Trachymyrmex* and *Sericomyrmex* along with the leaf cutters (*Acromyrmex* and *Atta*) comprise the *higher attine* group (Chapela *et al.*, 1994; Mueller *et al.*, 1998; Wetterer *et al.*, 1998). Recent phylogenetic analyses indicate that *T. septentrionalis* forms a sister clade to all leaf-cutting ants (*Acromyrmex* and *Atta*) (Schultz & Brady, 2008). Consequently, its use as a model system has been particularly enlightening in understanding the evolutionary ecology of the tribe (Seal & Tschinkel, 2007a, b, c). The purpose of this experiment was to observe the consequences of food supplementation over the course of 1 year on the production of ant (worker and sexual) and fungal biomass. A specific emphasis was made on salient life history traits that have been important in the evolutionary and ecological success of these unique insects. The results were specifically discussed in the context of the theoretical framework proposed by Bourke and Franks (1995) on ant life history evolution.

Materials and methods

Trachymyrmex septentrionalis is a common ant in sandy soils of eastern North America, occupying a region that extends to 40°N and west of the Mississippi River (Weber, 1972). *Trachymyrmex septentrionalis*, like many attini, is a highly abundant and conspicuous ant. It is among the most abundant ants in north Florida pine forests (>1000 nests ha⁻¹), making it presumably ecologically important (Seal & Tschinkel, 2006). This study was conducted in the Munson Sandhills of the Apalachicola National Forest (ANF) located in northern Florida, approximately 15 km south of Tallahassee. The study site was located in management compartment 219 of the ANF (30°20.8′ N, 84°17.6′ W), which was a focus of previous study (Seal & Tschinkel, 2006). This stand contained a mixture of scrubby oaks (*Quercus laevis* Walter, *Q. incana* Bartr. and *Q. geminata* Small) and longleaf pine (*Pinus palustris* Mill) of various immature and mature stages. This study site was neither too woody nor barren and was one of the more productive sites for *T. septentrionalis* colonies (Seal & Tschinkel, 2006). *Trachymyrmex septentrionalis* has been observed to collect caterpillar frass, oak (*Quercus* spp.) catkins (staminate flowers) and cut leaves from soft vegetation. In this forest, *T. septentrionalis* is somewhat sympatric with the introduced fungus gardening ant *Cyphomyrmex rimosus* Spinola; however, the latter appears to be more common in the wetter *flatwoods* where *T. septentrionalis* is not as abundant (Deyrup, 2000; Lubertazzi, 2003). Other than soil or litter fungi, this species appears to be free from inter-specific competitors.

In July 2002, 60 colonies were found, numbered and marked with flags. Colonies were randomly assigned to either a group that received supplemented food or a group that did not. Supplemented colonies received approximately 15 g of fungal substrate (fungus food) twice weekly starting 23 July 2002 and continued until October 2002. The fungal substrate was placed next to the nest entrance hole. Thirty grams is well in excess of the amount that laboratory colonies may consume on a weekly basis (Seal & Tschinkel, 2007a, b). Although one could argue that this is outside of what colonies would normally experience; both laboratory and field colonies will eventually cease collecting about 1 month before the sexuals mature. Periodically clumps of catkins and caterpillar nests can be blown down by strong winds and foragers have been noticed to recruit to such substrate piles (J. N. Seal, unpubl. obs.). Furthermore, foragers will sort through the pile and especially toward winter and again toward summer, only pick out certain items, although the basis of this selection is not clear. This species becomes dormant by mid-autumn (October–November); foraging trips become infrequent, gardens reduce in size, and the nest entrance becomes closed off. After colonies started depositing tumuli on the surface, food supplementation resumed on 24 March and continued until 16 May 2003. The fall and spring supplementations occurred during the period of worker and sexual production, respectively, in this species. Only 38 colonies (19 in each treatment group) were collected at the end of the experiment. The 22 remaining colonies had apparently moved or died. In some of these cases it was not clear whether colonies near the flags were actually colonies that were part of the experiment. The identity of a few colonies was also doubtful because a forest mammal had either moved or chewed the flags beyond recognition. However, the 38 colonies used in this study were beyond a reasonable doubt the same colonies marked and fed the preceding summer.

In 2002, the supplemented diet consisted of a mixture of oak catkins, frass from fall webworm (*Hyphantria cunea* Drury, Arctiidae) reared on persimmon leaves (*Diospyros virginiana* L.) and frass from the oleander moth caterpillar (*Syntomeida epilais* Walker, Arctiidae) reared on oleander (*Nerium oleander* L., Apocynaceae). In the 2003, however, the supplemented diet consisted entirely of frass from the eastern tent caterpillar, *Malacosoma americanum* Fabricius (Lasiocampidae) that were reared on black cherry leaves (*Prunus serotina* Ehrh.). These substrates are all considered to be items preferred by *T. septentrionalis* foragers (Seal & Tschinkel, 2007a, b).

Colony collection

Entire colonies were excavated (destructively) during a hot dry, relatively rainless period, 20 May–02 June 2003. Mating flights in this species generally occur after the first heavy convective thunderstorm of the summer, usually by early June (Seal & Tschinkel, 2007c). Soils of the ANF forest are deep, almost pure quartz sand entisols that contain very little organic matter (Brown *et al.*, 1990), a property very helpful for studies requiring the collection of complete colony contents (Tschinkel, 1999; Seal & Tschinkel, 2006, 2007a, b). Colonies were collected by

excavating a 1-m^3 pit approximately 30 cm from the nest entrance. Tunnels and fungus garden chambers were found by carefully removing soil from the face of the pit toward the entrance with a trowel and spoons. All tunnels were followed until all ants had been collected. The depth and volume of each chamber were recorded. Chamber volume was estimated by measuring the maximum width, length, and height of the inner dimensions with a measuring tape.

Measures of ant performance

The main response variables were the total weights of offspring. Initially, the energetic contents and average per cent fat of ant offspring were sought by extracting the body fat from adult ants in a Soxhlet extractor using diethyl ether for 48 h (Soxhlet, 1879). Energetic contents of ant biomass were obtained by multiplying lean weights by 18.87 J mg^{-1} and fat weights by 39.33 J mg^{-1} (Peakin, 1972) and summing. A maximum of 10 female, male and new worker offspring were chosen from each colony for extraction.

Measures of fungal performance

Fungus garden traits included fungus garden weight and an estimate of the amount of chitin (per cent and total amount). Chitin is the main constituent of fungal cell walls and its quantity in a substrate is frequently used as an indicator of fungal biomass in soil or wood, among other substrates (Plassard *et al.*, 1982). This method, summarised previously (Seal & Tschinkel, 2007a, b), estimates the total amount of fungal biomass that resulted from the experimental treatments in this study. Ant fungi do not appear capable of digesting chitin readily (Martin 1987), making this structural compound a metabolic dead-end. Thus, this method estimates the total amount of fungal biomass that was an outcome of the experimental manipulations in this study.

