UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE ECOLOGICAL IMPORTANCE OF ANT COLONY SIZE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By
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Norman, Oklahoma
2010

THE ECOLOGICAL IMPORTANCE OF ANT COLONY SIZE

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

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Dedication

To my parents

Acknowledgements

I would first like to thank my advisor, Mike Kaspari. Much of my growth as a scientist is to due to his high standards and honest evaluation of my proximity to them. He has spent countless hours revising my writing and clarifying my logic, has given me the freedom to explore my interests, and access to a respirometry system with which to answer important questions. My research was also made possible by support from an Alumni Fellowship in Zoology, Adams Summer Fellowships, a University of Oklahoma Biostation Summer Research Fellowship, and a Smithsonian Tropical Research Institute Short Term Fellowship. My labmate David Donoso has done much to help me focus, and he was always there to remind me that Americans practice just one of many kinds of science. Both David and Natalie Clay have made the ant lab a good place to be. I would also like to thank Matt Dugas for sharpening my axe since the very first day of graduate school and more importantly for his friendship. I am also indebted to all those who overlapped with me at the hardin house (including the pack of feral dogs). So many wonderful teachers have helped me along the way. Marty Condon showed me her quarantine room and then let me tag along on a trip to the tropics as a freshman in college. Martin Lechowicz set me on ants and let me wander around Mt. St. Hilaire trying to find them. Andre Francoeur, way up in Chicoutimi, helped me formalize my interests. Chris Buddle steered me towards my present graduate studies. Ernest Lo, Ben Gilbert, Don Shepard, Dan Spooner, and Puni Jeyasingh saw things far more clearly than me and showed me how to become a scientist. My committee members Ola Fincke, Rosemary Knapp, Jeff Kelly, and Jorge Mendoza have given me well timed kicks in the rear and have taken the time to help

me better frame my research. I would like to thank all of the professors, staff and graduate students in the Department of Zoology for making my six year stay in Sutton Hall so warm. I also thank the folks at Sable Systems International who taught me how to measure how ant respiration in Vegas, and then *very* patiently supported me by phone. I am also grateful to my experiences in Panama on Barro Colorado Island. It's hard to believe that I first rode the Jacana across Lake Gatun six years ago. Logistic support from Oris and Belkys simply made my research possible. So many other friends, colleagues, and island archetypes have helped me survive the late hours in the lab, the null results, and have made me wish for the summer field season when in wintry Oklahoma. For helping me keep my sanity during the long hours of ant sorting tedium, I would like to thank Joe Posnanski for writing about Kansas City and Denny Matthews for talking so laconically about it, Terry Gross for asking interesting people interesting questions, and Anthony Burgess and David Foster Wallace for timely inspiration. Most importantly, I would like to thank my family for their unconditional support of my unusual career choice, and for listening to me endlessly drone on about how I think ant colonies work. My parents have always given me the freedom to pursue my interests and have taught me how to work hard, be ethical, and think independently. My desire to figure things out comes from them. My sister is one of my best friends and her creative energy infuses my scientific reasoning more than she realizes. I am also grateful to have had the chance to develop my relationship my grandpa as an adult, and for his unwavering support of my endeavors.

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Abstract

Despite the rich diversity of life, all organisms are unified by the conserved biochemical pathways that drive metabolism and govern the rate at which resources are processed and used to perform biological work. Metabolic rate further scales as a positive decelerating function of body size, and biologists have long sought to explain why one allometry, with a slope of ¾, accounts for most of the variation in metabolic rate from unicells to whales. In my studies of ant colonies (*colonies of individuals*), I have extended scaling techniques long used to study unitary organisms (*colonies of cells*), plotting colony size as the independent variable instead of body size to test hypotheses about the costs and benefits of eusociality and the extent to which colonial and unitary organisms are unified by a shared metabolic currency.

Ants (Hymenoptera: Formicidae) rank among the planet's most ecologically dominant organisms, and owe much of their success to the organizational benefits of colony life. Like all organisms, ant colonies have life histories—they make decisions about when to reproduce and how much of a limited resource pool to allocate to reproduction. Unlike unitary organisms, colonies *grow* by producing sterile workers that care for developing brood, defend the nest, and harvest resources from the environment. Colonies *reproduce* when they allocate these resources to sexual alates that disperse, mate, and found new colonies. My research focused on the ecological and evolutionary forces shaping colony size at reproduction, which governs nearly every aspect of ant form and function, and which spans from 10^{-5} to 10^{9} g across >14,000 ant species. I used comparative analyses to test hypotheses about colony life

history evolution (Chapter 1), field experiments in a Panamanian rainforest to examine whether resource limitation constrains colony traits (Chapter 2), and respirometry experiments examining how worker traits impact both whole colony energy demands (Chapter 3) and agricultural production in fungus growing ant societies (Chapter 4).

A wide range of taxa exhibit allometries of reproductive effort—the trend towards decreasing fecundity as species mature at larger body sizes. Similar patterns have long been assumed for ants and theory has sought to explain evolutionary trends towards increasing colony size if workers in larger colonies yield relatively fewer alates for their work. The comparative analysis I performed in Chapter 1 was the first test of this assumption. I found that despite an allometry of reproductive number, species with larger colonies tend to package relatively equivalent biomass into relatively fewer alates, suggesting a classic size-number tradeoff.

Bergmann's Rule describes the positive trend in body size and latitude. Ant colonies exhibit a version of this relationship, with tropical forests containing, on average, the planet's smallest colonies. In Chapter 2, I used a variety of manipulative field experiments to test whether resource limitation constrains colony size and abundance in tropical ant communities. I found that added food stimulated microbial decomposition of leaf litter habitat rather than colony growth, and that colony densities increased only when I added both food and nest sites. These results suggest that tropical litter ants are simultaneously limited by the availability of food and patchy leaf litter that becomes increasingly ephemeral when saturated with food.

Across ant species, worker metabolism is governed by the classic metabolic allometry reflecting lower mass-specific metabolic demands with increasing size.

However, little is known about whether this allometry also applies to larger workers within colonies. If larger workers are energetically cheaper to maintain, gram for gram, they may provide savings to their colonies by using relatively little energy not only when performing specialized tasks, but also when resting. In Chapter 3, I used respirometry experiments to link whole colony energy demands with worker size, using large major and small minor castes from three species in the genus *Pheidole* from Oklahoma grasslands. I found that behaviorally specialized *Pheidole* majors may save their colonies energy not only through task performance, but also because of maintenance costs that scale allometrically with both body and group size.

The rise of fungus cultivation in the ant tribe Attini has been called one of the major breakthroughs in animal evolution and provides striking parallels with human agriculture. In Chapter 4, I used respirometry experiments to examine the energetic costs and benefits of attine fungal cultivation, and test whether energetic efficiencies accompanied an evolutionary trend towards larger, more complex societies with increasingly domesticated cultivars. I found that fungi dominate colony energy demands because their mass far exceeds that of their ant farmers. In addition, although domesticated cultivars of more derived attines do more metabolic work for greater agricultural returns, energetic economies of scale suggest that larger colonies net greater fungal production from relatively less metabolic work. This metabolic allometry appears to favor the evolution of larger agricultural systems, and lays the groundwork for the evolution from basal attine species with < 100 workers to the derived 1 million worker superorganisms of the genus *Atta*.

CHAPTER 1

Ant colony size and the scaling of reproductive effort

(formatted for *Functional Ecology*)

ABSTRACT

1. Reproductive effort typically scales as $mass^{0.75}$ in unitary organisms, but less is known about such scaling in colonial organisms.
2. I compiled data on worker and reproductive number at maturity for 65 ant species and found an interspecific allometry (*alate number* = $worker\ number^{0.73}$) whose exponent was significantly less than 1, even after a phylogenetic correction.
3. When I analyzed 15 species for which biomass data were available, I found an interspecific isometry (*alate biomass* = $worker\ biomass^{0.89}$) whose exponent was not significantly different from 1. Analysis of maximum species biomass values, rather than averages, strengthened this isometry, yielding a slope b = 1.01 that was also not distinguishable from 1.
4. Species with larger colony size at reproduction tended to couple investment in proportionately fewer alates with investment in larger male and female alates.
5. This comparative analysis suggests a tradeoff between alate size and number and provides a framework for studying the diversity of colony life histories and the mechanisms generating allometries.

INTRODUCTION

From an organism's body size, it is possible to accurately predict many aspects of its physiology (Kleiber 1932, Brown et al. 2004), ecology (Brooks & Dodson 1965, Peters 1983, Kaspari 1993) and life history (Blueweiss et al. 1978, Sibly & Brown 2007). An important correlate of body size is that females of larger species tend to invest proportionately less energy in their offspring per unit time (Reiss 1989). Measures of reproductive output typically scale as a power law (aM^b) where $b < 1$ (Blueweiss et al. 1978, Sibly & Brown 2007), and data from unitary organisms (*colonies of cells*) suggest that reproductive allometry arises from metabolic allometry (Brown & Sibly 2006). Interestingly, colonial organisms (*colonies of individuals*) show similar patterns (Michener 1964, Hughes & Hughes 1986, Karsai and Wenzel 1998) despite some evidence that larger colonies do not have metabolic constraints (Lighton 1989, Martin 1991). Colonial organisms may instead face unique constraints on reproductive allocation and provide insights into the causes of whole-organism scaling (Glazier 2005, Edmunds 2006). To date, however, interspecific analyses of colony size and the scaling of reproductive effort have been lacking (Tschinkel 1991).

Ant (Hymenoptera: Formicidae) colonies provide a model system for comparative life history studies because they include more than 12,000 species (Bolton et al. 2006), and reproduce at sizes spanning over half the range of all animals (10^{-5} to 10^{9}g; Kaspari & Vargo 1995). Like unitary organisms, ant colonies make decisions about when to reproduce, and how much of a limited resource pool to allocate to reproduction (Pamilo 1991, Backus 1995, Herbers, DeHeer & Foitzik

2001). Although alternative strategies exist (*e.g.* Peeters 1991), colonies of most species *grow* by allocating energy to the production of sterile workers that care for developing brood, defend the nest, and harvest resources from the environment (Oster and Wilson 1978). Colonies *reproduce* when they allocate these resources to sexual alates that disperse, mate, and found new colonies (Oster & Wilson 1978). Since Michener (1964) empirically found a social insect reproductive allometry, theory has sought to explain how the advantages of large colony size compensate for the corresponding decrease in reproductive output per worker (Wenzel & Pickering 1991, Naug & Wenzel 2006). Even so, few have tested whether this pattern applies across social insect taxa (*but see* Karsai & Wenzel 1998).

Here, I first quantify the scaling of reproductive effort across the ants. I supplement this with a phylogenetic analysis using independent contrasts to examine the relationship between the evolution of larger colony size and reproductive effort (*e.g.* Warton et al. 2006). Correcting for phylogeny removes the statistical problem of non-independence among closely related species (Harvey & Pagel 1991). Agreement between methods may also bolster interpretations of the scaling of life history variables (Berrigan et al. 1993).

I next contrast the scaling of alate size and number, because evidence suggests a tradeoff observed for unitary organisms (Lack 1954, Smith & Fretwell 1975, Stearns 1992), also applies to ant colonies (Rosenheim, Nonacs & Mangel 1996). First, reproduction is costly to colonies because alates are generally larger and contain more energy than workers (Peakin 1972). Reproducing colonies further divert resources to alates at the expense of workers that decline in both mass and number (Tschinkel

1987, Tschinkel 1993). Second, colonies benefit from investing more per alate because alates are typified by high mortality rates (Tschinkel 1992, Frederickson 2006) and a positive relationship between size and fitness (Davidson 1982, Wiernasz & Cole 2003, Fjerdingstadt & Keller 2004). I test the prediction that larger colonies package proportionately equivalent mass into relatively fewer alates, such that numerical allometry ($b < 1$) will be offset by biomass isometry ($b = 1$).

The scaling of ant colony reproductive effort may also be shaped by unique genetic and ecological factors (Herbers 1990, Pamilo 1991, Sundstrom 1995, Ruppell, Heinze & Holldobler 2001, Fjerdingstadt 2005, Linksvayer 2006). Unlike cells in a body, individuals in a colony are not genetically identical, and the resulting relatedness asymmetries (RA) cause conflict over reproductive allocation (Hamilton 1964, Trivers & Hare 1976). RA may shape reproductive scaling because its strength depends on aspects of colony structure that may vary with colony size, such as queen number (mono- vs. polygyny), queen mating frequency (mono- vs. polyandry), and worker reproduction (Trivers & Hare 1976, Herbers 1990, Keller & Vargo 1993, Crozier & Pamilo 1996). For example, colonies of sterile workers headed by a single, once-mated queen have high RA and are predicted to bias investment towards female alates (Boomsma & Grafen 1990, Chapuisat & Keller 1999). Because female alates are generally larger than male alates (*e.g.* Herbers 1984), increasing RA with colony size would generate numerical allometry with larger colonies packaging resources as fewer, larger female alates. To test for systematic changes in sex allocation with colony size, I contrast the scaling of male and female alate number and size.

Measurements of reproductive allocation may also depend on how colonies

spread reproductive effort throughout their life. Annual colonies are semelparous and do not divide investment between current and future reproduction (Oster & Wilson 1978, Pamilo 1991, Herbers et al. 2001). Mature annual colonies may thus allocate relatively more to reproduction for their size than perennial colonies. Unfortunately, limited data availability precludes comparative analyses of the effects of lifetime reproductive schedules. I focus instead on seasonal reproductive phenology, because this also shapes how colonies spread reproductive effort across time. I contrast the scaling of temperate species (that tend to release alates in single, pulsed flights; Dunn et al. 2007) and tropical species (that tend to release alates gradually, over longer periods; Kaspari, Pickering & Windsor 2001).

Combined, these comparative analyses fill gaps in the study of social insect life history and facilitate comparison with unitary organisms. Scaling may also reveal constraints on life history evolution (*e.g.* Brown & Sibly 2006), and thus inform theoretical predictions regarding how genetic and environmental factors shape colony phenotypes.

MATERIALS AND METHODS

I examined the interspecific scaling of reproductive effort by combining colonies collected from a tropical forest in Panama during the summers of 2005-2007 with a literature review (>250 articles). I sought studies reporting both alate output (number or dry biomass) and colony size (worker number or total worker dry biomass) (see Appendix S1 in supplementary material). For 5 species, I pooled data from multiple studies (see Appendix S1). Because sampling limitations may affect the

precision of colony size estimates, I applied the following criteria for data selection. I generally avoided estimates without methods and those not published in peer-reviewed journals. I focused on estimates of worker and alate number determined from whole colony collections, and generally avoided mark and recapture data, the results of manipulative field and lab experiments and longitudinal studies. I assumed equal probabilities of underestimating worker number (*e.g.* some foragers will have in the field when the nest was harvested) and alate number (*e.g.* some may have dispersed before the nest was harvested).

I defined colony size as the total number and, when available, total biomass of adult workers. I defined reproductive output as alate number and, when available, biomass. I computed both mean and maximum values when articles provided a range of values. Analyzing maximum values may better represent a reproductive constraint by showing the upper limits on reproductive effort (Tschinkel 1993, Porter & Hawkins 2001). Some species have colonies with ambiguous boundaries because they simultaneously nest in multiple, spatially discrete sites (e.g. polydomy; *see review* Debout et al. 2007). Removing the data of known polydomous species (N = 15 species; determined from the citations in Supplementary S1 and Debout et al. 2007), however, did not change the results. To further control for variation due to polydomy, I only used data from my collections if colonies were monodomous and had a queen. I also avoided published data from extremely polydomous species (*e.g. Formica yessensis*; Higashi & Yamauchi 1979).

I used least square regression to estimate a and b in the scaling equation $\log_{10}y = \log_{10}a + b\log_{10}M$. Scaling characterized the dependence of reproductive output

(*alate number* or *dry biomass*; *y*) on colony size (*worker number* or *total worker dry biomass*; *M*). To detect allometry ($\boldsymbol{b} \neq 1$), I calculated an *F*-statistic to test the null hypothesis of isometry (H_0: $b = 1$). Testing different hypotheses required slopes from separate analyses of how $\log_{10}$(worker number) shaped 7 measures of reproductive effort (Table 2). I therefore Bonferroni adjusted the significance level for these comparisons at 0.007 (0.05/7). For all measurements of colony size and reproductive output, I calculated pWR = $\log_{10}(M_{max}/M_{min})$ as a standard measure of size range (Prothero 1986). I analyzed the scaling of sexual allocation (males vs. females) and region (temperate vs. tropical) with ANCOVA. For analysis by region, I used locality information within articles to assign species to temperate (>23° latitude) or tropical (<23°) groups (*as per* Kaspari & Vargo 1995; see Appendix S1). For analyses of sex allocation and colony size, I used queen number data from the original citation and other published accounts if necessary (see Appendix S1). Where relevant, I also used SMATR software (Warton et al. 2006) to test for differences in the intercept of regression lines if slopes were found to be not significantly different. This method uses ANOVA on residual scores as a test for common intercept of regression lines (Warton et al. 2006).