Statistical methods

Data analysis followed a pathway of inferred causation that corresponded to the temporal sequence of worker and sexual production in this species. As the supplementations began during the period of worker production (late summer and autumn), it was expected that traits of old workers the following spring would be affected, as all colonies were excavated the following spring. Food manipulations conducted during the late winter and spring have the greatest effect on the production of sexuals (Seal & Tschinkel, 2007a, b).

Statistical analysis included standard linear models and measures of effect sizes. As we were comparing several means of colony-level traits, we ran the risk of inflating our type I error rate. The typical solution to this would be to perform a Bonferroni correction; however, recent reviews have illustrated undesirable consequences with this method and null hypothesis testing generally (Nakagawa, 2004; Garamszegi, 2006; Nakagawa &

Cuthill, 2007). These methods may in fact increase the type II error and have led to the under reporting of biologically important, yet statistically insignificant results. Therefore, we calculated Cohen's *d* statistic, which is a standardized measure of effect size that can be used to objectively compare means (Cohen, 1988). Accordingly, Cohen's *d* generally ranges from near zero to one, with values around 0.2 indicating small effects, 0.5, medium effects and greater than 0.8 indicating large effects. This value reported with the CI thus provides the amount of variation in a parameter and a measure of its biological relevance, whereas a *P*-value provides only a simple *yes-no* answer (Nakagawa & Cuthill, 2007). Nevertheless for informative purposes we report statistical tests; data were either log$_{10}$ transformed or non-parametric tests employed if parametric assumptions were violated.

As we were interested in the effect of nourishment on colony level traits and its consequences on population-level properties, colonies that did not produce a certain type of brood (e.g., males) were assigned a *0* in that category. An exception occurred with one colony that was excluded from analysis because it produced no brood of any kind (sexual, new worker, larvae, etc). Additionally, in the calculation of sex ratio (male brood weight/male and female brood weight), colonies that did not produce sexual brood were similarly excluded. In addition to the mathematical problem of division by zero, these colonies could have been immature; incipient colonies generally wait until their third year to produce sexual offspring in the laboratory (J. N. Seal, unpubl. obs.). Although the field colonies in this study were at least 2 years old, their age at sexual maturity is probably much longer.

As an inferential tool, stepwise procedures were performed to determine the relative contributions of colony size (weight of old workers), fungal biomass (total chitin amount), and supplementation treatment to the production of biomass in the spring. Although stepwise procedures have been criticised (James & McCulloch, 1990), the models employed here were relatively simple and contained a maximum of three independent variables. Nevertheless, the outcome of each analysis was checked by comparing correlation and regression coefficients of simple regressions. Colonies that did not produce males or females (i.e. those that were recorded as a *0*) were excluded from this analysis. This was justified because we were interested in how the data covaried with fungal biomass or colony size and the inclusion of zeros potentially obscures linear relationships. Visible outliers with standardised residuals > 2.5 SD were removed from the analysis. Effects were removed from the model (pooled) if they produced *P*-values > 0.20. All analyses were conducted with Statistica version 6.1 (Statsoft, 2003).

Results

Old worker traits

The effect of supplementing colonies during the period of worker production appeared to be small. Supplemented colonies did not differ significantly in size from unsupplemented colonies in terms of the number of old (dark) workers or worker

weight despite possessing medium effect sizes (Table 1). However, old workers were larger, fatter and consequently more energetically expensive in supplemented colonies than unsupplemented colonies; effect sizes for these variables approached one in most cases (Table 1).

Traits of fungus gardens

Supplementation during the period of sexual production had pronounced, obvious effects on fungus gardens. Supplementation clearly increased the performance of the symbiotic fungus, expressed as final fungus garden weight, per cent chitin and total amount of chitin (Table 2). Effect sizes for these traits exceeded one in all three cases (Table 2). Supplemented colonies were about two times heavier and contained three times the amount of chitin as unsupplemented colonies (Table 2). The linear relationship between total chitin content and fungus garden weight was identical in both treatments ($F_{1,36} = 0.7$, $P = 0.41$); both groups exhibited a common slope ($F_{1,36} = 17$, $P < 0.001$) (Fig. 1). This slope was negatively allometric (< 1 on a log-log scale) so that chitin content increased at a lower rate than fungus garden weight (B $= 0.803 \pm 0.071$) ($\pm$SE), (one-tailed t-test: $t_{36} = 2.78$, $P < 0.01$). In other words, this indicates that larger gardens have a lower per cent chitin than smaller gardens, which is independent of treatment.

To make room for the larger gardens, the ants in supplemented colonies appear to have made chambers about 25% larger (Table 2) but this value was not statistically significant [t-test on cube root, log transformed data, ($t_{34} = 1.82$, $P = 0.08$)]. Nevertheless, the effect size is somewhat high ($d = 0.53$) (Table 2). Although the ants in the supplemented group could have made their chambers larger to house their large gardens; the density of fungus garden material in the chamber (grams of fungus garden by cubic cm) indicated that supplemented fungus gardens were more densely packed into their chambers than unsupplemented colonies (Table 2). A similar finding was also observed with the density of fungal chitin in the chamber (Table 2). The physical appearance of these gardens was striking because they retained their shape during collection and were very firm to the touch, which is quite unlike normal/unsupplemented conditions where gardens are generally fragile and crumble easily (Fig. 2).

Traits of new ant biomass (sexuals and new workers)

Seventeen of the supplemented colonies produced sexual brood, whereas only 13 unsupplemented colonies produced sexual brood. Seventeen of the supplemented colonies produced female brood, whereas 15 of these produced male brood. In the unsupplemented group, only 8 produced females and 11 produced males. The pattern of new worker production was less obvious, as only 10 of the supplemented and 12 unsupplemented colonies produced a new worker brood. Only one colony, which occurred in the unsupplemented group, did not produce offspring of any kind. Data from this colony were excluded from further analysis.

Supplementation largely affected the production of sexual biomass. Supplemented colonies produced 2–4 times the amount of biomass and numbers of offspring as did unsupplemented colonies (Table 1). The larger amounts of total ant biomass produced in supplemented colonies appears to be attributed entirely to sexual production (both male and female), as no significant effects were observed in the weights or numbers of new workers or larvae, both of which had rather small effect sizes (Table 1).

Table 1. Mean ($\pm$SD) amounts of ant biomass and numbers produced, upper and lower CI, tests of significance and effect sizes.