I further removed phylogenetic non-independence from the comparative analysis using Comparative Analysis by Independent Contrasts (CAIC software by Purvis & Rambaut 1995) and the molecular phylogeny of the ants by Brady et al. (2006). CAIC tests hypotheses of correlated evolution using evolutionary relationships (the topology) and distances (branch lengths) from a phylogeny to calculate standardized contrasts for pairs of sister species (*e.g.* estimating the trait value of the

common ancestor) that can then be subjected to traditional statistical tests (Purvis & Rambaut 1995). I set branch lengths to 1, assuming a punctuated model of evolution (Harvey & Pagel 1991) and grouped congeners as soft polytomies, analyzing them as single comparisons. Data were $\log_{10}$ transformed prior to calculation of contrasts, assuming that different lineages were equally likely to make the same proportional changes in size (Purvis and Rambaut 1995). The scaling of contrasts was analyzed using linear regression through the origin because this forces the regression line to include both the point of no evolutionary divergence and the centre of standardized data (Warton et al. 2006).

RESULTS

The scaling of alate number included 65 ant species from 35 genera, with worker number spanning 4.6 orders of magnitude (18-650,000) and alate number spanning 3.4 orders of magnitude (2-4,673) (Table 1). *Alate number* scaled as *worker number*$^{0.73}$ (R^2=0.78; Fig. 1) and was significantly less than isometry (F-test for b =1: $F_{1,63} = 29.9$, p = 0.0001; Table 2). Analysis using maximum values instead of averages, did not change the result (b = 0.78, R^2 = 0.76; F-test for b = 1: $F_{1,63} = 15.3$, p = 0.0002; Table 2). This allometry remained significant after a phylogenetic analysis with independent contrasts (R^2 = 0.75, $F_{1,42} = 124.8$, p = 0.0001; Fig 2; Table 1) and was significantly less than isometry (F-test for b = 1: $F_{1, 42} = 46.3$, p = 0.0001).

The scaling of alate biomass included 15 ant species from 8 genera, with worker biomass spanning 4.6 orders of magnitude (0.88 – 34,385 mg) and alate biomass spanning 5.4 orders of magnitude (0.08 – 17,738 mg) (Table 1). *Alate*

biomass scaled as *worker biomass*$^{0.89}$ ($R^2 = 0.95$; Fig. 3) and was not significantly different from isometry (F-test for $b = 1$: $F_{1,14} = 3.70$; $p = 0.08$; Table 2). This two-tailed test of H_0: $b = 1$, however, included only 15 species and thus had low power (power = 0.50, Zar 1999; page 385). Further analysis using maximum values strengthened the isometric relationship ($b = 1.01$, $R^2 = 0.96$; F-test for $b = 1$: $F_{1,14} = 0.050$, $p = 0.830$; Table 2). Tschinkel (1993; Fig. 25) also scaled the reproductive biomass of 6 ant species and found that maximum values supported isometry. These results suggest that alate number accumulates more slowly with colony size than total mass. In other words, larger colonies tend to package proportionately equivalent mass into relatively fewer alates.

To analyze how sex allocation shapes reproductive scaling, I first quantified the scaling of male and female alate number. Data from most of the 65 species included colonies producing both alate sexes and the scaling of male and female number included averages from 53 male producing species and 52 female producing species (N = 105), with some species being used for both analyses. Male and female alate numbers were regressed against average worker numbers using only colonies producing that sex. Although alate number generally increased with worker number ($p = 0.0001$), the relative number of male and female alates did not change ($p = 0.108$) (Table 3). The scaling of *female number* = *worker number*$^{0.58}$ ($R^2 = 0.633$; Fig 4a) and *male number* = *worker number*$^{0.72}$ ($R^2 = 0.817$; Fig 4b) were not significantly different ($p = 0.0850$; Table 3). After removing the interaction term, alate sex remained non-significant ($F_{2,102} = 0.001$, $p = 0.99$).

To gather sufficient data to analyze the scaling of male and female size with worker number, I combined published accounts of alate dry biomass with conspecific colony size estimates (see Appendix S2 in Supplementary Material). Average alate size generally increased with worker number ($p = 0.0001$), but it did not vary between sexes ($p = 0.920$) (Table 3). The scaling of *male size = worker number*$^{0.28}$ ($R^2 = 0.569$; Fig. 5a) and *female size = worker number*$^{0.41}$ ($R^2 = 0.637$; Fig. 5b) were not significantly different (Table 3). After removing the non-significant interaction term, however, females tended to be larger than males ($F_{1,55} = 18.08$, $p = 0.0001$; least square means of log alate biomass: female = 0.710, male = 0.373). The intercept of the regression for female size was significantly greater than the intercept for male size ($F_{1,28} = 11.7$, $p = 0.001$).

I next examined the effects of region. Alate number tended to increase with worker number ($p < 0.0001$), although neither tropical nor temperate colonies tended to have more alates ($p = 0.097$) (Table 3). The scaling of temperate (*alate number = worker number*$^{0.68}$, $R^2 = 0.695$) and tropical (*alate number = worker number*$^{0.73}$, $R^2 = 0.853$) species was not significantly different (Table 3). This result suggests that reproductive scaling does not result from sampling colonies with different phenologies.

DISCUSSION

Large ant colony size may enhance the ability to discover and defend resources (Wenzel & Pickering 1991, Holway & Case 2001, Palmer 2004), confer protection against adverse environmental conditions (Kaspari & Vargo 1995), and facilitate

increasingly complex colony-level behaviours (Beckers et al. 1989, Gordon 1995, Pacala, Gordon & Godfray 1996, Karsai & Wenzel 1998, Anderson & McShea 2001). This comparative analysis provides support for an additional benefit—the production of larger alates (Figs 5a and 5b). Numerical allometry (Figs 1 and 2) and biomass isometry (Fig. 3) suggest that species maturing at larger colony sizes tend to package proportionately equivalent mass into relatively fewer alates. This correlation suggests a reproductive tradeoff—that alate size and number compete for the allocation of limited resources (Stearns 1992).

The tradeoff between alate number and size may depend on which resource limits parental investment (Rosenheim et al. 1996). Alate size may also be constrained by a combination of environmental and genetic effects (Fjerdingstad 2005). Colonies may further allocate to alates based on fixed schedules of development (Backus 1995) or by weighing the benefits of current and future reproduction against a backdrop of sex allocation conflict (Pamilo 1991, Herbers et al. 2001). This comparative analysis supports the notion that these factors are constrained in a general way by colony size (Tschinkel 1993). Deviation above or below the general allometry may represent the fitness consequences of ecological innovation (*e.g.* Sibly and Brown 2007). For example, if polydomy reduces foraging costs by placing nest fragments closer to resources (van Wilgenburg & Elgar 2007), polydomous species may have relatively more energy for reproduction than predicted from their colony size. This analysis also facilitates comparison of lineages with different life histories. The subfamily Ponerinae contains *ca.* 100 species whose colonies lack queens and instead have mated, egg-laying workers (Peeters 1991). In these colonies, physical conflicts over

who reproduces (Heinze, Holldobler & Peeters 1994) may divert energy from reproduction to posturing behaviours (Gobin et al. 2003).

These analyses did not detect systematic changes in sex allocation. First, the relative number of male and female alates did not change with colony size (Table 3). Second, female alates were generally larger than males, but they did not become increasingly so with increasing colony size (Table 3). Although comparative analyses provide limited insights into how RA shapes sex allocation conflict (Boomsma 1989), relatedness within colonies may nevertheless vary as species mature at larger colony sizes. Interestingly, although queen number may increase with colony size within facultatively polygynous species (Elmes & Keller 1993, Sundstrom 1995), a logistic regression of monogynous and polygynous colonies within this data set did not find a significant relationship with colony size (Wald statistic = 2.77, $p = 0.1$, d.f. = 1). Further comparative studies will be needed to determine whether the evolution of larger colony size increases the probability of polyandry (Cole 1983, Crozier & Fjerdingstadt 2001, Kronauer, Johnson & Boomsma 2007) and worker reproduction (Herbers 1990, Snyder & Herbers 1991, Crozier & Pamilo 1996).

The effects of ecological constraints on reproductive scaling remain uncertain. If large colonies are better equipped to discover and defend resources (*e.g.* Holway & Case 2001), they may be able to invest in relatively more expensive female alates (Nonacs 1986, Peterson & Roitberg 2006). Alternatively, due to limitations of central place foraging, larger colonies may increasingly deplete local resources (Oster & Wilson 1978) causing workers to make longer, more energetically expensive foraging trips (Fewell et al. 1996). If these workers yield diminishing returns per foraging trip,

larger colonies may have proportionately fewer resources available for reproduction. The ecology of resource harvesting may be further complicated by interactions between the scaling of worker and colony size (*e.g.* Bourke 1999) because larger workers tend to have greater foraging efficiency (Davidson 1978, Kaspari 1993). These dynamics may be offset by systematic changes in foraging strategies with colony size (Beckers et al. 1989).

Scaling from colonial organisms may also help evaluate models predicting that metabolic constraints unify diverse taxa (Glazier 2005). Biologists have long sought to explain why metabolic rate (I) scales with body mass as a quarter power ($I = M^{0.75}$) from the smallest microbes (10^{-14} g) to the largest homeotherms (10^{7} g) (West, Brown & Enquist 1997, Brown et al. 2004, Glazier 2005). This metabolic allometry may constrain the life histories of unitary taxa because the proportionately slower metabolic rate of larger species limits the rate they allocate resources to reproduction (Brown & Sibly 2006). Larger ant colonies may also have proportionately slower metabolic rates because, like larger unitary organisms, they tend to yield proportionately fewer reproductive individuals (Figs 1, 2). Similar constraints may be expected because unitary and colonial organisms are both composed of highly interdependent life forms that behave as a single organism (*e.g.* ant workers in ant colonies, Queller 2002; host and microbial cells in metazoan bodies, Li et al. 2008). Furthermore, whole-organism metabolic scaling for both these biological types arises from how the numbers, sizes, and metabolic rates of their subunits (*e.g.* cells or workers) scale with body (Savage et al. 2007) or colony (Lighton 1989) size. It remains unclear, however, whether colony-level energetics represents the allometric

decline in per-worker respiration ($b < 1$; Gallé 1978, Bartholomew, Lighton, & Feener 1988), or the additive sum of worker respiration ($b = 1$; Lighton 1989, Martin 1991).

Colony life history data have been published for relatively few of the more than 12,000 known ant species (Tschinkel 1991, Kaspari & Vargo 1995). Of those species for which data exist, we have much to learn regarding how colony attributes change during colony development and throughout a colony's lifetime (Wilson 1985). The preceding analyses suggest strong colony-size dependence of important life history traits despite the limited availability and variable precision of colony-level data. Nevertheless, some important implications of these analyses remain to be fully explored. For instance, although female size increased faster with worker number ($b = 0.41$) than male size ($b = 0.28$), the slopes were not significantly different (Table 3). A tradeoff should favour ever-greater investment in females relative to males because female size may be more closely tied to fitness (Rosenheim et al. 1996). This is because female traits relate to how they disperse (Nonacs 1993) and found new colonies (Stille 1996, Keller & Passera 1989, Johnson 2002, Hahn et al. 2004), while males have few sexually selected traits and die shortly after mating (Boomsma, Baer & Heinze 2005). The scaling approach used here provides a framework for such analyses as data continue to become available.

ACKNOWLEDGEMENTS

I would like to acknowledge the support and mentorship of M. Kaspari without which this research would not have been possible. R. Bixenmann, R. Cothran, O. Fincke, K. Kapheim, M. Kaspari, J. Kelly, R. Knapp, and J. Mendoza provided many excellent

suggestions for improving this manuscript. G. Costa and D. Shepard helped with the statistical analyses. D. Donoso and L. Jara provided help in the field and O. Acevedo and B. Jimenez provided invaluable logistical support in Panama. An Adams Summer Research Fellowship from the Department of Zoology of the University of Oklahoma and a Smithsonian Tropical Research Institute Short Term Fellowship supported this research.

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Tables

Table 1 Summary Statistics for species averages of linear measurements. The measure pWR = $\log_{10}$ (max value / min value) is a standardized measure of the size range. * denotes dry biomass (mg)—otherwise, values are numbers of individuals.

Level	Species	Genera	Worker values		Alate values	
Alate Number	65	35	Mean	17,671	Mean	345
			Range	18 – 650,000	Range	2 – 4,673
			pWR	4.61	pWR	3.4
Alate Biomass	15	8	Mean	5,714*	Mean	1,632*
			Range	0.88 – 34385*	Range	0.08 – 17,738*
			pWR	4.59*	pWR	5.35*
Male Number	52	29	Mean	25,207	Mean	407
			Range	19 – 775,000	Range	1 4,880
			pWR	4.61	pWR	3.69
Female Number	51	27	Mean	6,432	Mean	154
			Range	17 – 109,143	Range	1 – 1,560
			pWR	3.81	pWR	3.19
Male Size	29	15	Mean	5,688	Mean	2.43*
			Range	24 – 58,112	Range	0.035 – 10.6*
			pWR	3.38	pWR	2.48*
Female Size	29	16	Mean	7,129	Mean	10.59
			Range	14 – 58,112	Range	0.09 – 59.5*
			pWR	3.62	pWR	2.82*

Table 2 Least squares mean regression for scaling relationships. For regressions of alate output against $\log_{10}$(worker number), deviation from isometry (b = 1.0) denoted by *(P < 0.007), the critical value set by a Bonferroni adjustment (see methods). *Avg.* and *Max.* refer to average and maximum species values. The *biomass* regressions denote the analysis of summed alate vs. summed worker biomass and used a critical value 0.05. *CAIC* is the phylogenetically independent contrasts regression, and its parameters have been calculated with the intercept (a) set to 0 (see methods).

Group	N	MS model	MS error	F	R^2	*a*	SE	*b*	SE	±95% CI
Numerical avg.	65	40.63	0.182	223.0	0.780	-0.400	0.145	0.73*	0.049	0.098
Numerical max.	65	45.00	0.231	195.0	0.756	-0.311	0.170	0.78*	0.056	0.113
CAIC	43	4.283	0.034	124.8	0.748	0.000	n/a	0.62*	0.056	0.111
Biomass avg.	15	31.46	0.121	260.2	0.952	-0.379	0.141	0.89	0.055	0.120
Biomass max.	15	38.14	0.122	313.2	0.960	-0.453	0.153	1.01	0.057	0.124
Male number	53	32.34	0.142	228.2	0.817	-0.441	0.147	0.72*	0.048	0.095
Female number	52	18.42	0.241	86.23	0.633	-0.061	0.182	0.58*	0.063	0.126
Male size	29	2.148	0.060	35.68	0.569	-0.407	0.137	0.28*	0.047	0.096
Female size	29	5.498	0.116	47.40	0.637	-0.430	0.179	0.41*	0.059	0.121

Table 3 Comparing the scaling of alate number and alate size against worker number for male and female alates and across regions. The result of ANCOVAs using worker number as a covariate.

Effect	Factor	df	Type III SS	F	p > F
Alate number	Log_{10} (worker number)	1	49.22	277	0.0001
	Alate Sex	1	0.467	2.63	0.1077
	Log_{10} (W #) x Sex	1	0.535	3.02	0.0850
	Error	101			
Alate Size	Log_{10} (worker number)	1	7.094	80.5	0.0001
	Alate Sex	1	0.001	0.01	0.9200
	Log_{10} (W #) x Sex	1	0.250	2.83	0.0980
	Error	54			
Region	Log_{10} (worker number)	1	36.89	229	0.0001
	Region	1	0.457	2.84	0.0970
	Log_{10} (W #) x Sex	1	0.054	0.34	0.5640
	Error	61			

Figure Captions

Figure 1 Log transformed relationship between alate number and worker number. Dashed line represents isometry. Each data point represents a species average.

Figure 2 Log transformed relationship between alate number and worker number using phylogenetically independent contrasts. See methods for details.

Figure 3 Log transformed relationship between total alate dry biomass and total worker dry biomass. Dashed line represents Isometry. Each data point represents a species average.

Figure 4a Log transformed relationship between male alate number and worker number.

Figure 4b Log transformed relationship between female alate number and worker number.

Figure 5a Log transformed relationship between individual male alate biomass and worker number.

Figure 5b Log transformed relationship between individual female alate biomass and worker number.