	Supplemented	CI (lower, upper)	Unsupplemented	CI (lower, upper)	Mann–Whitney U-test, P-value	Cohen's d
Ant biomass	**260 $\pm$ 183 mg**	**183, 337**	**115 $\pm$ 75 mg**	**75, 155**	**$U = 74$,****	**1.35**
Sexual biomass	**149 $\pm$ 87 mg**	**107, 190**	**48 $\pm$ 58 mg**	**20, 77**	**$U = 63$,****	**1.36**
Female biomass	**58 $\pm$ 48 mg**	**35, 81**	**26 $\pm$ 37 mg**	**8, 45**	**$U = 93.5$,***	**0.74**
Male biomass	**90 $\pm$ 72 mg**	**56, 125**	**22 $\pm$ 33 cm³**	**6, 39**	**$U = 72.5$,****	**1.21**
% male biomass	56 $\pm$ 29%, $n = 17$	41, 71	57 $\pm$ 43%, $n = 13$	31, 82	$U = 103$,n.s.	**0.01**
New worker biomass	13 $\pm$ 21 mg	3, 23	15 $\pm$ 18 mg	6, 24	$U = 150.5$,n.s.	0.13
Larval biomass	98 $\pm$ 109 mg	46, 151	52 $\pm$ 50 mg	27, 77	$U = 126.5$,n.s.	0.46
Sexual No.	**150 $\pm$ 101**	**101, 199**	**46 $\pm$ 55**	**18, 73**	**$U = 65.5$,****	**1.27**
Female No.	**50 $\pm$ 49**	**30, 78**	**24 $\pm$ 36**	**6, 42**	**$U = 95.5$,***	**0.62**
Male No.	**96 $\pm$ 84**	**55, 137**	**22 $\pm$ 31**	**7, 38**	**$U = 80$,****	**1.17**
New worker No.	31 $\pm$ 52	6, 56	32 $\pm$ 37	14, 50	$U = 150$,n.s.	0.38
Larvae No.	128 $\pm$ 113	74, 183	69 $\pm$ 38	50, 88	$U = 120$,n.s.	**0.60**
Dark worker No.	226 $\pm$ 102 ants	177, 275	174 $\pm$ 92 ants	127, 219	$U = 116$,n.s.	0.56
Weight	122 $\pm$ 48 mg	99, 146	96 $\pm$ 52 mg	70, 122	$U = 116$,n.s.	0.57
Mean weight	**0.568 $\pm$ 0.041 mg**	**0.55, 0.59**	**0.50 $\pm$ 0.06**	**0.47, 0.53**	**$U = 61$,*****	**1.32**
Lean weight	**0.485 $\pm$ 0.034**	**0.47, 0.50**	**0.442 $\pm$ 0.054 mg**	**0.42, 0.47**	**$U = 92$,***	**0.98**
Fat content	**0.083 $\pm$ 0.023**	**0.07, 0.09**	**0.06 $\pm$ 0.027**	**0.05, 0.07**	**$U = 85.5$,****	**0.94**
% fat	**15 $\pm$ 4%**	**12.9, 16.3**	**12 $\pm$ 5%**	**9.4, 14.2**	**$U = 105$,***	**0.66**
Energetic content	**12.4 $\pm$ 1.1 J**	**11.9, 13**	**10.7 $\pm$ 1.5J**	**10, 11.4**	**$U = 55$,*****	**1.35**

Except where noted, there were 19 colonies in the supplemented group and 18 that were unsupplemented. ****$P < 0.0001$. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, n.s. $= P > 0.05$.

Table 2. Mean (±SD) amounts of fungal biomass produced, upper and lower CI, tests of significance and effect sizes.

	Supplemented	CI (lower, upper)	Unsupplemented	CI (lower, upper)	Mann–Whitney U-test, P-value	Cohen's d
Fungus garden weight	**7.2 ± 4.1 g**	**5.2, 9.2**	**3.4 ± 1.6 g**	**2.6, 4.2**	$U = 55$,***	1.21
Percent chitin	**2.4 ± 0.6%**	**2.1, 2.7**	**1.8 ± 0.4%**	**1.6, 2.0**	$U = 76$,**	1.01
Total chitin content	**157 ± 74 mg**	**121, 193**	**58 ± 26 mg**	**46, 71**	$U = 28$,****	1.76
Chamber volume	422 ± 175 cm³ ($n = 18$)	335, 509	329 ± 170 cm³ ($n = 17$)	242, 417	$U = 99$,n.s.	0.53
Fungus garden weight: chamber volume	**1.65×10^{-2} ± 0.65 g cm⁻³** ($n = 17$)	**1.3×10^{-2}, 2×10^{-2}**	**1.12×10^{-2} ± 0.5 g cm⁻³** ($n = 17$)	**0.9×10^{-2}, 1.4×10^{-2}**	$U = 77$,*	0.82
Chitin: chamber volume	**4×10^{-4} ± 1.2 mg cm⁻³** ($n = 17$)	**3×10^{-4}, 5×10^{-4}**	**2.08×10^{-4} ± 1 mg cm⁻³** ($n = 17$)	**1.5×10^{-4}, 2.75×10^{-4}**	$U = 51$,***	1.16

Except where noted, there were 19 colonies in the supplemented group and 18 that were unsupplemented. ****$P < 0.0001$. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, n.s. = $P > 0.05$.

The sex ratio was also not significantly affected and had a miniscule effect size (Table 1). The amount of new worker production was not correlated with the amount of sexual production, which would have indicated a trade-off ($r = -0.3$, $P > 0.25$). The supplemented colonies also did not have a higher rate of production (ant biomass per gram of chitin) than unsupplemented colonies (1.68 ± 1.01 vs 1.98 ± 1.04, $F_{1,35} = 0.76$, $P = 0.40$). Likewise the rate of sexual production (per gram of chitin) was similar in both groups (0.94 ± 0.55 vs 0.74 ± 0.86, Mann–Whitney $U = 122$, $P = 0.14$). Both of these production rates had effect sizes of approximately 0.28, which are low. These data indicate that the primary consequence of the experiment was to increase the amount of fungus, which caused a linear increase in the amount of ant production.

Most individual male and female offspring were incompletely developed; only 10 supplemented and 5 unsupplemented colonies contained adult females. Similarly, only 12 colonies and 4 unsupplemented colonies contained adult males. The remainder of the sexual brood in these colonies were present as pupae. It is very likely that the female alates were not ready to fly, because

the average female fat content of both treatment groups [supplemented: $14 \pm 5\%$, unsupplemented $14 \pm 8\%$ (mean ± 1SD)] was considerably less than the 25% found in individuals immediately after mating flights (Seal & Tschinkel, 2007c). Consequently energetics and fat contents are reported only for old (dark) workers (Table 1).

Colonies that produced sexuals of any kind, females or males, were not significantly different in size (the number of old workers) (Table 3). However, colonies that produced these types of offspring had significantly greater amounts of fungal biomass than colonies that did not (Table 4). The effect sizes for fungal biomass were much higher than those for old worker number (Tables 3 and 4, respectively). Sexual colonies contained triple the amount of chitin as colonies that failed to produce a single sexual.