Figure 1

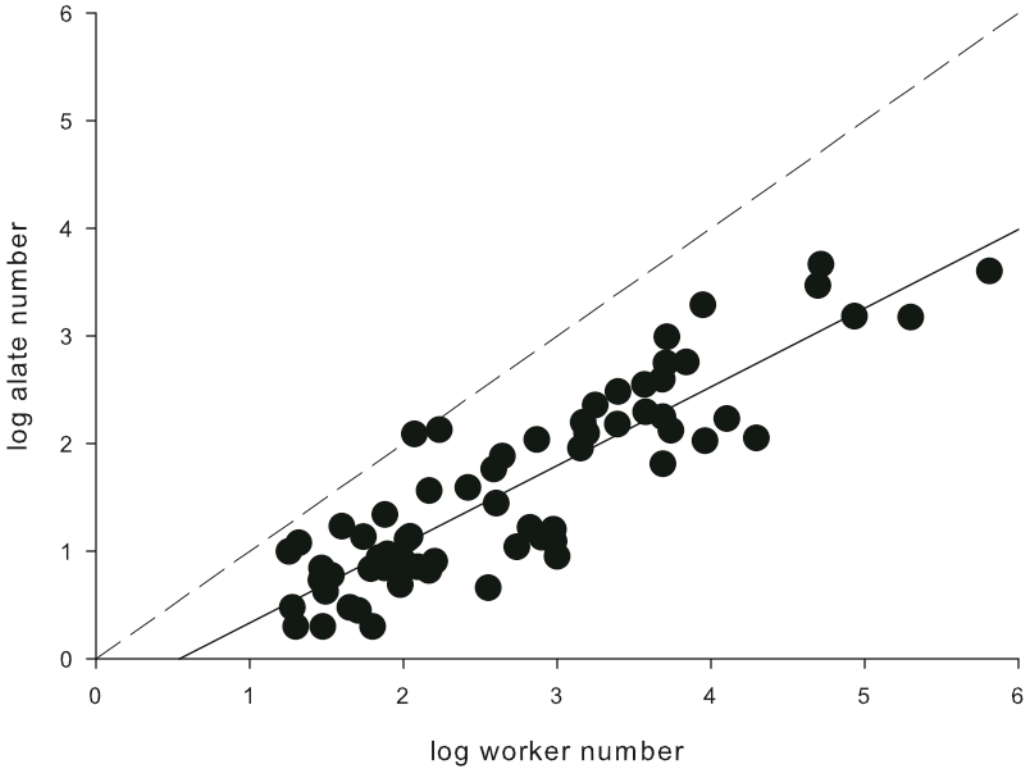


Figure 2

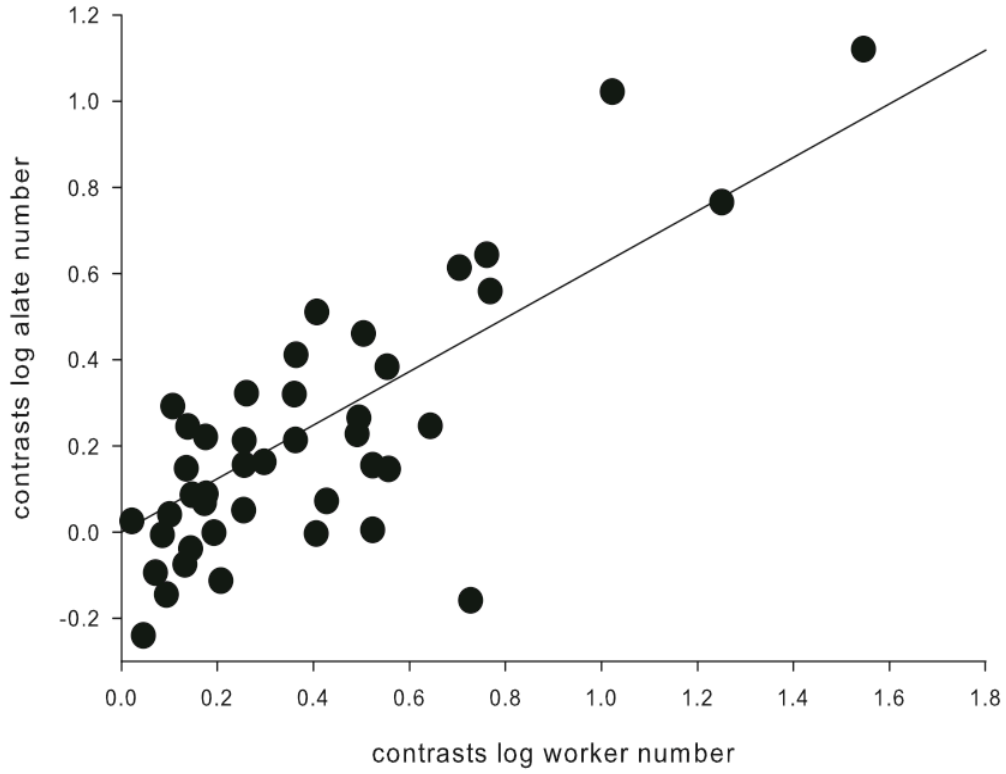

contrasts log alate number
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
contrasts log worker number

Figure 3

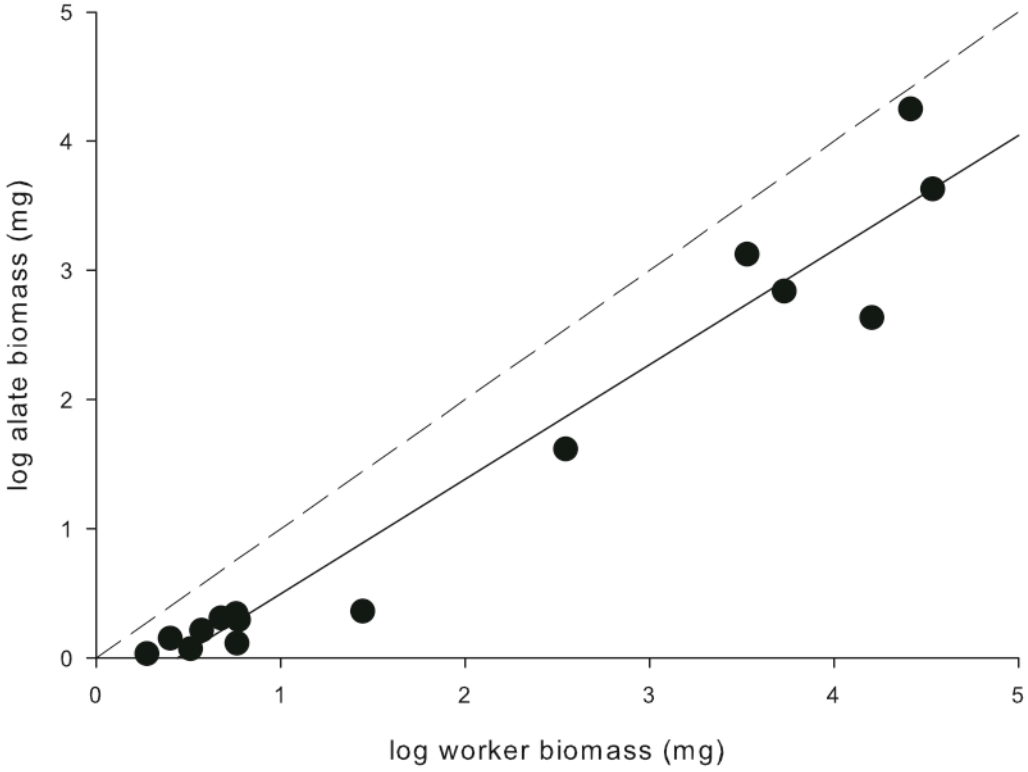


Figure 4a

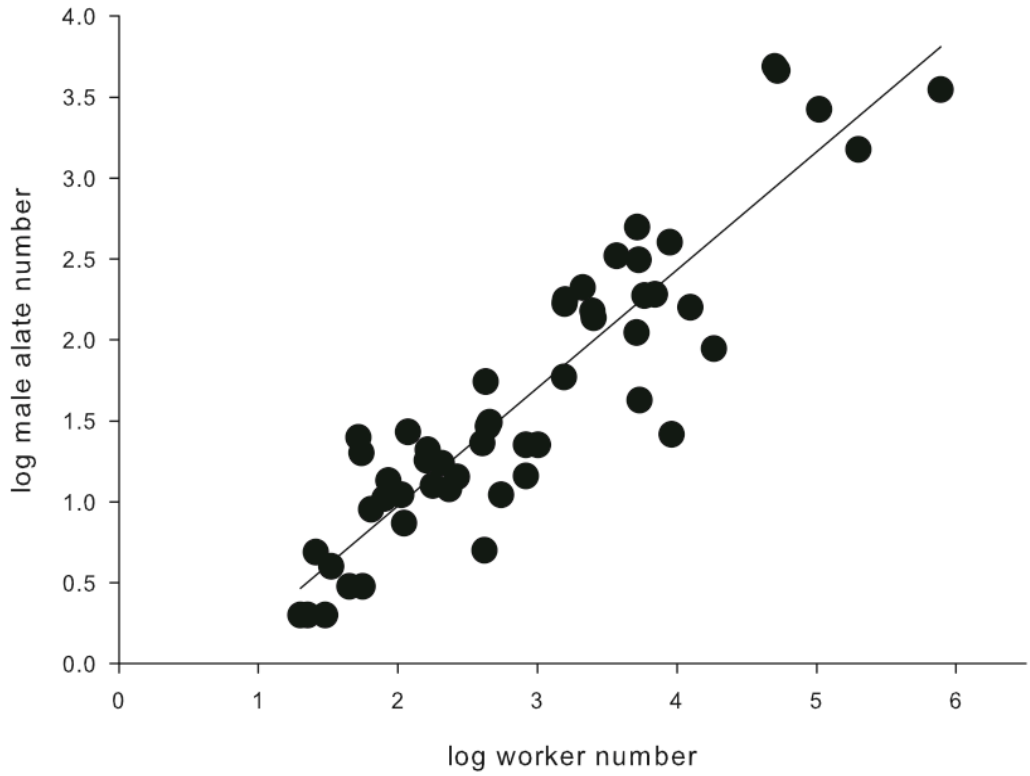

log male alate number
log worker number
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
1
2
3
4
5
6

Figure 4b

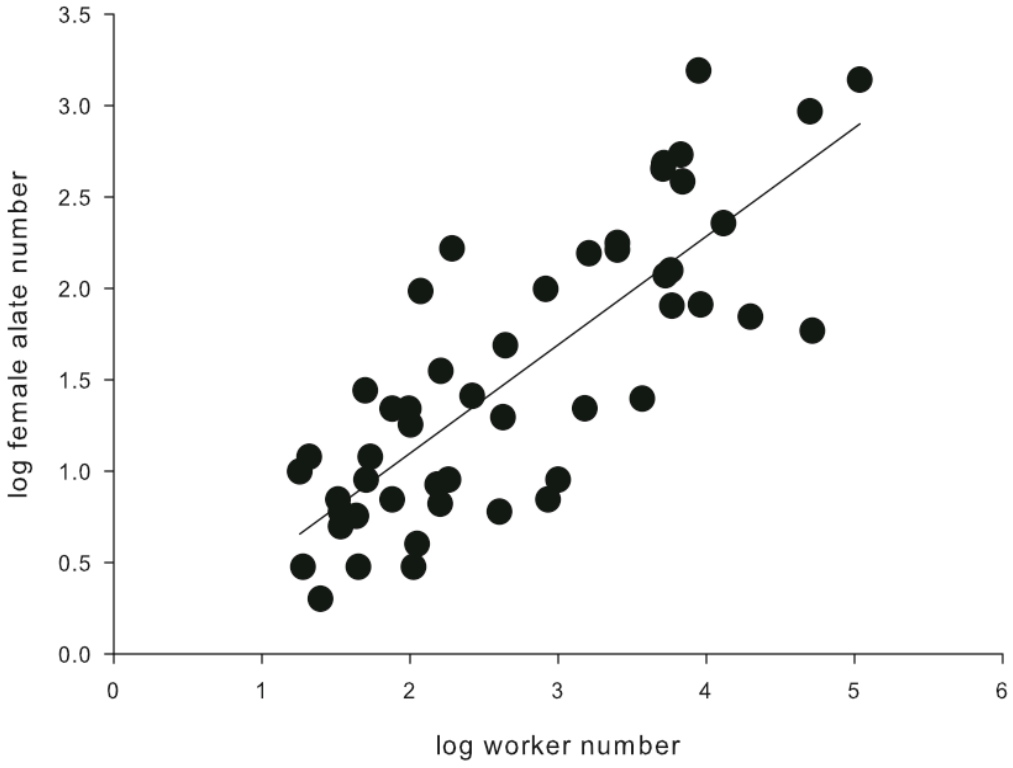

log female alate number
log worker number
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
1
2
3
4
5
6

Figure 5a

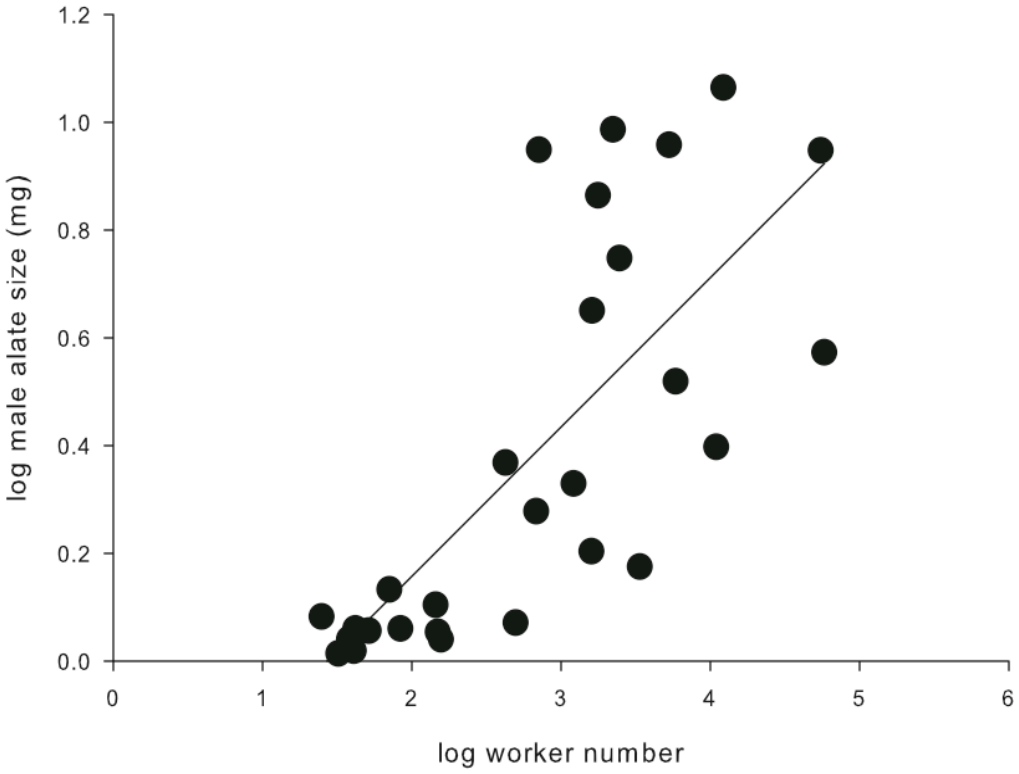
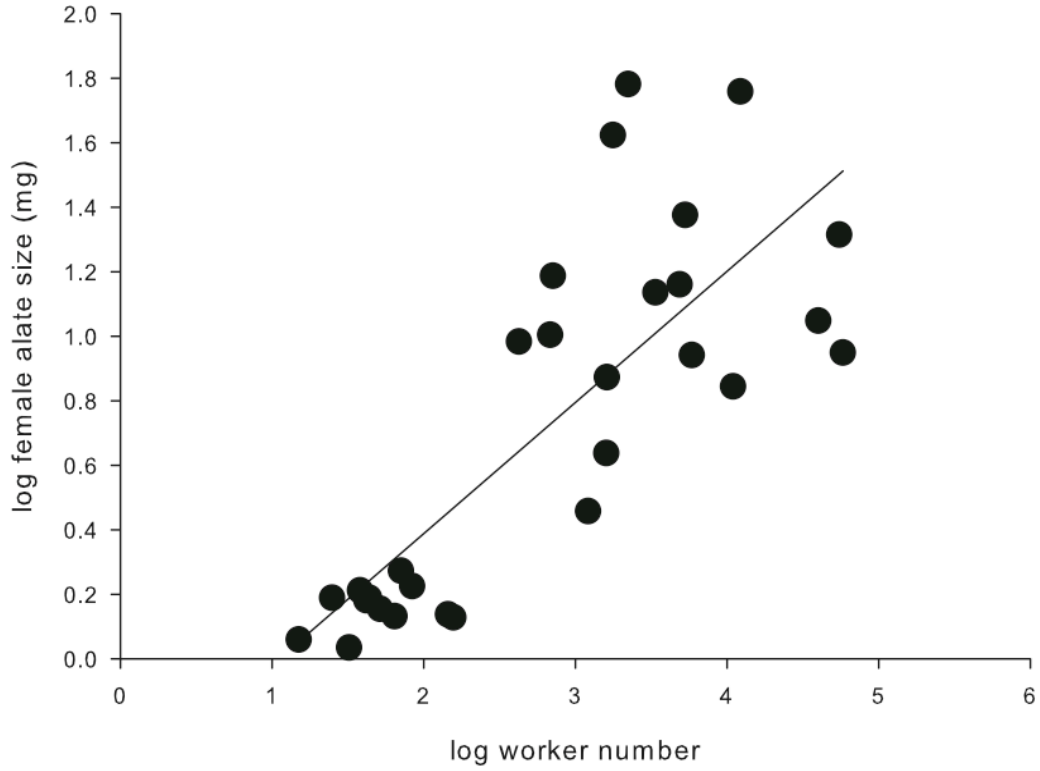

log male alate size (mg)
log worker number
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
1
2
3
4
5
6