The above analyses indicate that fungal biomass was a more important variable than old worker number. This conclusion was further supported by the multiple regression analyses. Most variation in the production of ant biomass was largely attributable to the amount of fungal biomass in the colony; once the variable of fungal biomass was removed, neither the weight of dark workers nor the categorical variable of treatment were generally significant explanatory variables (Table 5). This stresses the fact that the additional food had a quantitative effect on the production of ant biomass. New ant (sexual, new worker and larvae combined) biomass increased positively with the amount of fungal biomass in the fungus garden (Table 5, Fig. 3). This relationship was isometric; a proportional increase in fungal biomass was met by a similar proportion of ant production ($H_0 = 1$, $t_{33} = 0.673$, $P = 0.25$). The production of sexual biomass on the other hand appeared to be best explained by a model indicating that the weight old workers was the best explanatory variable (Table 5). Again this slope was not significantly different than one ($H_0 = 1$, $t_{26} = 0.77$, $P = 0.22$). These patterns appear to be driven by higher amounts of male and larval biomass on supplemented colonies, as neither female nor new worker biomass appear to have been affected by the supplementation (Table 5). Moreover the isometry of these slopes appears to be a consequence of positive and negative allometries
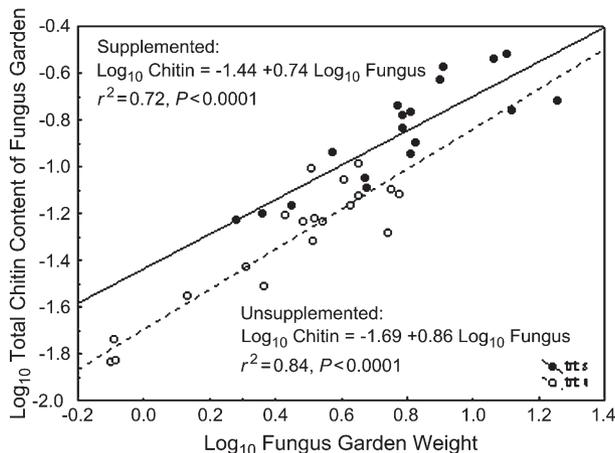

Fig. 1. Log-log plot of total chitin content versus the weight of fungus gardens.
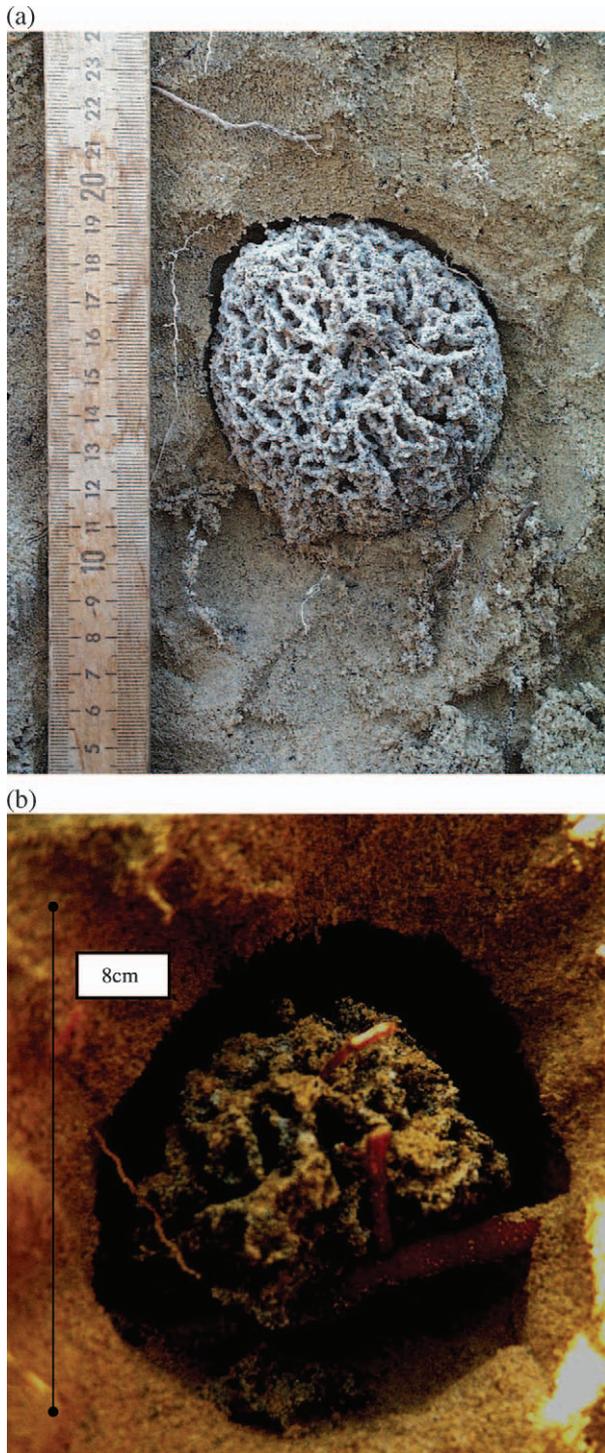
Fig. 2. Photographs of (a) supplemented, and (b) unsupplemented fungus gardens. Note the free space inside the unsupplemented fungus garden chamber.

among the slopes with male and larval biomass, respectively. The weight of male biomass was positively allometric with respect to total chitin content, so not only did male biomass increase with fungus, but proportionally more males were produced by larger fungus gardens than smaller gardens ($H_0 = 1$, $t_{25} = 2.46$, $P = 0.01$). The weight of larval biomass was negatively allometric with respect to total chitin content, so that larval biomass increased at a lower rate than fungal biomass ($H_0 = 1$, $t_{33} = 1.86$, $P = 0.04$).

Discussion

The population of *T. septentrionalis* that lives in these pine forests appears to be quite food limited, just like other ant populations in other ecosystems (Deslippe, 1994; Deslippe & Savolainen, 1995; Herbers & Banschbach, 1998; Morales & Heithaus, 1998; Bono & Heithaus, 2002; Sorvari & Hakkarainen, 2007). Populations are living under suboptimal conditions. Food supplementation increased the size of the fungus garden and the amounts of fungal and ant biomass. Although *T. septentrionalis* would appear to be free of inter-specific competitors and seemingly an abundance of catkins and caterpillar frass from oak trees and other shrubs, the population does not seem to be as high as it could be in these nutrient poor forests (Abrahamson & Harnett, 1990; Brown *et al.*, 1990). This is quite unlike the situation in the fire ant, *Solenopsis invicta* Buren, which saturates its habitat (Tschinkel *et al.*, 1995; Adams & Tschinkel, 2001).