Figure 5b


log female alate size (mg)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
log worker number

Appendix S1 Data used for scaling. Abbreviations: *log w #* = $\log_{10}$(worker number + 1), *log (a #)* = $\log_{10}$(alate number +1), *log (w bm)* = $\log_{10}$(summed dry worker biomass + 1), *log (a bm)* = $\log_{10}$ (summed dry alate biomass + 1). All measurements dry biomass in mg. N signifies number of colonies in the sample. TE refers to temperate region (>23° latitude), TR refers to tropical region (<23° latitude). Q #: queen number for colonies used in this analysis (1 = 1 queen, 2 = >1 queen, B = both 1 and multiply queened colonies).

subfamily	genus	species	N	log (w #)	log (a #)	log (w bm)	log (a bm)	Q #	region	source
Amblyoponiinae	*Amblyopone*	*sp.*	1	2.0043	0.9031	0.3305		1	TR	18
Amblyoponiinae	*Amblyopone*	*pluto*	1	1.2788	0.4771			1	TR	13
Amblyoponiinae	*Apomyrma*	*stygia*	1	1.8808	1.3424	0.3145		2	TR	5
Amblyoponiinae	*Onychomyrmex*	*hedleyi*	11	2.9789	1.0921	0.5549		1	TR	32
Aneuretinae	*Aneuretus*	*simoni*	16	1.4636	0.7333			B	TR	20
Dolichoderinae	*Iridomyrmex*	*purpureus*	7	4.6969	3.4704			B	TE	15
Ecitoninae	*Eciton*	*burchellii*	1	5.8129	3.6028	6.7672		1	TR	12, 40
Ecitoninae	*Eciton*	*hamatum*	1	5.3010	3.1781			1	TR	40
Ectatomminae	*Rhytidoponera*	*chalybaea*	21	2.6442	1.8871	0.5867		1	TE	44
Ectatomminae	*Rhytidoponera*	*confusa*	65	2.4228	1.5922	0.5603		1	TE	44
Ectatomminae	*Rhytidoponera*	*metallica*	37	2.0458	1.1386			2	TE	42
Formicinae	*Camponotus*	*ferrugeneus*	6	3.2497	2.3592			1	TE	35
Formicinae	*Camponotus*	*herculeanus*	4	3.5759	2.2986	0.7553		1	TE	2, 38
Formicinae	*Camponotus*	*laevigatus*	1	3.0004	0.9542			1	TE	2
Formicinae	*Camponotus*	*modoc*	7	4.1035	2.2308			B	TE	2
Formicinae	*Camponotus*	*noveboracensis*	1	3.9494	3.2925			1	TE	37
Formicinae	*Camponotus*	*pennsylvanicus*	4	3.3958	2.4853			1	TE	35
Formicinae	*Camponotus*	*solon*	1	3.5680	2.5502	5.1377		n/a	TR	26

Formicinae	*Formica*	*japonica*	1	3.3939	2.1790	3.7301	2.8382	1	TE	23
Formicinae	*Formica*	*podzolica*	24	3.1934	2.0990			n/a	TE	39
Formicinae	*Lasius*	*carniolicus*	4	2.2348	2.1320			1	TE	8
Formicinae	*Lasius*	*flavus*		3.6889	1.8137			1	TE	33
Formicinae	*Polyrhachis*	*hodgsoni*	1	3.9635	2.0294			1	TR	10
Formicinae	*Prenolepis*	*imparis*	10	3.1534	1.9565			B	TE	41
Myrmeciinae	*Myrmecia*	*desertorum*	1	2.0719	2.0899			2	TE	14
Myrmeciinae	*Myrmecia*	*dispar*	2	1.8973	0.9771			1	TE	14
Myrmeciinae	*Myrmecia*	*mandibularis*	1	2.6053	1.4472			1	TE	14
Myrmeciinae	*Myrmecia*	*nigrocincta*	2	2.8669	2.0384			1	TE	14
Myrmeciinae	*Myrmecia*	*picta*	1	1.3222	1.0792			1	TE	14
Myrmeciinae	*Myrmecia*	*varians*	2	2.1682	1.5652			1	TE	14
Myrmeciinae	*Myrmecia*	*vindex*	2	2.2037	0.9065			1	TE	16
Myrmeciinae	*Myrmecia*	*pilosula*	2	2.8239	1.2236			1	TE	14, 16
Myrmicinae	*Aphaenogaster*	*rudis*[1]	16	2.5895	1.7634	2.7431	2.1518	B	TE	4
Myrmicinae	*Basiceros*	*manni*	2	1.6532	0.4771	0.5356		1	TR	49
Myrmicinae	*Crematogaster*	*artifex*	1	4.7183	3.6696			1	TR	36
Myrmicinae	*Crematogaster*	*dohrni rogenhoferi*	1	3.7100	2.7505			n/a	TR	36
Myrmicinae	*Cyphomyrmex*	*rimosus*	1	1.2553	1.0000			B	TR	Shik unpub.
Myrmicinae	*Erebomyrma*	*urichi*	1	2.7396	1.0414	0.3382		2	TR	47
Myrmicinae	*Leptothorax*	*curvispinosus*	2	1.7403	1.1371			1	TE	17
Myrmicinae	*Leptothorax*	*longispinosus*	14	1.4700	0.8431			B	TE	17
Myrmicinae	*Myrmicaria*	*eumenoides*	2	4.2975	2.0535	4.5425		n/a	TR	28
Myrmicinae	*Pheidole*	*dentata*	2	2.9770	1.2041	0.4893		1	TE	9
Myrmicinae	*Pheidole*	*multispina*	8	1.9810	0.6886	0.1795	0.1633	1	TR	21, Shik unpub.
Myrmicinae	*Pheidole*	*nigricula*	28	1.7908	n/a	0.4008	0.1527	1	TR	21
Myrmicinae	*Pheidole*	*rugiceps*	9	2.1662	0.8216	0.3038	0.2814	1	TR	21, Shik unpub.
Myrmicinae	*Pheidole*	*ruida*	23	2.0945	0.8544	0.6764	0.3128	1	TR	Shik unpub
Myrmicinae	*Pheidole*	*specularis*	11	1.8784	n/a	0.5727	0.2151	1	TR	21
Myrmicinae	*Pogonomyrmex*	*californicus*	1	3.6882	2.2480			1	TE	11
Myrmicinae	*Pogonomyrmex*	*montanus*	39	3.1711	2.1986	3.5287	3.1232	1	TE	30

Myrmicinae	*Pogonomyrmex*	*occidentalis*	1	3.7145	2.9917			1	TE	24
Myrmicinae	*Pogonomyrmex*	*rugosus*	4	3.6852	2.6006	4.5364	3.6290	1	TE	30
Myrmicinae	*Pogonomyrmex*	*subnitidis*	7	3.7404	2.1238	4.2057	2.6363	1	TE	30
Myrmicinae	*Procryptocerus*	*scabriusculus*	1	1.7993	0.3010			2	TR	45
Myrmicinae	*Pyramica*	*emmae*	1	1.3010	0.3010			1	TR	Shik unpub.
Myrmicinae	*Solenopsis*	*MEK-005*	4	1.8430	0.9428			B	TR	Shik unpub.
Myrmicinae	*Solenopsis*	*invicta*[2]	57	4.9351	3.1836	4.6382	3.8379	1	TE	43
Myrmicinae	*Stenamma*	*debile*	108	1.6004	1.2345			B	TE	7
Myrmicinae	*Terataner*	*alluaudi*	2	1.7081	0.4515			B	TR	92
Myrmicinae	*Wasmannia*	*auropunctata*	1	1.8808	0.8451			1	TR	Shik unpub.
Ponerinae	*Gnaptogenys*	*hartmani*	1	1.5315	0.7782	0.3300		1	TR	Shik unpub.
Ponerinae	*Leptogenys*	*chinensis*	2	2.5545	0.6611	0.6014		2	TR	31
Ponerinae	*Pachycondyla*	*krugeri*	6	1.7890	0.8395			2	TR	46
Ponerinae	*Pachycondyla*	*marginata*	7	2.9019	1.1312			B	TR	25
Ponerinae	*Platythyrea*	*quadridenta*	4	1.4949	0.6276			B	TR	19
Ponerinae	*Ponera*	*pennsylvannica*	1	1.4771	0.3010			1	TE	34
Ponerinae	*Proceratium*	*goliath*	1	2.0253	1.1139	0.5107		1	TR	Kaspari unpub.
Pseudomyrmecinae	*Tetraponera*	*sp.*	1	3.8422	2.7604			1	TR	6

[1] Biomass values for *A. rudis* calculated as follows. Worker and alate numbers calculated from Table using values from Table 1 and 2 from Bono and Heithaus (2002). Dry worker biomass (1.3 mg) taken from Lynch, Balinsky and Vail (1980) and multiplied by worker number. Dry male alate (0.48 mg) and female alate (6.1 mg) biomass values taken from Trivers and Hare (1976) and multiplied by alate number.

[2] Biomass values for *S. invicta* are average of June colonies (*i.e.* peak reproduction during an annual cycle *as per* Tschinkel 1993; Fig. 25).

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Appendix S2 Data used to scale individual male and female biomass with worker number. Abbreviations: *log w #* = $\log_{10}$(worker number + 1), *log (m bm)* = $\log_{10}$(individual male biomass + 1), *log (f bm)* = $\log_{10}$(individual female biomass + 1). All measurements dry biomass in mg.

subfamily	genus	species	log w#	log (m bm)	log (f bm)	source
Formicinae	*Camponotus*	*ferrugineus*	3.2497	0.8645	1.6251	4, 9
Formicinae	*Camponotus*	*herculeanus*	4.0878	1.0645	1.7597	4, 9
Formicinae	*Camponotus*	*pennsylvanicus*	3.3469	0.9868	1.7818	4, 9
Formicinae	*Formica*	*japonica*	3.3939	0.7482		6
Formicinae	*Formica*	*lugubris*	4.6021		1.0492	4, 5
Formicinae	*Formica*	*pallidefulva*	2.8537	0.9494	1.1875	4, 9
Formicinae	*Lasius*	*flavus*	3.6889		1.1614	4, 5
Formicinae	*Prenolepis*	*imparis*	3.5278	0.1761	1.1367	4, 9
Myrmicinae	*Acromyrmex*	*octospinosus*	4.7404	0.9479	1.3151	4, 9
Myrmicinae	*Aphaenogaster*	*rudis*	2.6294	0.1720	0.8510	1, 9
Myrmicinae	*Aphaenogaster*	*treatae*	2.8344	0.2788	1.0043	4, 9
Myrmicinae	*Cyphomyrmex*	*rimosus*	1.1761		0.0611	Shik unpub.
Myrmicinae	*Leptothorax*	*ambiguus*	1.5798	0.0414	0.2122	4, 9
Myrmicinae	*Leptothorax*	*curvispinosus*	1.9243	0.0607	0.2253	4, 9
Myrmicinae	*Leptothorax*	*longispinosus*	1.6397	0.0573	0.1903	2
Myrmicinae	*Megalomyrmex*	*sp.*	1.6128	0.0197		Shik unpub.
Myrmicinae	*Myrmecina*	*americana*	1.3979	0.0828	0.1903	4, 9
Myrmicinae	*Myrmica*	*ruginodis*	3.0853	0.3304	0.4579	4, 9
Myrmicinae	*Pheidole*	*multispina*	2.1968	0.0407	0.1283	Shik unpub.
Myrmicinae	*Pheidole*	*pallidula*	3.2044	0.2041	0.6385	4, 9
Myrmicinae	*Pheidole*	*radzoskowskii*	2.6982	0.0718		Shik unpub.
Myrmicinae	*Pheidole*	*rugiceps*	2.1750	0.0548		Shik unpub.
Myrmicinae	*Pheidole*	*ruida*	2.1615	0.1050	0.1378	Shik unpub.

Myrmicinae	*Pogonomyrmex*	*montanus*	3.2097	0.6513	0.8739	7
Myrmicinae	*Pogonomyrmex*	*rugosus*	3.7252	0.9581	1.3771	7
Myrmicinae	*Pogonomyrmex*	*subnitidis*	3.7673	0.5198	0.9420	7
Myrmicinae	*Pyramica*	*gundlachi*	1.5098	0.0148	0.0364	Shik unpub.
Myrmicinae	*Solenopsis*	*invicta-june*	4.7643	0.5736	0.9502	9
Myrmicinae	*Solenopsis*	*MEK-005*	1.8107		0.1332	Shik unpub.
Myrmicinae	*Stenamma*	*brevicorne*	1.8513	0.1335	0.2742	4, 9
Myrmicinae	*Stenamma*	*debile*	1.7127	0.0569	0.1553	2
Myrmicinae	*Stenamma*	*diecki*	1.6232	0.0607	0.1818	4, 9
Myrmicinae	*Tetramorium*	*caespitum*	4.0404	0.3979	0.8451	4, 9

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CHAPTER 2

More food, less habitat: how necromass and leaf litter decomposition combine to regulate a litter ant community

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ABSTRACT

1. In brown food webs of the forest floor, necromass (*e.g.* insect carcasses and frass) falling from the canopy feeds both microbes and ants, with the former decomposing the homes of the latter. In a tropical litter ant community, we added necromass to 1-m^2 plots, testing if it added as a net food (increasing ant colony growth and recruitment) or destroyer of habitat (by decomposing leaf litter).

2. Maximum, but not mean, colony growth rates were higher on +food plots. However, neither average colony size, nor density was higher on +food plots. In contrast, +food plots saw diminished availability of leaf litter and higher microbial decomposition of cellulose, a main component of the organic substrate that comprises litter habitat.

3. Furthermore, necromass acted as a limiting resource to the ant community only when nest sites were supplemented on +food plots in a second experiment. Many of these +food +nest plots were colonized by the weedy species *Wasmannia auropunctata*.

4. Combined, these results support the more food-less habitat hypothesis and highlight the importance of embedding studies of litter ant ecology within broader decomposer food web dynamics.

INTRODUCTION

Food limitation is the basic assumption underlying bottom-up regulation of populations (Hairston *et al.* 1960; Oksanen *et al.* 1981; Power 1992), biomass (Sterner & Elser 2002; Brown *et al.* 2004; Kooijman *et al.* 2004), and diversity (MacArthur 1972; Tilman 1982; Rosenzweig 1996). Evidence for food limitation comes when individuals with more access to food increase rates of growth or reproduction, increase recruitment to rich patches, or both. However, studies of food limitation among terrestrial consumers rarely distinguish between growth and recruitment (*but see* Power 1984; Osenberg & Mittelbach 1996; Letourneau & Dyer 1998).

Tropical litter ants (Hymenoptera: Formicidae) are an ideal system in which to explore the mechanisms of food limitation. Litter ants nest in twigs and between leaves that fall from the forest canopy (Kaspari 1996a; McGlynn 2006). Their small colony size (many mature with <100 workers; Wilson 1959; Kaspari 1996b) and frequent nest relocation (*e.g.* every 34-100 days; Byrne 1994) promotes their patchiness at small scales (*e.g.* 0 to 23 colonies m^{-2}; Kaspari 1996b). Litter ant colony growth and reproduction can be measured as the number or biomass of pupae relative to workers (Foitzik *et al.* 2004; Gammans *et al.* 2005; Fokuhl *et al.* 2007) and the presence of new reproductives (winged alates; MacKay 1985; Deslippe & Savolainen 1994; Aron *et al.* 2001; Bono & Herbers 2003; Brown & Keller 2006). If litter ants are food limited, one would predict higher growth rates, reproduction, and recruitment to supplemented plots.

However, the necromass (*e.g.* faeces and dead insects) that falls to the forest floor feeds the entire brown food web in which litter ants are embedded. This can lead

to indirect interactions that trump direct food limitation. For example, microbes that attack necromass may also attack the ant's leaf litter habitat. As a consequence, microbes may be both direct competitors for food and indirect competitors via the decomposition of leaf litter (Kaspari & Yanoviak *in press*). In short, any increase in decomposition—even as it feeds the brown web by converting detritus to microbial biomass—comes at the cost of decreased habitat space.

Currently, there is little evidence that food limitation accounts for the patchiness and small size of tropical litter ant colonies (Kaspari 1996a; *but see* McGlynn & Owen 2002, McGlynn 2006). Toward a partial remedy, we report the results of a two-month press experiment in a tropical litter food web, contrasting +food necromass plots with control plots, and those later supplemented with nest sites. We find that necromass acts most strongly as a limiting resource by enhancing recruitment when nest sites are also available.

MATERIALS AND METHODS

Experiments were performed from 11 May to 15 Aug 2007 on Barro Colorado Island (9° 09′ N, 79° 51′ W), a lowland tropical forest managed by the Smithsonian Tropical Research Institute in Panama. BCI receives *ca*. 2600 mm of annual rainfall with nearly 90% falling from May to December (Croat 1978; Leigh *et al.* 1982). Sampling thus occurred from early to mid wet season on BCI—a period of high ant activity (Levings 1983; Kaspari 1996b).

Food press experiments were performed at two sites on BCI matched for habitat type, plant community and flat topography—Conrad Catchment and Barbour 9

(Leigh *et al.* 1982). At each site, 20 control and 20 +food plots were arrayed equally along 4 transects, 15 m apart. The 1-m^2 plots were 8 m apart on each transect, and control and +food treatments were randomly assigned. Every third day, +food plots received 6 g of homogenized insects (mostly katydids, cicadas and scarab beetles) collected at light traps, frozen, and homogenized for 1 minute in a small Black and Decker food processor. Half the plots were randomly selected for harvest (equal numbers of control and +food) after one month and the rest were sampled after two months.

Litter invertebrates (*e.g.* Acari, Araneae, Collemobola, Coleoptera, Diplopoda) were extracted with Berlese funnels from the leaf litter of 10 control plots harvested on 12 Aug 2007. The standing crop dry biomass of these organisms ranged from 0.09 g to 1.2 g m^{-2}. Thus, 6 g insect necromass addition m^{-2} represented a substantial increase over ambient conditions.

Prior to food addition, baiting trials were performed to confirm that ants harvested insect necromass. At both sites, 20 baiting stations were placed adjacent to experimental plots at 5 m intervals along 2 parallel transects. Each baiting station consisted of 4 g insect necromass, placed on a 3 x 5 inch note card and checked for ants after 45 min and 1.5 hr.

Ant colonies were harvested after 1 and 2 months by searching all leaf litter in plots and cracking all twigs and seedpods, using a headlamp when necessary (*ca.* 1 plot harvested hr^{-1}; *as per* Kaspari 1996a,b). Colonies were stored in plastic bags. Back at the lab, colonies were identified and surveyed for larvae, pupae, workers, queens, male and female alates using a dissecting scope. After counting, all

components were frozen, dried at 60°C for 2 days and then weighed to the nearest 1 μg. Vouchers of species and morphospecies (henceforth species) are deposited in the collection of MK at the University of Oklahoma.

Do ants grow and reproduce more on +food plots?

To test for higher growth rates on +food plots, pupae-worker curves were constructed (*as per* Kaspari 1996b) for species with ≥ 15 colonies on both food and control plots and for all colonies with queens. These curves characterized the dependence of pupae number (y) on worker number (M) using ordinary least square regression to estimate a and b in the scaling equation $\log_{10}y = \log_{10}a + b\log_{10}M$. To test the null hypothesis of isometry (H_0: $b = 1$), F-statistics were calculated. ANCOVAs were used to examine variation in scaling exponents (b) across feeding treatments. The relationships between worker number and pupae number sometimes yielded triangular relationships suggesting constraint functions (Brown 1995; Cade & Noon 2003). To test whether food addition removed an upper constraint on colony growth, least absolute deviation regression was used to describe scaling for the upper ninetieth quantiles of these plots (Koenker 2005; Cade & Richards 2005).

Using $\log_{10}$ transformed data, we also tested for increasing colony size (worker number or dry biomass of workers, pupae, and queens) with food addition among the common species and across all colonies with queens. ANOVA was used to test whether colony size increased on +food plots from month 1 to 2 and ANCOVA was used to test whether food addition increased mean colony size m^{-2} after controlling for litter depth. Finally, as reproductives were relatively rare, the biomass of reproductives

on control and +food plots was compared using a non-parametric Mann-Whitney (MW) test.

Do ant colonies accumulate on +food plots?

Using $\log_{10}$ transformed data, ANOVA was performed to test whether food addition increased the density of nests m^{-2} after 1 and 2 months. ANCOVA was then performed with litter depth as a covariate.

Does litter habitat decompose more quickly on +food plots?

Litter depth (two corners and plot centre) was recorded at the outset and harvest of each plot using a ruled pvc rod inserted through litter to mineral soil. In each plot, decomposition rates were estimated during the first month by measuring mass lost from two discs (96 mg) of coarse filter paper (100% cellulose, Fisher 09-795C) inserted in 10 x 10 cm polyester litterbags and placed under leaf litter (*as per* Milton & Kaspari 2007). These closed litterbags excluded taxa larger than 100 microns and thus provided estimates of microbial decomposition. After 1 month in the field, the remaining cellulose was harvested, dried for 2 days at 60ºC, and mass loss measured to the nearest 0.01g. We tested if mass loss of cellulose increased (ANOVA), and litter depth decreased (paired t-test on *initial – final* litter depth) on +food plots.

Next, artificial nest sites were added to test whether providing important components of habitat structure would enhance recruitment to +food plots. Once plots were harvested after both 1 and 2 months, we 1) returned litter (without colonies and

their nest sites) to these plots and 2) added 4 artificial nests (8cm x 0.25cm hollow bamboo internodes) to 0.25-m^2 zones within the original plots. Previous experiments have shown that litter ants readily colonize hollow internodes (*e.g.* Herbers 1986; Kaspari 1996b; Armbrecht *et al.* 2004). These bamboo-seeded plots were maintained as +food (4 g homogenized insects added every 3 days) or control. Half of these plots were harvested after 2 weeks and the rest after 1 month. Colonies were collected from bamboo and processed as above.

RESULTS

Overall, 634 colonies (298 control, 335 +food) were harvested from 80 1-m^2 plots. Of the 63 species collected from 24 genera (Table S1), 20 occurred only once (11 on +food, 9 on control plots). Species richness did not differ between control (mean: 4.6 ± 2.0; range: 1-9 species) and +food plots (mean ± S.D.: 5.6 ± 2.9; range: 1-15 species) (ANOVA: $F_{1,77} = 2.61$; $p = 0.11$). Seven species (*Cyphomyrmex rimosus* Spinola 1851, *Solenopsis* sp. 2, *Solenopsis* sp. 3, *Pheidole multispina* Wilson 2003, *Pheidole rugiceps* Wilson 2003, *Pheidole ruida* Wilson 2003, and *Pyramica brevicornis* Mann 1922) were found ≥ 15 times on both +food and control plots and met our criteria as "common".

Ants rapidly mobilized at necromass baits and 95% were occupied after 45 minutes. All common species except *P. brevicornis* and *C. rimosus* were observed at baits. Overall, baiting once per site (for only 1.5 hr) yielded 24 species (60% of all harvested colonies) and 12 genera (76% of all harvested colonies).

Of all sampled colonies, 394 had queens (control plots: 189; +food plots: 205) and 43 of these were lone foundress queens (control plots: 18; +food plots: 25). The queen colony subset was used to measure population-level responses to food addition, and all harvested colonies were used for community-level analyses of biomass. Because ant community structure and colony responses to food addition were consistent across the two sites on BCI, data were combined for analyses.

Do ants grow and reproduce more on +food plots?

Least square regression analyses of all harvested colonies with queens suggested that growth did not increase with food addition. First, pupa-worker curves did not differ between colonies from control ($b = 0.70$) or +food ($b = 0.79$) plots (Table 2). Second, the slope for the growth curve remained significantly less than isometry after food addition (F-test for $b = 1$: $F_{1,203} = 21.22$, $p = 0.0001$) (Table 1). However, the triangular nature of these relationships (Fig. 1) suggested that worker number was necessary, but not sufficient to explain variation in pupae number across colonies. At the ninetieth quantile, the upper edge of this triangle, the slope ($b \pm 95\%$ C.I) from +food plot colonies (0.91 ± 0.13) overlapped with isometry and was 0.2 greater than the slope for control colonies (0.71 ± 0.2) (Table 1).

Growth curves following food addition varied considerably among common species (Fig. 2), including slopes significantly less than ($b = 0.49$; *C. rimosus*) and significantly greater than ($b = 1.62$; *S.* sp. 3) isometry (Table 1). Colonies of *P. multispina* and *P. ruida* had steeper growth curves on + food plots, but these were not significantly different from their respective control plot curves (Table 2). Regressions

through ninetieth quantiles yielded slopes that were generally within <0.10 of slopes from least square regressions (Table 1). Notable exceptions were colonies of *S.* sp. 3 and *P. ruida* from +food plots (Fig. 2) that, respectively had ninetieth quantile slopes 0.43 and 0.33 greater than control plot colonies (Table 1). For these species, food addition may have relieved an upper constraint on colony growth.

The number of workers per colony and mean colony biomass increased on all plots by month 2, but colonies on +food plots were not larger than those from control plots (Table 3). Likewise, total colony biomass summed per m^2 increased 31% across all plots by month 2 (from 21.7 ± 18.9 mg m^{-2} to 31.5 ± 23.2 mg m^{-2}; ANOVA: $F_{1,77}$ = 5.12, p = 0.03). However, none of the focal species had significantly larger colonies after two months.

Overall, 54 colonies had reproductive adults or pupae (24 control, 30 +food). Colonies on +food plots (lower quartile, median, upper quartile: 0.00, 0.00, 0.85 mg) did not yield more reproductive biomass than those from control plots (0.00, 0.06, 0.97 mg) (MW U = 1604; n = 40,41; p = 0.72). In addition, control (18 of 40) and +food (22 of 40) plots were equally likely to house reproducing colonies ($\chi^2_{0.05,1}$ = 0.8, p = 0.37).

Do ant colonies accumulate on +food plots?

Nest density did not increase with food addition (Table 3) and averaged 7.5 ± 4.4 nests m^{-2} (range: 1-20) on control plots and 8.2 ± 4.6 (range: 2-22) on +food plots. Litter depth was not a significant covariate explaining nest density (Table 3). Among

common species, only nest densities of *P. ruida* increased with litter depth (ANCOVA: $F_{1,75} = 10.12$, $p = 0.002$).

Does litter habitat decompose more quickly on +food plots?

Three pieces of evidence suggest that habitat space declined on +food plots. First, microbial decomposition of cellulose averaged 15% higher on +food plots (mean mass loss +food: 687 mg vs. control: 599 mg; $F_{1,76} = 4.17$, $p = 0.045$). Second, food addition accelerated a seasonal decline in habitat availability: litter depth decreased 30% on +food plots (from 2.5 to 1.7 cm, $t_{39, 0.05} = 5.22$, $p = 0.0001$), but decreased only 12% on control plots (from 2.3 to 2.1 cm, $t_{37, 0.05} = 1.62$, $p = 0.11$). Finally, although nest densities did not previously increase on +food plots (*see above*), artificial nests were colonized twice as frequently when combined with food addition than when added alone ($\chi^2_{0.05,1} = 12.5$, $p = 0.0001$; Fig. 3). Nearly half of all colonization on +food plots was by queenless satellite nests of *Wasmannia auropunctata* (Roger 1863).

DISCUSSION

Contrary to the food limitation hypothesis, colony size, number, and total biomass were not enhanced on +food plots relative to controls. Instead, food addition may have indirectly impacted this tropical litter ant community in ways that support the "more food, less habitat" hypothesis. First, food addition simultaneously increased rates of microbial decomposition and decreased litter availability. Second, we only detected food limitation when we added nest sites and thus reduced the ability of

decomposer taxa to destroy habitat. In sum, the picture of how resources limit tropical litter ant colonies should increasingly come into focus as studies integrate decomposer food web dynamics, tracking how food flows from microbes to ants.

The present results contrast with other studies reporting enhanced colony growth (*e.g.* Fokuhl *et al.* 2007) and reproduction (*e.g.* Deslippe & Savolainen 1994; Aron *et al.* 2001; Brown & Keller 2006) following experimental food addition. However, many of the previously studied ecosystems are in temperate regions, including English heath lands (Gammans *et al.* 2005), southern Californian chaparral (MacKay 1985), and temperate woodlands (Bono & Herbers 2003). Species in these studies excavate nests as subterranean galleries that are unlikely to be degraded by decomposer taxa, such that food addition may be less closely tied to the availability of habitat space.

McGlynn (2006) performed the only other food addition experiment in a tropical litter ant community, adding 5g of termites covered in vegetable oil m^{-2} at 2-day intervals. After 4 months, a total of *ca.* 300 g necromass m^{-2} increased nest densities relative to controls. In this study, adding both food and nests more than doubled colonization relative to control after only one month and *ca.* 40 g necromass ¼-m^{-2}. Thus, although food limitation appears to limit litter ant colony density within tropical forests, the availability of leaf litter habitat may constrain colony access to food patches.

In tropical forests, food limitation appears necessary, but not sufficient to explain patterns of colony size. Although least square regressions did not detect treatment differences between colony growth curves (Table 2), the triangular shape of

many of these relationships suggested an upper constraint on relative investment in worker pupae, with unmeasured variables generating scatter below this line (Brown 1995, Cade & Noon 2003). At the ninetieth quantile, colonies yielded increasingly more pupae on +food plots ($b = 0.91 \pm 0.13$) relative to those from control plots (0.71 ± 0.20) (Fig. 1).

In this study, at least three additional factors independent of colony size may have reduced growth below the 90^{th} quantile. The first is natural interspecific variation in growth rate (Fig. 2), some of which may be due to dietary variation among species in diverse tropical litter ant communities (Tobin 1994; Wilson 2005). Second, raids by predators like army ants occur daily at the m^2 scale in the most productive tropical forests (Kaspari & O'Donnell 2003), and may empty nests of their brood. Third, soil-nesting species with aggressive workers (*e.g. Odontomachus bauri* Emery 1892 and *Ectatomma ruidum* Roger 1860) may also have harvested some fraction of added food. At both sites, foraging workers of *E. ruidum* recruited to 58% of necromass baits after 1.5 hr. Experimental removal of these colonies should go far in quantifying their influence (Gibb & Hochuli 2004).

Although diversity did not change with food addition, habitat loss on these plots may have confounded recruitment dynamics. Interestingly, satellite nests of *W. auropunctata* increasingly recruited to +food plots when we mitigated habitat loss by also adding nests. Although BCI is likely within the native range of *W. auropunctata* (Tennant 1994; Wetterer & Porter 2003), it is a highly invasive weedy species that displaces native taxa when introduced into novel tropical habitats (Clark *et al.* 1982; Le Breton *et al.* 2003). Although foraging workers in native populations of *W.*

auropuntata do not appear numerically or behaviourally dominant at resource baits (Tennant 1994, Orivel et al. 2009), whole colonies may be predisposed for invasive success because their decentralized nests quickly colonize ephemeral resource patches. Furthermore, the environmental heterogeneity hypothesis predicts decreasing diversity when resource addition diminishes niches of low resource use (Rosenzweig *et al.* 1984; Tilman & Pacala 1993). Further studies should shed light on whether *W. auropunctata*, a species with low nitrogen use efficiency (Davidson 2005), excludes other taxa in native forests by dominating available nitrogen (*e.g.* Wedin & Tilman 1993).

Conclusions

The quality of +food plots in this study may have been diminished by enhanced decomposer activity that degraded the structure of leaf litter habitat (*e.g.* Jonasson *et al.* 1996). Experimental nutrient addition has previously enhanced decomposition rates across tropical forests with weathered soils (Hobbie & Vitousek 2000; Kaspari *et al.* 2008). When we added nutrients, packaged as insect necromass, decomposer activity increased and colonies recruited to +food plots only when we also added nests (Fig. 3). Thus, tropical litter ants may simultaneously be limited by the availability of food (McGlynn 2006) and patchy leaf litter that becomes increasingly ephemeral when saturated with food (Kaspari & Yanoviak *in press*). Needed now is a more detailed picture of the specific aspects of litter habitat (*e.g.* nest sites) most impacted by decomposer microbes, and the biological pathways through which this occurs.

ACKNOWLEDGEMENTS

N Clay, D Donoso, O Fincke, J Kelly, R Knapp, J Mendoza, and the members of the Zoology Journal Club provided many excellent suggestions for improving this manuscript. D Donoso assisted with species identification and construction of litter bags. O Acevedo and B Jimenez of STRI provided invaluable logistical support in Panama. This project was funded by the National Science Foundation under Grant no. 0212386 to MK, and an Adams Summer Research Fellowship and Alumni Fellowship, both from the University of Oklahoma, to JZS. The experiments in this study comply with current laws of the Republic of Panama.

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Tables

Table 1 Ordinary least squares (OLS) and least absolute deviation (LAD) regression for scaling of pupae number against worker number ($y = aM^b$). For OLS regressions, significant deviation of slope from isometry ($b = 1.0$) denoted by *(P = 0.05), **(P=0.01), ***(P=0.001), ****(P=0.0001). For LAD regressions, p values indicate whether slope b differs significantly from zero. N denotes number of samples in analysis. Treatment groups are colonies harvested from control (C), or +food (F) plots. Queen colonies include all harvested colonies with queens. N.S. indicates non-significance of overall model.