The data suggest that larger colonies have a fitness advantage, as sexual production is a linear function of the amount of standing worker biomass. It is tempting also to include that the slightly larger colonies and larger workers in supplemented colonies contributed to the higher amounts of sexual output relative to unsupplemented colonies. While this finding is generally in accord with results in other species of social insect (Tschinkel, 1993, 1999; Bouwma *et al.*, 2006), the results are similar to the descriptive study by Seal and Tschinkel (2006) in that only male production appears to be a function of colony size and is partly responsible for its significance. In other words, colonies appear to allocate a quantity of energy towards female production that is independent of colony size or fungal biomass. One possibility is that if the experiment had proceeded for a longer duration, female production may have been a significant function of the three explanatory variables, because they would have had time to develop and fatten up.

Generally it does not appear that colony size is an important variable in predicting the amount of ant biomass that a colony produces each spring. Rather the amount of fungal biomass may be the best indicator of the magnitude of overall production in ant, male, and larval biomass. Colony size *per se* may not be as important a factor as the amount of food or energy stores, which may be correlated with colony size, that predict the performance of a colony. This may especially be the case if colonies have a means to temporarily store food, such as fungus gardens, and foraging behaviour and colony performance are largely decoupled from each other. For example, *T. septentrionalis* may collect substrate quantities in excess of what they can use for brood production (Seal & Tschinkel, 2007b); foraging behaviour is probably stimulated by fungal growth and not simply larval hunger as reported in other social insects (Cassill & Tschinkel,

Table 3. Mean colony size (numbers $\pm$ SD of dark workers), upper and lower CI, tests of significance and effect sizes of colonies that produced sexual, female, male or new worker biomass versus colonies that did not.

	Mean ($\pm$ 1 SD) number of workers in unproductive colonies	CI (lower, upper)	Mean ($\pm$ 1 SD) number of workers in productive colonies	CI (lower, upper)	Mann–Whitney U-test, P-value	Cohen's d
Sexual	173 ± 117, $n = 7$	65, 281	207 ± 96, $n = 30$	171, 243	$U = 81$,[n.s.]	0.34
Female	169 ± 93, $n = 12$	110, 227	215 ± 101, $n = 25$	174, 257	$U = 111$,[n.s.]	0.48
Male	181 ± 125, $n = 11$	97, 265	208 ± 89, $n = 26$	173, 244	$U = 102$,[n.s.]	0.27
New worker	218 ± 111, $n = 15$	157, 280	188 ± 92, $n = 22$	147, 229	$U = 157$,[n.s.]	0.30

****$P < 0.0001$. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, n.s. $= P > 0.05$.

1999). Therefore, if foragers are capable of making return trips when a cache of food is discovered, overall numbers and weight of workers may be less important. Recruitment in *T. septentrionalis* has been observed on numerous occasions and recruitment trails may be longer than 3 m (J. N. Seal, unpubl. data). Consequently, a small colony composed of relatively few workers could produce a similarly sized garden and thus the same amount of sexuals as a larger colony, if food is abundant. Typically after colonies produce sexuals and undergo mating flights, workers appear to take down the fungus gardens and deposit the excess and refuse outside the nest or in old nest chambers (J. N. Seal, unpubl. field and laboratory obs.). Of course there is probably an upper limit to the size of fungus garden that a colony can maintain without suffering pathogen attack. Although the ecology of attine pathogens remains poorly understood (Currie *et al.*, 1999; Currie *et al.*, 2003) and laboratory colonies of *T. septentrionalis* can succumb to *Escovopsis* infections, in no cases in this study or in previous experiments (Seal & Tschinkel, 2007a, b) did colonies experience a pathogen attack.

Food limitation in this species, as is likely in most non-leaf cutting attini, is probably driven by the rarity of suitable fungal substrates, which are relatively ephemeral, seasonally available and/or difficult to discover (Leal & Oliveira, 2000). For example, oaks catkins, a preferred substrate (Seal & Tschinkel, 2007a, b), are produced only in late March until early April. Frass of all types would only be available when caterpillars are present. *Malacasoma americanum* is strictly univoltine and active only in the early spring. Frass from other caterpillar species are not available until mid to late April after the (deciduous) oaks have flowered, and flushed out new leaves. As soils of this forest are nearly pure sand and contain few nutrients (Waite, 1984; Brown *et al.*, 1990), it is probable that the nutrients in frass quickly leach out, leaving only dried plant fibre. Dried frass pellets can easily be found by sorting through the leaf litter, but *T. septentrionalis* foragers will reject these pellets (J. N. Seal, unpubl. data).

Non-leaf cutting attini are known to harvest fresh leaves and flowers (Leal & Oliveira, 2000). Foragers of *T. septentrionalis* have been observed in this study site each April (2001–2006) to cut leaves from bracken fern [*Pteridium aquilinum* L. (Polypodiaceae)] and short oak seedlings in the understory (J. N. Seal unpubl. data). Leaf-cutting in the true leaf-cutters (*Atta* spp.) is among the most energetically expensive animal behaviours (Roces & Lighton, 1995). The costs of leaf-cutting are thought to be offset by the production of highly polymorphic workers with large, muscular and mineralised mandibles (Wilson, 1980a, b; Schofield *et al.*, 2002), traits which non-leaf-cutting attines such as *T. septentrionalis* lack. It is thus not clear how efficient of a food source fresh vegetation could be for these ants. Nevertheless, oak leaves can be a genuine source of nourishment in *T. septentrionalis*, at least in early spring when the leaves are still soft (Seal & Tschinkel, 2007a, b).

Because fungal substrates are rare, one could expect to find evidence of intra-specific competition in *T. septentrionalis*. However, nests of this species avoid interactions, although foragers of *T. septentrionalis* will act aggressively with individuals from foreign colonies (J. N. Seal, unpubl. data). Colonies would appear to avoid each other generally by orienting their mound, nest entrance, and trunk trail in different directions (Tschinkel & Bhatkar, 1974). Evidence of competition could have also come in the form of an inverse relationship between colony number and colony size or other biomass equivalent per unit area. However, an earlier descriptive study on *T. septentrionalis* did not find such a relationship in similar sites to the one in the present study, where colonies exhibited a positive relationship between colony size and colony number in 400 m^2 transects. This suggested recruitment limitation because where

Table 4. Mean weights ($\pm$ SD) of total fungus garden chitin content of colonies that produced sexual, female, male or new worker biomass versus colonies that exhibited no production. Bold text indicate significant differences ($\alpha = 0.05$).

	Mean ($\pm$ SD) chitin content of unproductive gardens	CI (lower, upper)	Mean ($\pm$ 1 SD) chitin content of productive gardens	CI (lower, upper)	Mann–Whitney U-test, P-value	Cohen's d
Sexual	**0.049 ± 0.028, n = 7**	**0.023, 0.074**	**0.123 ± 0.075, $n = 30$**	**0.095, 0.151**	**$U = 36$,****	**1.08**
Female	**0.052 ± 0.026, n = 12**	**0.036, 0.069**	**0.136 ± 0.075, $n = 25$**	**0.105, 0.167**	**$U = 37$,*****	**1.31**
Male	**0.073 ± 0.050, n = 11**	**0.039, 0.107**	**0.124 ± 0.079, $n = 26$**	**0.093, 0.156**	**$U = 87$,***	**0.72**
New worker	0.124 ± 0.067, n = 15	0.087, 0.161	0.099 ± 0.079, $n = 22$	0.064, 0.134	$U = 138$,[n.s.]	0.34

Table 5. Results from linear regression analysis of the effects of colony size (weight of dark workers), total chitin content and the effect of supplementation on the production of new ant, sexual, female, male, new worker and larval biomass.

Dependent variable	Independent variable	Partial correlation coefficient (*P*-value)	In model?	r^2	Adj. r^2	Slope (SE)	*F* statistic, *P*-value
Ant biomass	Treat	−0.09 (*P* = 0.63)	No				
	Chitin	**0.72 (*P* < 0.0001)**	**Yes**	**0.68**	**0.66**	**0.80 (0.14)**	$\mathbf{F_{2,32} = 34.6, P < 0.001}$
	Colony size	0.29 (*P* = 0.10)	Yes			0.31 (0.18)	
Sexual biomass	Treat	−0.26 (*P* = 0.20)	No				
	Chitin	0.28 (*P* = 0.16)	Yes			0.61 (0.19)	
	Colony size	**0.43 (*P* = 0.03)**	**Yes**	**0.52**	**0.49**	**0.50 (0.22)**	$\mathbf{F_{2,25} = 13.8, P < 0.001}$
Female biomass	Treat	—	No	0.17	0.03	0.05 (0.18)	$F_{3,21} = 0.21, P = 0.88$
	Chitin	—	No			0.19 (0.38)	
	Colony size	—	No			0.12 (0.33)	
Male biomass	Treat	0 (*P* = 0.21)	No			−0.27 (21)	
	Chitin	**0.76 (*P* < 0.001)**	**Yes**	**0.57**	**0.56**	**1.74 (0.30)**	$\mathbf{F_{1,25} = 33.6, P < 0.001}$
	Colony size	−0.02 (*P* = 0.92)	No				
New worker biomass	Treat	−0.12 (*P* = 0.61)	No				
	Chitin	−0.06 (*P* = 0.79)	No				
	Colony size	−0.25 (*P* = 0.27)	No	0.25	0.06	−0.49 (0.44)	$F_{1,20} = 1.28, P = 0.27$
Larval biomass	Treat	0.31 (*P* = 0.08)	Yes			0.3 (0.17)	
	Chitin	**0.37 (*P* = 0.03)**	**Yes**	**0.62**	**0.38**	**0.73 (0.33)**	$\mathbf{F_{3,31} = 6.38, P = 0.03}$
	Colony size	0.22 (*P* = 0.13)	Yes			0.51 (0.33)	

Bold-faced text indicate significant differences ($\alpha = 0.05$). Chitin = total chitin content, Colony size = weight of dark workers, Treat = supplementation treatment.

colonies were larger (and presumably more productive), there were more nests (figure 5 of Seal & Tschinkel, 2006).

According to the scenario outlined by Bourke and Franks (1995), which states that species, such as most attini, which found colonies independently (i.e. by single foundresses) and non-claustrally (newly-mated queens forage during the founding phase), should be favoured in environments that have either low densities of conspecific nests or high densities of otherwise non-interacting nests. The rationale here is that intense intraspecific competition has led to the evolution of territoriality (Hölldobler, 1974, 1979; Skinner, 1980; Bourke & Franks,
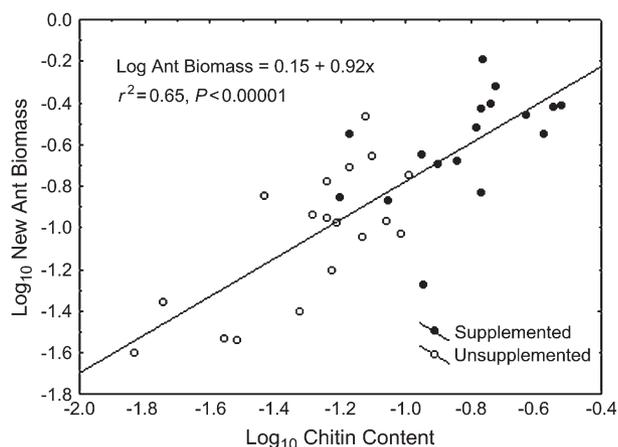

Fig. 3. Log-log plot of new ant biomass as a function of the total chitin content of fungus gardens. Data have been log transformed. Slopes for the treatment have been pooled.

1995; Tschinkel, 2006). Except for *Atta* spp., the attini reproduce by independent non-claustral founding and are not known to defend spatial territories (Whitehouse & Jaffe, 1996; Fernández-Marín *et al.*, 2004).

The question then becomes, if food is limiting, why do most attini neither exhibit traits such as claustral founding nor territoriality? We believe that an explanation derives from the evolution of fungus gardening in that founding queens of *T. septentrionalis* are poorly adapted to survive in competitive environments. Most basal attines produce small queens and it is their small size that prevents claustral founding (Seal & Tschinkel, 2007c). Our hypothesis is that queen fat content and dry weight are allometrically constrained, so that for fatter queens to evolve, queen bodies had to become larger (Seal & Tschinkel, 2007c; J. N. Seal, unpubl. data). Specifically for a queen to exhibit claustral founding, queens must have approximately 50% body fat (Keller & Passera, 1989; McInnes & Tschinkel, 1995; Tschinkel, 1996). The only claustral attines are *Atta* (Fernández-Marín *et al.*, 2004), which are also the heaviest and fattest (40% body fat; J. N. Seal, unpubl. data). More basal attines, such as *Trachymyrmex*, are approximately 200 times smaller and contain much less fat (25% body fat (Seal & Tschinkel, 2007c); J. N. Seal, unpubl. data).