			Linear regression							Quantile Regression (upper 90th quantile)		
Species	**TRT**	**N**	**F**	**R^2**	***a***	**SE**	***b***	**SE**	**± 95% CI of *b***	***b***	**SE**	**p**
C. rimosus	C	25	5.90	0.20	-0.30	0.29	0.54*	0.22	0.46	0.51	0.41	0.04
	F	22	5.35	0.21	-0.13	0.26	0.49*	0.21	0.44	N.S.	N.S.	N.S.
Solenopsis sp. 2	C	19	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	F	29	7.10	0.21	-0.18	0.45	0.86	0.32	0.66	0.84	0.34	0.02
Solenopsis sp. 3	C	28	40.75	0.61	-0.77	0.36	1.28	0.20	0.41	0.73	0.42	0.002
	F	29	50.25	0.65	-1.45	0.41	1.62*	0.23	0.47	1.16	0.40	0.02
P. multispina	C	24	8.57	0.28	-0.22	0.38	0.72	0.25	0.51	0.75	0.20	0.02
	F	21	26.58	0.58	-0.29	0.26	0.91	0.18	0.37	0.88	0.32	0.04
P. rugiceps	C	17	25.57	0.63	-0.07	0.26	0.80	0.16	0.34	0.88	0.17	0.05
	F	25	14.83	0.40	-0.16	0.31	0.75	0.19	0.40	0.85	0.27	0.04

P. ruida	C	32	32.20	0.52	-0.14	0.24	0.78	0.14	0.28	0.66	0.20	0.02
	F	28	34.58	0.57	-0.30	0.25	0.89	0.15	0.31	0.99	0.14	0.02
P. brevicornis	C	15	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	F	16	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Queen colonies	C	189	162.11	0.46	-0.09	0.08	0.70****	0.05	0.11	0.71	0.08	0.0001
	F	205	318.31	0.61	-0.15	0.06	0.79****	0.04	0.08	0.91	0.05	0.0001

Table 2 Results from ANCOVAs testing effects of Treatment (food addition vs. control) on the scaling of $\log_{10}$pupae number on $\log_{10}$worker number. In no case did average colony growth rate (i.e., pupae worker ratio) increase on food plots.

Species	**Factor**	**df**	**Type III SS**	**F**	**p > F**
C. rimosus	Treatment	1	0.025	0.20	0.66
	Worker number	1	1.429	11.16	0.002
	TRT x Worker number	1	0.004	0.03	0.87
	Error	43			
Solenopsis sp.2	Treatment	1	0.785	2.63	0.11
	Worker number	1	0.529	1.77	0.19
	TRT x Worker number	1	0.814	2.73	0.11
	Error	44			
Solenopsis sp.3	Treatment	1	0.342	1.55	0.22
	Worker number	1	20.160	91.25	0.0001
	TRT x Worker number	1	0.271	1.23	0.27
	Error	53			
P. multispina	Treatment	1	0.006	0.02	0.88
	Worker number	1	7.324	28.66	0.0001
	TRT x Worker number	1	0.098	0.38	0.54
	Error	41			
P. rugiceps	Treatment	1	0.009	0.04	0.84
	Worker number	1	7.380	33.89	0.0001
	TRT x Worker number	1	0.009	0.04	0.84
	Error	38			
P. ruida	Treatment	1	0.037	0.21	0.65
	Worker number	1	11.755	67.13	0.0001
	TRT x Worker number	1	0.045	0.26	0.61
	Error	56			
P. brevicornis	Treatment	1	0.102	0.66	0.42
	Worker number	1	0.857	5.16	0.03
	TRT x Worker number	1	0.082	0.49	0.49
	Error	26			
Colonies with queens	Treatment	1	0.093	0.43	0.51
	Worker number	1	97.415	452.97	0.0001
	TRT x Worker number	1	0.396	1.84	0.18
	Error	393			

Table 3 Effects of food limitation on colony growth (mean worker number m^{-2} or mean colony biomass m^{-2}) and recruitment (nest density m^{-2}) using log_{10} transformed data. Colony biomass measured per colony as sum of worker, pupae, queen dry mass (mg).

Effect	Test	Factor	df	Type III SS	F	p > F
Mean worker number	ANOVA	Month	1	0.408	5.28	0.02
		Treatment	1	0.051	0.67	0.42
		Month x Treatment	1	0.070	0.90	0.35
		Error	77			
	ANCOVA	Log_{10}(Litter Depth)	1	0.085	1.09	0.30
		Treatment	1	0.050	0.63	0.43
		Log_{10}(Litter Depth) x Treatment	1	0.073	0.93	0.34
		Error	75			
Mean colony biomass	ANOVA	Month	1	0.096	4.94	0.03
		Treatment	1	0.000	0.01	0.92
		Month x Treatment	1	0.046	2.36	0.13
		Error	77			
	ANCOVA	Log_{10}(Litter Depth)	1	0.011	0.54	0.46
		Treatment	1	0.067	0.32	0.58
		Log_{10}(Litter Depth) x Treatment	1	0.006	0.31	0.58
		Error	75			
Nest density	ANOVA	Month	1	0.070	1.36	0.25
		Treatment	1	0.040	0.77	0.38
		Month x Treatment	1	0.001	0.00	0.99
		Error	77			

	ANCOVA	Log_{10}(Litter Depth)	1	0.124	2.47	0.12
		Treatment	1	0.066	1.31	0.26
		Log_{10}(Litter Depth) x Treatment	1	0.102	2.03	0.16
		Error	75			

Figure Captions

Figure 1 Scaling of $\log_{10}$ (pupae number) against $\log_{10}$ (worker number) across all colonies with queens, with slopes (*b*) from OLS regressions. Each data point represents a single colony. Open circles and dashed lines: C = control. Closed circles and solid lines: F = +food colonies. Gray lines are LAD regressions through the ninetieth quantile drawn as per treatment.

Figure 2 Scaling of pupa number against worker number for common species. See Fig. 1 caption for details.

Figure 3 Colonization of artificial ant nests on +food and control 0.25-m^2 plots after 2 weeks and 1 month. Each bar represents the mean (± 1 SE) of 20 plots.

Figure 1

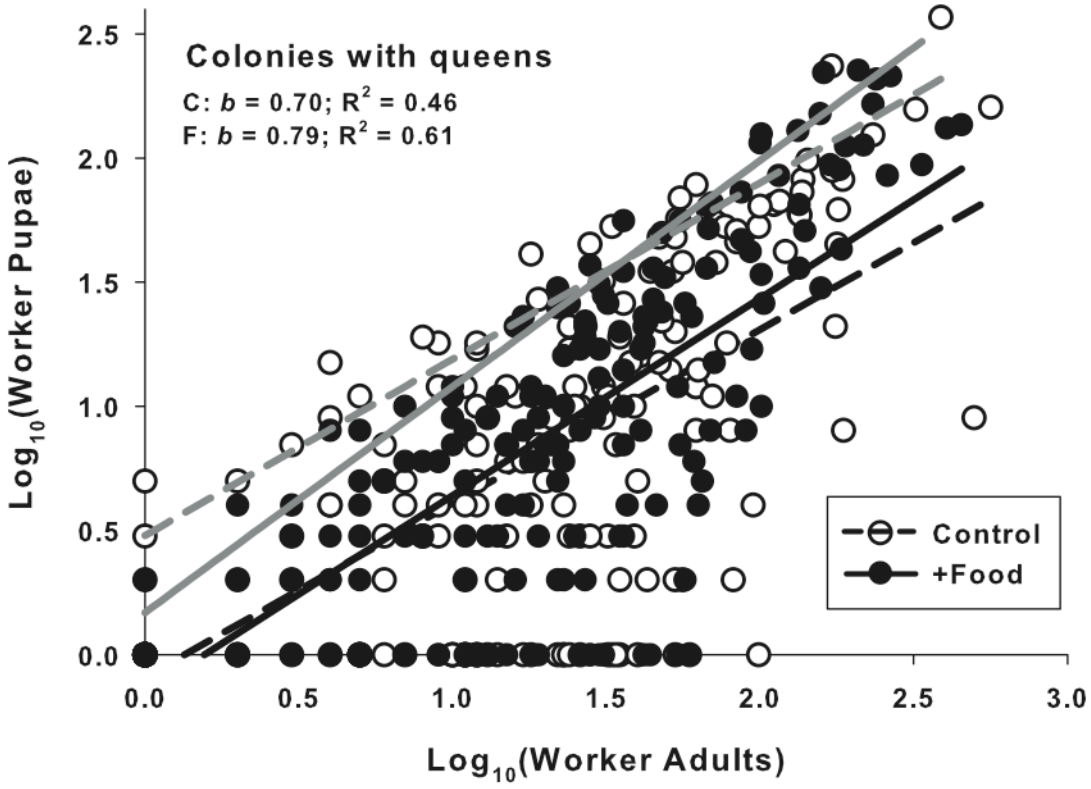

Colonies with queens
C: b = 0.70; R2 = 0.46
F: b = 0.79; R2 = 0.61
Control
+Food
Log10(Worker Pupae)
Log10(Worker Adults)
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Figure 2

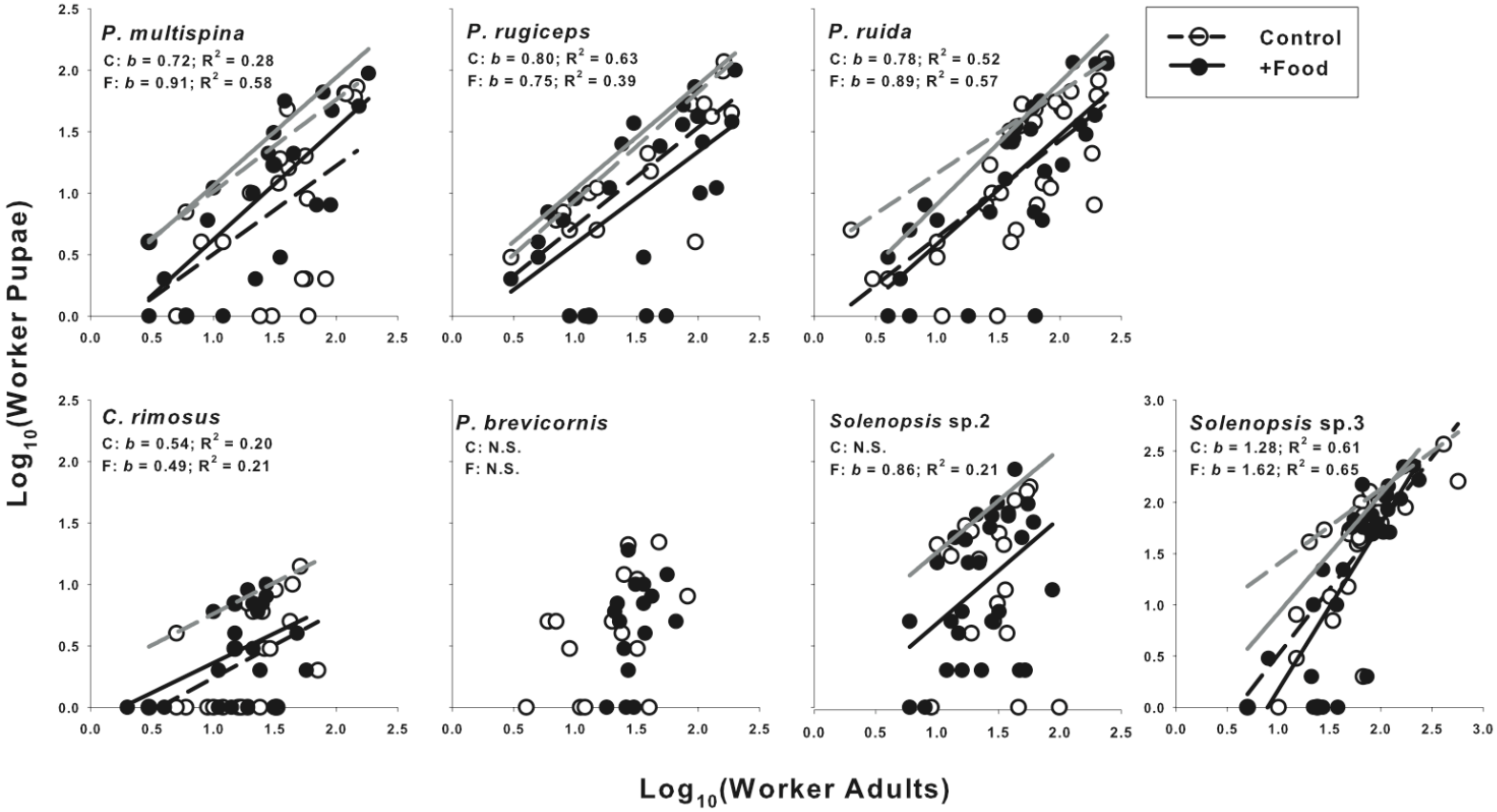
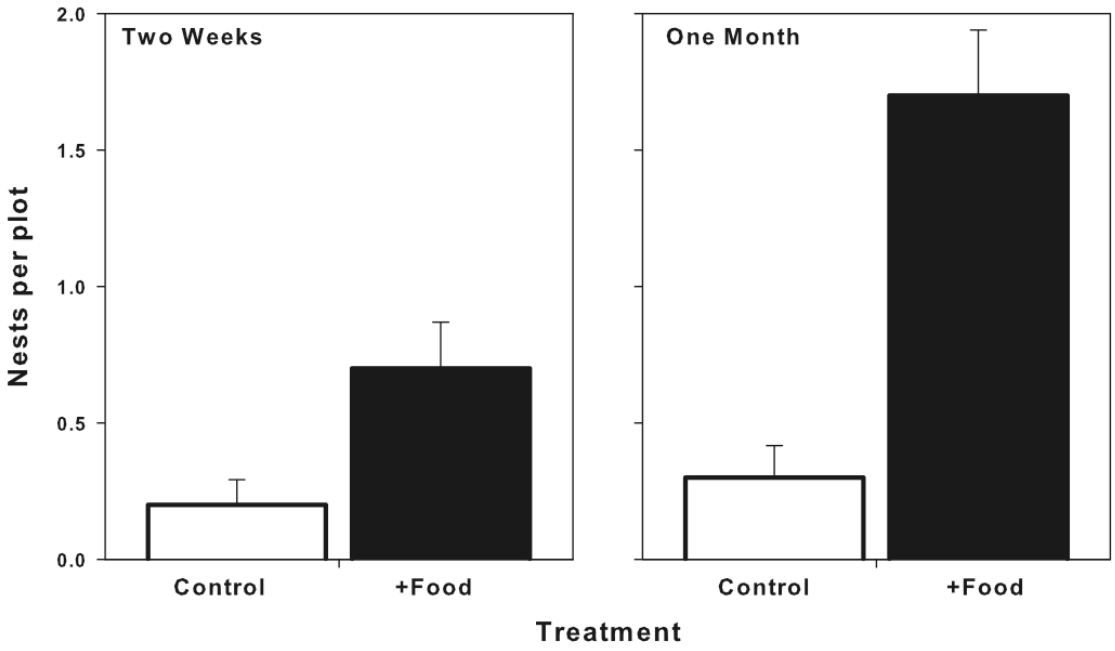

P. multispina
C: b = 0.72; R2 = 0.28
F: b = 0.91; R2 = 0.58
P. rugiceps
C: b = 0.80; R2 = 0.63
F: b = 0.75; R2 = 0.39
P. ruida
C: b = 0.78; R2 = 0.52
F: b = 0.89; R2 = 0.57
Control
+Food
C. rimosus
C: b = 0.54; R2 = 0.20
F: b = 0.49; R2 = 0.21
P. brevicornis
C: N.S.
F: N.S.
Solenopsis sp.2
C: N.S.
F: b = 0.86; R2 = 0.21
Solenopsis sp.3
C: b = 1.28; R2 = 0.61
F: b = 1.62; R2 = 0.65
Log10(Worker Pupae)
Log10(Worker Adults)

Figure 3


Two Weeks
One Month
Nests per plot
2.0
1.5
1.0
0.5
0.0
Control
+Food
Control
+Food
Treatment

Appendix S1 Species list

Subfamily	Genus	Species
Ectatomminae	*Gnamptogenys*	*cf. horni*
	Gnamptogenys	*cf. interrupta*
	Gnamptogenys	*cf. porcata*
	Gnamptogenys	*mecotyle*
	Gnamptogenys	sp. 1
	Gnamptogenys	sp. 2
	Gnamptogenys	*triangularis*
Formicinae	*Acropyga*	sp. 1
	Brachymyrmex	sp. 1
	Camponotus	*cf. simillimus*
	Paratrechina	*cf. Para_m1_*
Myrmicinae	*Apterostigma*	sp. 1
	Basiceros	*balzani*
	Carebara	*urichi*
	Crematogaster	*sumichrasti*
	Cyphomyrmex	*cf. dixus*
	Cyphomyrmex	*costatus group*
	Cyphomyrmex	*rimosus group*
	Solenopsis	sp. 1
	Solenopsis	sp. 2
	Solenopsis	sp. 3
	Solenopsis	sp. 4
	Solenopsis	sp. 5
	Solenopsis	sp. 6
	Solenopsis	sp. 7
	Solenopsis	sp. 8
	Solenopsis	sp. 9
	Megalomyrmex	*silvestrii*
	Pheidole	*cf. JTL-103*
	Pheidole	*cf. radoszkowskii*
	Pheidole	*dasypyx*
	Pheidole	JTL-100
	Pheidole	*mendicula*
	Pheidole	*multispina*
	Pheidole	*punctatissima*
	Pheidole	*rugiceps*
	Pheidole	*ruida*
	Pheidole	sp. 1
	Pheidole	*specularis*
	Pyramica	*brevicornis*
	Pyramica	*gundlachi*
	Pyramica	sp. 2
	Pyramica	sp. 3
	Pyramica	sp. 4
	Pyramica	sp. 5

	Rogeria	*belti*
	Rogeria	*foreli*
	Strumigenys	*elongata*
	Tranopelta	*gilva*
	Wasmannia	*auropunctata*
	Wasmannia	*rochai*
Ponerinae	*Anochetus*	*diegensis*
	Hypoponera	cf. JTL-001
	Hypoponera	*cf. opacior*
	Hypoponera	sp. 1
	Hypoponera	sp. 2
	Hypoponera	sp. 3
	Leptogenys	*cf. pusilla*
	Pachycondyla	*harpax*
Proceratiinae	*Discothyrea*	sp. 1
	Problomymrex	*cf. petiolatus*
	Problomymrex	sp. 1
	Problomyrmex	*bolivensis*

CHAPTER 3

The metabolic costs of building ant colonies from variably-sized subunits

(formatted for *Behavioral Ecology and Sociobiology*)

ABSTRACT

Ant colonies are superorganisms with emergent traits that, for some species, reflect the combined activity of physically distinct worker castes. Although larger castes have high production costs, they are thought to save their colonies energy by efficiently performing specialized tasks. However, because workers are generally idle until sensing specific stimuli, their maintenance costs may be an important component of colony-level investment. I used metabolic scaling to examine the maintenance costs of dimorphic major and minor *Pheidole* castes across levels of colony organization (*e.g.* individual, group, colony). Majors from three species had lower mass specific metabolic rates than minors because of allometries at both individual and group levels, and subsequently lived longer when starved. Thus, large major castes may offset their production costs in both their idle and active states. The slope scaling metabolic rate from incipient to reproductive colonies of *P. dentata* ($\sim colony\ mass^{0.89}$) fell between the slopes for minor groups ($\sim group\ mass^{1.04}$) and major groups ($\sim group\ mass^{0.79}$) and appears to reflect developmental shifts in subunit mass and number and their offsetting effects on per-capita energy demands. These results highlight how metabolic scaling may help visualize the energetic correlates of emergent behavior and unravel the mechanisms governing colony organization.

1. INTRODUCTION

The social insects (e.g. ants, bees, and termites) are abundant and conspicuous consumers in terrestrial habitats across the planet (Hölldobler and Wilson 2008). Division of labor between reproductive queens and more or less sterile workers is the lynchpin of colony life and may account for much of this success (Oster and Wilson 1978). In 15% of ant genera, colonies further divide labor among physically distinct worker castes (Hölldobler and Wilson 2008). Larger and often morphologically specialized castes tend to have high production costs (Wilson 1968; Calabi and Porter 1989; Tschinkel 1993; Kaspari and Byrne 1995), but they are thought to offset these costs by more efficiently performing their specialized tasks (Wilson 1976; Lighton *et al.* 1987; Kay and Rissing 2005; Powell and Franks 2005; Powell 2009). Within nests however, workers (especially specialized castes) typically remain idle, becoming active only when exposed to a specific stimulus of sufficient strength (Wilson 1968; Wilson 1976; Robinson and Page 1989; Detrain and Pasteels 1991; Gordon 2002; Beshers and Fewell 2001; Fewell *et al.* 2009). Thus, worker maintenance costs represent an important, but rarely studied aspect of colony-level investment in castes (Oster and Wilson 1978, *but see* Wilson 1980; Calabi and Porter 1989).

Species in the ant genus *Pheidole* have dimorphic worker castes, with big-headed *majors* that perform narrow sets of behaviors (*e.g.* nest defense: Detrain and Pasteels 1991; Passera *et al.* 1996) and smaller *minor* workers with much wider repertoires (*e.g.* brood care, nest excavation, foraging: Wilson 1984; Seid 2006; Mertl and Traniello 2009). Majors are a diagnostic colony trait of the *ca.* 900 species in this globally distributed genus that have radiated to fill most niches occupied by ants

(Wilson 2003; Moreau 2008). Interestingly, majors may have fuelled this ecological success despite being uniformly larger than minors (Wilson 2003; Pie and Traniello 2007), and thus more expensive to produce (Oster and Wilson 1978). This is because majors tend to ensure nest safety and enable colonies of many species to produce relatively cheap minors (rapidly if needed) that have reduced defensive traits (Wilson 2003). However, colonies may also recoup production costs if majors are cheaper to maintain, gram for gram, than minors. Three crucial components must be considered to address the metabolic costs of different castes. These are outlined below and examined in turn in this study.

(a) ***Body size and maintenance demands***

The allometric scaling of metabolic rate with body size from the smallest unicells to the largest metazoans is one of the most robust patterns in biology (Kleiber 1932; Peters 1983; Brown *et al.* 2004). Although larger bodied ant species also appear to have lower mass-specific metabolic demands than smaller species (Chown *et al.* 2007), little is known about whether this allometry also applies to variably-sized castes within colonies. Larger castes appear to have lower mass-specific metabolic rates (*e.g. Atta laevigata*, Hebling-Beraldo and Mendes 1981; *Pogonomyrmex badius,* Porter 1986; and *Solenopsis invicta*, Porter and Calabi 1989), and may provide savings to their colony by using relatively less energy than minors not only in their active states, but also in their idle states. Here, I test this possibility by comparing the mass-specific metabolic rates of majors and minors from three species of *Pheidole.* To examine

impacts on caste performance, I then ask whether majors predicted to have lower mass-specific maintenance demands live longer than minors when starved.

(b) ***Group size and maintenance demands***

Despite the allometric scaling of metabolic rate with ant body size (Chown *et al.* 2007), many ant behaviors related to metabolism also depend on group size (*e.g.* rates of nest excavation, food sharing, and antennal contact with nestmates; Chen 1937; Howard and Tschinkel 1980; Gordon 1993; Buhl *et al.* 2004). These group behaviors scale worker activity, and thus metabolic rate, to generate the emergent traits that define colonies as superorganisms. As behavioral specialists, *Pheidole* majors can be expected to maximize group performance differently than minors, rapidly scaling up activity for their specialized tasks when needed (*e.g.* nest defense: Wilson 1976; or foraging: Burkhardt 1998), while scaling down activity during other times to minimize their maintenance costs to their colonies. For three species of *Pheidole*, I tested whether major maintenance costs are reduced relative to those of minors by the allometric scaling of metabolic rate with group size, just as they may be with body size of individuals.

(c) ***Colony size and subunit maintenance demands***

An organism's metabolic rate ultimately reflects the combined energetic demands of its subunits, whether they be a body's cells (Davidson 1956; Darveau *et al.* 2002; Kozlowski *et al.* 2003; Savage *et al.* 2007) or a colony's ants (Gallé 1978; Lighton 1989; Fonck and Jaffe 1996). As ant colonies age, they tend to increasingly

produce larger and more variably-sized worker subunits (Wilson 1985; Tschinkel 1993; Wetterer 1999). The energetic costs of these ontogenetic changes may be studied using intraspecific metabolic scaling (Glazier 2005). Although conserved scaling exponents may reflect general energetic constraints on development (*e.g.* Moses *et al.* 2008), a broad range of empirically observed slopes (*e.g.* $b < 1$, $b = 1$, $b > 1$, and multiphasic) may also reflect species-specific growth patterns (Ricklefs 2003; Glazier 2005).

For colonies with dimorphic castes, developmental shifts in the abundance of majors and minors (Oster and Wilson 1978; Walker and Stamps 1986; Kaspari and Byrne 1995) should yield shifting combinations of per-capita metabolic demands. If developing *Pheidole* colonies increasingly allocate mass to majors (*caste mass* = *colony mass*^{b}; $b_{major\ mass} > b_{minor\ mass}$), they will increasingly be composed of larger subunits with lower mass-specific metabolic rates. Thus, whole colony metabolic rate will scale as *colony mass*$^{b<1}$. However, minors may increasingly outnumber majors in larger colonies (*major number* = *minor number*$^{b<1}$), even if larger colonies produce equal masses of majors and minors. Thus, larger colonies may have relatively higher metabolic rates ($b > 1$) if they are increasingly composed of smaller subunits with higher per-capita metabolic rates. I thus examined how metabolic rate scaled across colonies of *P. dentata* ranging from incipient to reproductive and examined energetic costs of ontogenetic changes in caste structure.

2. METHODS

(a) Harvesting and culturing ants

I harvested ants from grassland sites in central and southern Oklahoma from 17 May to 20 September 2008 and 27 May to 15 Aug 2009 (Appendix S1). Ants were housed in plastic nest boxes lined with fluon for at least 48 hr prior to experiments and were provided *ad lib* with freshly immobilized crickets, Bhatkar's diet (egg, honey, and vitamin set in agar: Bhatkar and Whitcomb 1971), and a 1.5-ml vial filled with water-soaked cotton. Complete colonies of *Pheidole dentata* for whole colony scaling were harvested from a population inhabiting an oak forest at the University of Oklahoma Biological Station (33° 49' N, 96° 34' W) in south central Oklahoma. Nest entrances were located by following foragers returning from baits of pecan sandies. Colonies were generally situated in the roots of small clumps of grasses, around which a 1 m diameter disk was cut and the walls of the pit inspected for additional tunnels. Back at the lab, I sorted through all soil and collected all ants and brood. All harvested colonies were monogynous, and ants were returned to the forest if the queen could not be located. The largest colony had two male pupae that were removed prior to metabolic measurements.

(b) Recording individual and group $\dot{V}CO_2$

I used equipment from Sable Systems International (SSI; Las Vegas, Nevada, USA) to perform constant volume respirometry and measure metabolic rate (μl CO_2 hr^{-1}, hereafter, $\dot{V}CO_2$). Before all trials, a CA-10 CO_2 analyzer (accuracy of 1%, resolution of 0.00001%) was zeroed with N_2 gas and then spanned with a gas of known CO_2 concentration (1,000 p.p.m. CO_2 in N_2 ± 1%). For individual and group

samples, seven chambers were attached to an RM8 multiplexer—six contained ants, one remained empty as a control. Hourly CO_2 values from the empty chamber were subtracted from all experimental chambers to correct for extrinsic CO_2. To baseline measures of ant respiration, air scrubbed of CO_2 was passed through tubing affixed to the eighth position on the multiplexer between each experimental trial. Sample sizes for individual (Table 1) and group (Table 2) data are provided in the results section.

Incurrent air was first scrubbed of H_2O and CO_2 using a drierite/ascarite/drierite column (Lighton 2008). Flow rate was set to 50 ml min^{-1} using an SS-3 subsampler pump and regulated by a 200 ml min^{-1} Sierra Valve set by an MFC unit. This air was scrubbed by a second drierite/ascarite/drierite column and then sent to the multiplexer, programmed to switch between chambers using SSI Expedata software. Ambient temperature was continuously recorded adjacent to respirometry chambers using a Thermistor cable. All equipment was interfaced with a computer using a SSI UI-2.

In this way, ants in chambers were placed in air free of CO_2. After 1 hr, this air was flushed out for 200 s at 50 ml min^{-1}, passed through a 10-cc column of magnesium perchlorate (Cl_2MgO_8) to remove any remaining moisture, and then to the CO_2 analyzer. Trials were run for at least 6 hr, yielding six measurements per chamber. The first hour of data included extrinsic CO_2 and was never used. Thereafter, ants were generally inactive and CO_2 readings stabilized at a lower value (*as per* Lighton 2008). Each data point was the mean of five hourly measurements taken during this time. Due to the small size of *P. bicarinata* minors (0.07 mg; Table 1), 2 hr

were necessary to generate stable CO_2 measurements. For this species, data points were the average of four hourly measurements (hr 3-6).

Respirometry chambers for individual and group trials were 10-cc syringe barrels fitted with a rubber stopper. Chambers were cleaned with 95% EtOH between trials. Gradients of mass were generated using groups of 1, 2, 5, 10, 20, and 40 workers, replicating each group size six times per species. A larger group, whose size depended on available ants, was also included (Table 1). After experimental measurements, ant groups were frozen, dried at 60°C for 24 hr, and weighed to the nearest 10^{-3} mg. Individual ants were always used for only 1 experimental trial. Although each worker group consisted of only nestmates, the results of trials from multiple colonies were pooled to generate regressions.

(c) Recording colony $\dot{V}CO_2$

Colony-level $\dot{V}CO_2$ was generally recorded as described above. An exception was that colony respirometry chambers—10 cm x 2.75 cm clear cylindrical acrylic tubes (*ca.* 238 ml) set lengthwise with plaster filling 1/3 of the volume—were designed for long-term ant habitation. The queen, her brood, and retinue of workers always moved into a small nest disk—a 15 ml petri dish painted green with wide exits facilitating airflow cut into the walls, and capped with a transparent red plexiglass lid. At biweekly intervals, I hydrated the plaster nest floor by inserting 0.5 ml dH_2O through a plastic tube that was set into the plaster and extended through the chamber ceiling.

All edges where plaster met the acrylic tube were lined with aquarium sealant. Both ends of the colony tube were plugged with large rubber stoppers (#12 Cole-Parmer) into which a hole was drilled and the end of a 35-ml syringe affixed, sealed, and screened from the inside by fine mesh. The syringe tips could be locked into the multiplexer, and air pushed through diffusely through a wide opening to ensure complete mixing. Flow rates were set to 320 ml min^{-1}, with data recorded hourly over 400 s for each colony chamber attached to the multiplexer. Peltier Effect Air Driers (Sable Systems PC-3) were used to reduce the dew point of incurrent and excurrent air to 1ºC. At the completion of each trial, ants comprising each measured colony were dried and weighed as before. Colony chambers were not axenic, and I recorded empty chamber CO_2 following all experimental trials and subtracted these values from the respective colony-level values.

(d) Data analysis

SSI ExpeData software was used to subtract the empty chamber CO_2 from each experimental measurement of individual ants or group of ants and to correct for small variations in flow rate (generally ± 0.1ml min^{-1}). I then used ExpeData software to generate a new variable $\dot{V}CO_2$ by transforming CO_2 measurements from p.p.m. to µl hr^{-1} and integrating these values for trial intervals. $\dot{V}CO_2$ measurements were then standardized to a metabolic rate at 25°C assuming $Q_{10} = 2$ (*as per* Lighton 2008). Minimal temperature corrections were necessary, however, because the mean of 778 hourly temperature measurements was 23.8 ± 1.6°C (± 1 SD).

Ordinary least square regression was used to estimate *a* and *b* in the scaling equation $\log_{10}y = \log_{10}a + b\log_{10}M$. Scaling characterized the dependence of $\dot{V}CO_2$ ($\mu l\ CO_2\ hr^{-1}$; *y*) on group or colony size (*dry biomass mg*; *M*). To detect allometry ($b \neq 1$), an *F*-statistic was calculated to test the null hypothesis of isometry (H_0: $b = 1$). ANCOVA was used to examine variation in scaling exponents (*b*) between castes. For each scaling relationship for group or colony mass, I calculated a standardized measure of size range, pWR: $pWR = \log_{10}(M_{max}/M_{min})$ (Prothero 1986).

(e) Caste longevity experiment

Majors (n = 45) and minors (n = 45) were removed on 15 August 2009 from a *P. dentata* colony harvested on 14 August at the University of Oklahoma Biological Station. Each ant was placed in a separate petri dish and housed at 25ºC in a room exposed to ambient photoperiods. Ants were supplied with 0.2 ml H_2O soaked into cotton placed in small foil cups. Water was added every 3rd day, and cotton was changed every 10 days. Ants were checked daily and considered dead if they failed to right themselves when placed on their backs. Because data (days alive) were not normally distributed, a non-parametric Mann-Whitney test was used to test for caste differences in longevity. Although initial age was unknown, selection bias was controlled by randomly selecting ants from the natal colony. Upon death, all ants were frozen, dried and then weighed to the nearest 1 µg.

3. RESULTS

(a) ***Body size and maintenance costs***

Majors of *P. dentata*, *P. bicarinata*, and *P. morrisi* were 3.7, 6.3 and 2.9 times larger than their respective conspecific minors (Table 1). Majors had significantly lower mass-specific metabolic rates than minors in *P. dentata* ($t_{16, 0.05}$ = -6.26; p = 0.0001), *P. bicarinata* ($t_{12, 0.05}$ = -4.50; p = 0.01), and *P. morrisi* ($t_{11, 0.05}$ = -2.24; p = 0.046) (Fig. 1). Majors (mean ± 1 SD: 22 ± 19 days; range: 1 – 72 days) also lived significantly longer than minors (8 ± 8 days; range: 2 – 36 days) when provided only water (MW U = 2456.5; n = 44, 44; p = 0.0001; Fig. 2).

(b) ***Group size and maintenance costs***

For each species tested, the slopes of regressions for majors were significantly lower than those for minors (Table 3). Although only the slope for *P. dentata* majors differed significantly from isometry (Table 2), the group size ranges matched those found in chambers of harvested colonies, but generally spanned *ca.* 2 o.m. of mass (Table 1) and thus yielded slope tests of relatively low power. Here, group size—not body size differences between castes—accounted for scaling differences ($b_{minor} \neq b_{major}$). Slopes for majors were consistently less than isometry: *P. dentata* ($M^{0.79}$), *P. bicarinata* ($M^{0.90}$), and *P. morrisi* ($M^{0.92}$). For minors of each species, $\dot{V}CO_2$ scaled isometrically with group size following an abrupt increase after samples with one or two ants (Table 2).