What could cause populations to be substrate and recruitment limited? Perhaps temperature is an important variable that influences foraging and ultimately colony performance, population dynamics, and evolution. Soil temperature, insolation, and/or food sources have long been implicated in the performance of ant colonies (Brian & Brian, 1951; Brian & Elmes, 1974; Elmes & Wardlaw, 1982b; Bollazzi & Roces, 2002; Sorvari & Hakkarainen, 2007). The earlier descriptive study surveyed colonies from a variety of sites in the ANF and found that the

least productive colonies were in shady and cool stands of pine forest, typically containing a closed canopy of oaks (Seal & Tschinkel, 2006). On the other hand, the largest and deepest nests were found in relatively barren, open, warm if not hot, sandy sites. The site used in this study lay between these extremes. In the barren areas, the slope between nest number and colony size appeared negative and was consistent among years, but was not statistically significant (Seal & Tschinkel, 2006; $r = 0.47$, $P = 0.6$) yet it had a *medium* effect size (Nakagawa & Cuthill, 2007). Given the ambiguity of this relationship, it seems quite possible that if density dependent competition ever occurred in *T. septentrionalis*, it would occur in these open, sandy sites, which contain little plant cover and thus fewer sources of food. The lack of statistical significance of that slope could be evidence that this species is not capable of competition because its queens, colony size, and founding mode are poorly adapted to do so. As the *T. septentrionalis* group is thought to be the sister group to the leaf cutting clade (Schultz & Brady, 2008), it is nevertheless tempting to conclude that marginally competitive environments such as these may have selected for large queen size, large colony size, territoriality or the evolution of the leaf-cutting ants generally. This conclusion will remain speculative until we can compare the survivorship of founding queens and overall probability of colony success in these extreme habitats. Evidence is mounting that further studies on this species will become vital in understanding not only the evolutionary ecology of these ecologically important insects, but the conditions under which complex societies have evolved.

Acknowledgements

References

Abrahamson, W.G. & Harnett, D.C. (1990) Pine flatwoods and dry prairies. *The Ecosystems of Florida* (ed. by R. L. Myers and J. J. Ewel), pp. 103–149. The University of Central Florida, Orlando, Florida.

Adams, E.S. & Tschinkel, W.R. (2001) Mechanisms of population regulation in the fire ant *Solenopsis invicta:* an experimental study. *Journal of Animal Ecology*, **70**, 355–369.

Agosti, D., Majer, J.D., Alonso, L.E. & Schultz, T.R. (2000) *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. Smithsonian Institution, Washington, D.C.

Bollazzi, M. & Roces, F. (2002) Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. *Insectes Sociaux*, **49**, 153–157.

Bono, J.M. & Heithaus, E.R. (2002) Sex ratios and the distribution of elaiosomes in colonies of the ant, *Aphaenogaster rudis*. *Insectes Sociaux*, **49**, 320–325.

Bourke, A.F.G. & Franks, N.R. (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey.

Bouwma, A.M., Nordheim, E.V. & Jeanne, R.L. (2006) Per-capita productivity in a social wasp: no evidence for a negative effect of colony size. *Insectes Sociaux*, **53**, 412–419.

Brian, M.V. & Brian, A.D. (1951) Insolation and ant populations in the west of Scotland. *Transactions of the Royal Entomological Society of London*, **102**, 303–330.

Brian, M.V., Clark, R.T. & Jones, R.M. (1981) A numerical model of an ant society. *Journal of Animal Ecology*, **50**, 387–405.

Brian, M.V. & Elmes, G.W. (1974) Production by the ant *Tetramorium caespitum* in a southern English heath. *Journal of Animal Ecology*, **43**, 889–903.

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. (2002) Yoyo-bang: a risk-aversion investment strategy by a perennial insect society. *Oecologia*, **132**, 150–158.

Cassill, D.L. & Tschinkel, W.R. (1999) Regulation of diet in the fire ant, *Solenopsis invicta*. *Journal of Insect Behavior*, **12**, 307–328.

Chapela, I.H., Rehner, S.A., Schultz, T.R. & Mueller, U.G. (1994) Evolutionary history of the symbioses between fungus-growing ants and their fungi. *Science*, **266**, 1691–1694.

Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Erlbaum, Hillsdale, New Jersey.

Currie, C.R., Bot, A.N.M. & 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. & Malloch, D. (1999) The agricultural pathology of ant fungus gardens. *Proceedings of the National Academy of Science U.S.A*, **96**, 7998–8002.

Davidson, D.W., Cook, S.C., Snelling, R.R. & Chua, T.H. (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science*, **300**, 969–972.

Deslippe, R.J. (1994) Role of food supply in structuring a population of *Formica* ants. *Journal of Animal Ecology*, **63**, 756–764.

Deslippe, R.J. & Savolainen, R. (1995) Sex investment in a social insect: the proximate role of food. *Ecology*, **76**, 375–382.

D'Ettorre, P., Mora, P., Dibangou, V., Rouland, C. & Errard, C. (2002) The role of the symbiotic fungus in the digestive metabolism of two species of fungus-growing ants. *Journal of Comparative Physiology B Biochemical, Systematic, and Environmental Physiology*, **172**, 169–176.

Deyrup, M.A. (2000) Exotic ants in Florida. *Transactions of the American Entomological Society*, **126**, 293–326.

Elmes, G.W. & Wardlaw, J.C. (1982a) A population study of the ants *Myrmica sabuleti* and *Myrmica scabrinodis*, living at two sites in the south of England. I. A comparison of colony populations. *Journal of Animal Ecology*, **51**, 651–664.

Elmes, G.W. & Wardlaw, J.C. (1982b) A population study of the ants *Myrmica sabulleti* and *Myrmica scabrinodis* living at two sites in the south of England. II. Effect of above-nest vegetation. *Journal of Animal Ecology*, **51**, 665–680.

Farji Brener, A.G. & Ruggiero, A. (1994) Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range size. *Journal of Biogeography*, **21**, 391–399.

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.

Garamszegi, L.Z. (2006) Comparing effect sizes across variables: generalization without the need for Bonferroni correction. *Behavioral Ecology*, **17**, 682–687.

Herbers, J.M. & Banschbach, V.S. (1998) Food supply and reproductive allocation in forest ants: repeated experiments give different results. *Oikos*, **83**, 141–151.

Hölldobler, B. (1974) Home range orientation and territoriality in harvesting ants. *Proceedings of the National Academy of Science U.S.A*, **71**, 3274–3277.

Hölldobler, B. (1979) Territoriality in ants. *Proceedings of the American Philosophical Society*, **123**, 211–218.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Harvard University Press, Cambridge, Massachusetts.

Hunt, J.H. & Amdam, G.V. (2005) Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science*, **308**, 264–267.

Hunt, J.H. & Dove, M.A. (2002) Nourishment affects colony demographics in the paper wasp *Polistes metricus*. *Ecological Entomology*, **27**, 467–474.

James, F.C. & McCulloch, C.E. (1990) Multivariate analysis in ecology and systematics: panacea or pandora's box? *Annual Review of Ecology and Systematics*, **21**, 129–166.

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.

Korb, J. & Heinze, J. (2004) Multilevel selection and social evolution of insect societies. *Naturwissenschaften*, **91**, 291–304.

Leal, I.R. & Oliveira, P.S. (2000) Foraging ecology of attine ants in a Neotropical savanna: seasonal use of fungal substrate in the cerrado vegetation of Brazil. *Insectes Sociaux*, **47**, 376–382.

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) The symbioses between the attine ants and the fungi they culture in their nests. *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.

Morales, M.A. & Heithaus, E.R. (1998) Food from seed-dispersal mutualism shifts sex ratios in colonies of the ant *Aphaenogaster rudis*. *Ecology*, **79**, 734–739.

Mueller, U.G. & Gerardo, N. (2002) Fungus-farming insects: multiple origins and diverse evolutionary histories. *Proceedings of the National Academy of Science U.S.A*, **99**, 15247–15249.

Mueller, U.G., Rehner, S.A. & Schulz, T.R. (1998) The evolution of agriculture in ants. *Science*, **281**, 2034–2038.

Nakagawa, S. (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology*, **15**, 1044–1045.

Nakagawa, S. & Cuthill, I.C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, **82**, 591–605.

Oster, G.F. & Wilson, E.O. (1978) *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, New Jersey.

Peakin, G.J. (1972) Aspects of productivity in *Tetramorium caespitum* L. *Ekologia Polska*, **20**, 55–63.

Plassard, C.S., Mousain, D.G. & Salsac, L.E. (1982) Estimation of mycelial growth of basidiomycetes by means of chitin determination. *Phytochemistry*, **21**, 345–348.

Richard, F.-J., Mora, P., Errard, C. & Rouland, C. (2005) Digestive capacities of leaf-cutting ants and the contribution of their fungal cultivar to the degradation of plant material. *Journal of Comparative Physiology B*, **175**, 297–303.

Roces, F. (2002) Individual complexity and self-organization in foraging by leaf-cutting ants. *Biological Bulletin*, **202**, 306–313.

Roces, F. & Lighton, J.R.B. (1995) Larger bites of leaf-cutting ants. *Nature*, **373**, 392–393.

Schofield, R.M.S., Nesson, M.H. & Richardson, K.A. (2002) Tooth hardness increases with zinc-content in mandibles of young adult leaf-cutter ants. *Naturwissenschaften*, **89**, 579–583.

Schultz, T.R. & Brady, S.G. (2008) Major evolutionary transitions in ant agriculture. *Proceedings of the National Academy of Science U.S.A.*, **105**, 5435–5440.

Seal, J.N. & Hunt, J.H. (2004) Food supplementation affects colony-level life history traits in the annual social wasp *Polistes metricus* (Hymenoptera, Vespidae). *Insectes Sociaux*, **51**, 239–242.

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.

Seal, J.N. & Tschinkel, W.R. (2007a) Co-evolution and the superorganism: switching cultivars does not alter the performance of fungus-gardening ant colonies. *Functional Ecology*, **21**, 988–997.

Seal, J.N. & Tschinkel, W.R. (2007b) Complexity in an obligate mutualism: do fungus-gardening ants know what makes their garden grow? *Behavioral Ecology and Sociobiology*, **61**, 1151–1160.

Seal, J.N. & Tschinkel, W.R. (2007c) Energetics of newly mated queens and colony founding in the fungus-gardening ants *Cyphomyrmex rimosus* and *Trachymyrmex septentrionalis* (Hymenoptera: Formicidae). *Physiological Entomology*, **32**, 8–15.

Skinner, G.J. (1980) Territory, trail structure, and activity patterns in the wood-ant *Formica rufa* (Hymenoptera: Formicidae) in limestone woodland in North-West England. *Journal of Animal Ecology*, **49**, 381–394.

Sorvari, J. & Hakkarainen, H. (2005) Deforestation reduces nest mound size and decreases the production of sexual offspring in the wood ant *Formica aquilonia*. *Annales Zoologici Fennici*, **42**, 259–267.

Sorvari, J. & Hakkarainen, H. (2007) The role of food and colony size in sexual offspring production in a social insect: an experiment. *Ecological Entomology*, **32**, 11–14.

Soxhlet, F. (1879) Die Gewichtsanalytische Bestimmung des Milchfettes. *Polytechnisches Journal*, **232**, 461–465.

Statsoft (2003) *STATISTICA (Data Analysis Software System)*. Tulsa, Oklahoma.

Tobin, J.E. (1994). Ants as primary consumers: diet and abundance in the Formicidae. *Nourishment and Evolution in Insect Societies* (ed. by J. H. Hunt and C. A. Nalepa), pp. 279–307. Westview, Boulder, Colorado.

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.

Tschinkel, W.R. (2006) *The Fire Ants*. Harvard University Press, Cambridge, Massachussetts.

Tschinkel, W.R., Adams, E.S. & Macom, T. (1995) Territory area and colony size in the fire ant *Solenopsis invicta*. *Journal of Animal Ecology*, **64**, 473–480.

Tschinkel, W.R. & Bhatkar, A. (1974) Oriented mound building in the ant, *Trachymyrmex septentrionalis*. *Environmental Entomology*, **3**, 667–673.

Waite, W.R. (1984) *Soils and Vegetation of the Apalachicola National Forest*. United States Department of Agriculture, Forest Service, Southern Region, Atlanta, Georgia.

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. (1991) The developmental basis of worker caste polymorphism in ants. *American Naturalist*, **138**, 1218–1238.

Wheeler, D.E. (1986) Developmental and physiological determinants of caste in social hymenoptera: evolutionary implications. *American Naturalist*, **128**, 13–34.

Whitehouse, M.E.A. & Jaffe, K. (1996) Ant wars: combat strategies, territory and nest defence in the leaf-cutting ant *Atta laevigata*. *Animal Behaviour*, **51**, 1207–1217.

Wilson, E.O. (1971) *The Insect Societies*. Harvard University Press, Cambridge, Massachussetts.

Wilson, E.O. (1980a) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). I. The overall pattern in *Atta sexdens*. *Behavioral Ecology and Sociobiology*, **7**, 143–156.

Wilson, E.O. (1980b) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). II. The ergonomic optimization of leaf cutting *Atta sexdens*. *Behavioral Ecology and Sociobiology*, **7**, 157–165.

Wilson, E.O. (1983) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*) IV. Colony ontogeny of A. *cephalotes*. *Behavioral Ecology and Sociobiology*, **14**, 55–60.

Wilson, E.O. (1985) The sociogenesis of insect colonies. *Science*, **228**, 1489–1495.

Wirth, R., Herz, H., Ryel, R.J., Beyschlag, W. & Hölldobler, B. (2003) Herbivory of leaf-cutting ants: a case study on *Atta colombica* in the tropical rainforest of Panama. Springer Verlag, Berlin, Germany.