For minor workers of *P. dentata* ($M^{1.04}$) isometric scaling occurred for samples containing more than one ant (Fig. 3a); for *P. bicarinata* ($M^{1.07}$; Fig. 3b) and *P. morrisi* ($M^{1.07}$; Fig. 3c), isometric scaling occurred for samples containing more than

one or two ants (Table 2). Scaling $\dot{V}CO_2$ across all minor samples yielded allometry for *P. dentata* (**$M^{1.16}$**), *P. bicarinata* ($M^{1.38}$), and *P. morrisi* ($M^{1.38}$). However, the transitions from 1 ant to 2 ants, and 2 ants to multiple ants appear to be different phenomena, and I focus here on the latter. Thus, subsequent analyses for these species were performed without values for one or two ants. Although regression parameters for majors did not change with the inclusion of these samples, they were excluded for both castes in the ANCOVAs.

(c) *Colony size and subunit maintenance demands*

The mass of 17 colonies of *P. dentata* spanned 1.20 orders of magnitude, and included colonies that were incipient (15.25 mg: 31 minors, 9 majors, 1 queen, plus worker brood) and reproductive (242.38 mg: 841 minors, 165 majors, 1 queen, worker brood, plus 2 male pupae). Colonies contained from 31 to 841 minors (pWR = 1.43) that weighed from 4.62 to 133.42 mg (pWR = 1.46), and from 9 to 167 majors (pWR = 1.27) that weighed from 4.87 to 134.94 mg (pWR = 1.44).

Metabolic rate scaled as $colony\ mass^{0.89}$ across this range of colony size (Table 2; Fig. 4). Although major mass (= $colony\ mass^{1.11}$) accumulated more rapidly with colony size than minor mass (= $colony\ mass^{1.02}$), major individuals were increasingly outnumbered by minors ($major\ number = minor\ number^{0.76}$) (Table 2). Thus, although larger colonies invested relatively more mass into large majors with lower mass-specific $\dot{V}CO_2$, these colonies were increasingly composed of smaller minors with higher mass-specific $\dot{V}CO_2$. Whole colony metabolic scaling (b = 0.89) was centered

between minor groups (b = 1.04) and major groups (b = 0.79), such that the slope may reflect these offsetting changes in subunit mass and number (Fig. 4).

4. DISCUSSION

A body's metabolic rate reflects the combined energetic demands of morphologically and physiologically diverse cells (Davidson 1956; Darveau *et al.* 2002; Kozlowski *et al.* 2003; Savage *et al.* 2007). A *Pheidole* colony's metabolic rate appears to similarly reflect the relative mass and number of major and minor subunits (Fig. 4) whose metabolic demands are set by the scaling of metabolic rate with body size (Fig. 1) and group size (Fig. 3). Although factors like predation risk (*e.g.* Passera *et al.* 1996; Yang *et al.* 2004) and developmental stage (*e.g.* Oster and Wilson 1976; Walker and Stamps 1986) may drive production of majors in *Pheidole* colonies, resource limitation may also play an important role (McGlynn and Owen 2002), especially as majors may also be used for food storage in some species (Yang 2006). The present results highlight how colonies may trade high production costs for reduced maintenance demands and higher starvation tolerance when allocating limited resources to majors. Furthermore, given the costs to colonies of producing specialized majors and the possible energetic savings of lower respiration in larger groups (Fig. 3), selection may act not only on majors' physical traits, but also on their behavior in groups.

Colonies and bodies may thus be linked not only by the molecules and biochemical pathways that govern metabolism (Hochachka and Somero 2002), but also by how natural selection targets individual subunits and subunits collectively

(Partridge 1994; Foster *et al.* 2006; Reeve and Hölldobler 2007; Hölldobler and Wilson 2008; Starostova *et al.* 2009). For instance, colony size, like body size, is an evolutionary labile trait (Kaspari and Vargo 1995) that varies with latitude (Kaspari 2005) and governs features as varied as foraging behavior (Beckers *et al.* 1989; Gordon 1995) and reproductive effort (Shik 2008). Ants thus appear physiologically linked to their colonies in the ways that cells are linked to their bodies. Crowding is one proximate mechanism known to influence colony metabolic rate, possibly by regulating the flow of information among nestmates (Cao and Dornhaus 2008). This social mechanism may also account for biphasic scaling observed for minors (Fig. 3). Lacking interactions with nestmates, and thus information about task demands, isolated minors may remain idle, consuming relatively little energy.

Further study is needed to determine the mechanisms generating the differences between minors and majors in the scaling of metabolic rate on mass. It is possible that because specialized majors have smaller behavioral repertoires than minors (*e.g.* Wilson 1976, Brown and Traniello 1998), demands for major activity are reduced at faster rates with increasing group size (*e.g.* Beshers and Fewell 2001). In addition, these results may provide an alternative to the notion that morphological specialization of majors limits their behavioral range. Perhaps, smaller repertoires are an adaptive, energy saving characteristic of larger castes that have higher production costs. Regardless of the mechanism, it will be of interest to examine the generality of this phenomenon across the genus *Pheidole* and then across lineages of ants with dimorphic castes.

If metabolic allometries reduce major maintenance costs and thus the costs of morphological specialization, colonies with such caste systems may ultimately benefit by converting relatively more energy to reproduction than those colonies without castes. Furthermore, this maintenance cost hypothesis yields two basic scaling predictions. First, major metabolic rate will exhibit stronger allometries with group mass ($b << 1$) among species with stronger caste dimorphism (*i.e.* major mass >> minor mass) to offset relatively greater major production costs. Second, the uses of specialized majors are predicted to show greater interspecific variation relative to the more generalized tasks performed by minors that govern day-to-day colony functioning. Thus, major slopes scaling metabolic rate with group mass are predicted to be more variable across species than those for minors.

Allometric scaling of metabolic rate with colony size also has important implications for ecosystem studies that seek to predict patterns of ant colony energy flux, but are limited because most ant activity occurs hidden in underground nest chambers (Petal 1972; Brian 1978; Seal and Tschinkel 2006). To estimate colony respiration, most studies thus scale laboratory measurements of individual ant metabolism up to the entire colony tacitly assuming metabolic rate remains constant with group size (Golley and Gentry 1964; Nielsen 1972; Jensen 1978; MacKay 1985; Tschinkel 1993). This assumption may be confounded if, for instance, behavioral roles and thus *per capita* energetic demands are set by interactions within the nest (*e.g.* Brian and Carr 1960; Horn-Mrowzowska 1976; Martin 1991; Gobin *et al.* 2003). Similar complexities accompany inference of a body's metabolic rate from measurements of cell cultures *in vitro* (Kozlowski *et al.* 2003, Savage *et al.* 2007).

Thus, as shown by the data here, isometric scaling is an assumption that should be tested before estimating a colony's metabolic rate.

Respirometry experiments require caveats. First, because ants freely interacted within the nest, colony $\dot{V}CO_2$ may reflect activity absent from measures of a body's standard metabolic rate. However, it should be noted that a mammal's cellular subunits freely interact even when the animal's basal metabolic rate is measured within its thermoneutral zone. Second, castes within *P. dentata* colonies are divided into temporal castes that divide labor by age class (Seid 2006). For practical reasons, temporal castes were combined in this study, although they may further divide per-capita energy demands. Third, other traits of specialized castes (*e.g.* diminished task flexibility; Mertl and Traniello 2009) may also govern their energetic costs to their colonies. Quantifying these aspects of task performance will be critical for testing the extent to which colonies balance caste production and maintenance costs. Fourth, because behavioral studies of *Pheidole* castes have been performed for few of the *ca.* 900 species in the genus, the present results provide but a first look at the ways in which majors may offset their production costs.

It will be important to extend the present scaling results across ant species, each with a characteristic colony size at maturity. Interspecific scaling may be used to examine constraints on the evolution of colonial organisms (colonies of individuals) and facilitate comparisons with unitary organisms (colonies of cells) for which scaling has long guided theory (Peters 1983; Brown *et al.* 2004). Similar scaling for both biological types may reflect unifying constraints on biological form and function (Hou et al. 2010). However, scaling exponents may also reflect the basic differences

between ant colonies and unitary bodies. For instance, colonies must coordinate the behaviors of independent subunits that have competing genetic interests (Trivers and Hare 1976; Heinze *et al.* 1994; Mehdiabadi *et al.* 2003) and may face systemic energetic costs absent from unitary organisms that package genetically identical subunits (*i.e.* cells) within one body (*e.g.* Gobin *et al.* 2003).

We have learned much about how genetic, physiological, and ecological factors govern the organization of social insect colonies (*for recent reviews, see* Hölldobler and Wilson 2008; Gadau and Fewell 2009). More recently, researchers have sought to explain self-organization—how the decentralized actions of workers yield colonies with highly coordinated behaviors (Beshers and Fewell 2001; Fewell *et al.* 2009). Currently, however, much remains unknown about how worker traits scale up to yield traits of whole colonies (Tschinkel 1991). Metabolic scaling links the physiology of individual ants to that of their colony, and slopes differing from isometry ($b \neq 1$) suggest that these linkages are strengthened in larger societies. Future studies are bound to uncover even greater scaling variation given the diversity of ant colony form and function observed in nature.

ACKNOWLEDGEMENTS

D. Donoso, O. Fincke, D. Glazier, M. Kaspari, J. Khalili, R. Knapp, S. Powell, and the members of the ZEEB Reading Group, and 3 anonymous reviewers, provided excellent suggestions for improving this manuscript. I thank M. Kaspari for providing access to respirometry equipment. Technical support from B. Joos and J.R.B Lighton at Sable Systems International made this research possible. Thanks to L. Davis for

help with species identification. I benefited from participation in the 2008 Gordon Conference on the Metabolic Basis for Ecology. A University of Oklahoma Biostation Summer Fellowship, an Adams Summer Research Scholarship from the Department of Zoology and an Alumni Fellowship from the University of Oklahoma funded this research.

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Tables

Table 1 Summary of individual and group traits for the *Pheidole* species used in this study. Dry mass of individual ants averaged for n individuals. The value pWR = $\log_{10}$ (maximum mass / minimum mass) describes the range of mass analyzed.

species	caste		individual worker traits		group traits		
		n	worker mass (mg) (± 1 SE)	$\dot{V}CO_2$ (µl CO_2 hr^{-1}) (± 1 SE)	worker number range	group mass range (mg)	group mass pWR
P. dentata	minor	9	0.21 (± 0.01)	1.09 (± 0.12)	1-92	0.166-19.832	2.08
	major	9	0.78 (± 0.05)	1.74 (± 0.22)	1-73	0.577-90.15	2.19
P. bicarinata	minor	9	0.07 (± 0.01)	0.40 (± 0.04)	1-96	0.048-8.179	2.23
	major	8	0.44 (± 0.05)	0.73 (± 0.08)	1-20	0.214-9.297	1.64
P. morrisi	minor	9	0.28 (± 0.05)	1.33 (± 0.20)	1-101	0.166-20.298	2.09
	major	4	0.81 (± 0.04)	1.99 (± 0.60)	1-105	0.713-86.808	2.09

Table 2 Results from least squares mean regression for scaling of metabolic rate ($\dot{V}CO_2$) against group mass (caste metabolism), and for the accumulation of caste subunits with colony size (caste demography). Deviation of slope from isometry (b = 1.0) denoted by *(P=0.0001). N denotes number of samples in analysis. Castes are either minors (m) or majors (M). Scaling of minor $\dot{V}CO_2$ with group mass performed without one (*P. dentata*) or two (*P. bicarinata*, *P. morrisi*) samples (*see results section for details*).

comparison	N	MS model	MS error	F	R^2	*a*	SE of *a*	*b*	SE of *b*	± 95% CI of *b*
caste metabolism										
P. dentata (m)	36	3.540	0.007	514.2	0.94	0.40	0.03	1.04	0.05	0.09
P. dentata (M)	36	5.290	0.007	811.4	0.96	0.20	0.03	0.79*	0.03	0.06
P. bicarinata (m)	27	1.452	0.007	215.5	0.89	0.36	0.03	1.07	0.07	0.15
P. bicarinata (M)	27	1.342	0.011	126.7	0.84	0.12	0.04	0.90	0.08	0.16
P. morrisi (m)	29	3.123	0.006	504.2	0.95	0.44	0.04	1.07	0.05	0.09
P. morrisi (M)	18	4.824	0.018	263.8	0.94	0.27	0.06	0.92	0.06	0.12
caste demography for colonies of *P. dentata*										
colony $\dot{V}CO_2$ vs. colony mass	17	1.862	0.016	117.5	0.89	0.63	0.15	0.89	0.08	0.17
minor mass vs. colony mass	17	2.583	0.038	67.7	0.82	-0.44	0.23	1.02	0.12	0.27
major mass vs. colony mass	17	3.029	0.010	313.7	0.95	-0.52	0.11	1.11	0.06	0.13
major number vs. minor number	17	1.879	0.070	26.8	0.64	-0.06	0.33	0.77	0.15	0.32

Table 3 Results of ANCOVAs testing for caste differences in metabolic scaling with group mass.

species	factor	df	type III SS	F	p > F
P. dentata caste comparison	$\log_{10}$(group mass)	1	6.98	1096.37	0.0001
	caste	1	0.18	28.19	0.0001
	$\log_{10}$ (mass) x caste	1	0.10	16.37	0.0002
	error	59			
P. bicarinata caste comparison	$\log_{10}$(group mass)	1	8.79	108.65	0.0001
	caste	1	0.00	0.06	0.80
	$\log_{10}$(mass) x caste	1	0.07	8.15	0.007
	error	36			
P. morrisi caste comparison	$\log_{10}$(group mass)	1	3.45	447.39	0.0001
	caste	1	0.005	0.65	0.43
	$\log_{10}$ (mass) x caste	1	0.11	14.73	0.0005
	error	34			

Figure Captions

Figure 1 Majors had significantly lower mass-specific maintenance costs than conspecific minors. Data are means ± 1 SE.

Figure 2 Majors of *P. dentata* lived significantly longer than conspecific minors when starved.

Figure 3 Scaling of metabolic rate on worker group mass for castes of *Pheidole dentata* (a), and *P. bicarinata* (b), and *P. morrisi* (c). Minors denoted by black circles. Majors denoted by grey circles. Major slopes significantly lower than minor slopes for each species. Open circles are the average of one or two minor worker data (bidirectional error bars ± 1 SD). Scale bars above images = 1mm.

Figure 4 Intraspecific scaling of whole colony $\dot{V}CO_2$. The colony slope for *P. dentata* is between major and minor groups and may reflect offsetting developmental shifts in caste mass and number.

Figure 1

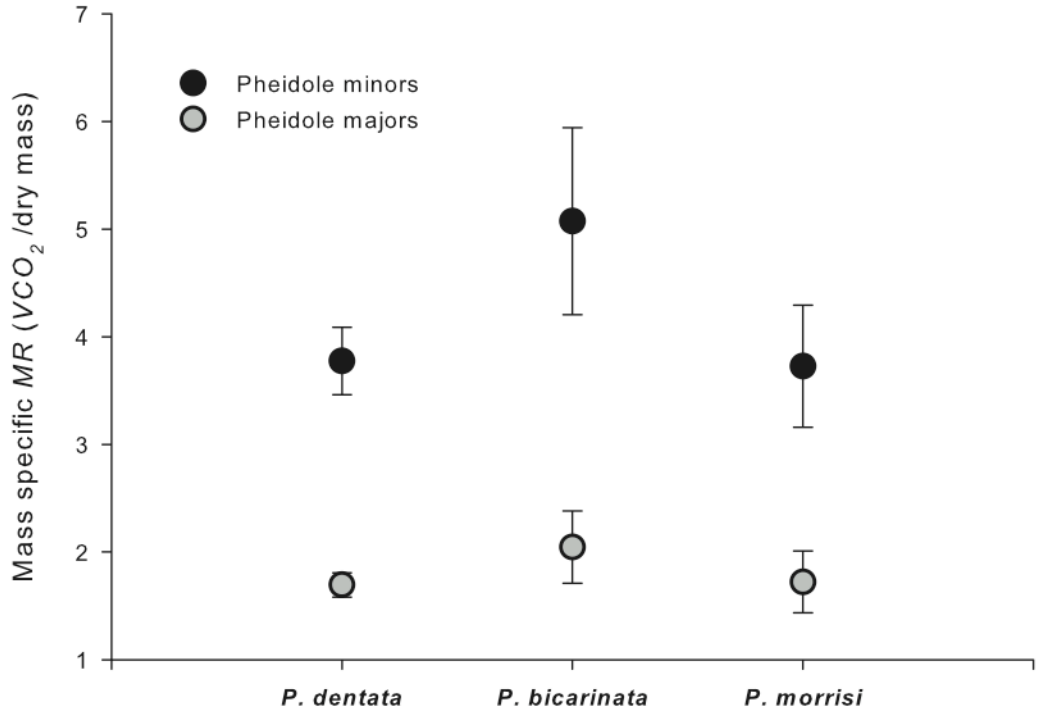

Pheidole minors
Pheidole majors
Mass specific MR (VCO_2 /dry mass)
7
6
5
4
3
2
1
P. dentata
P. bicarinata
P. morrisi

Figure 2

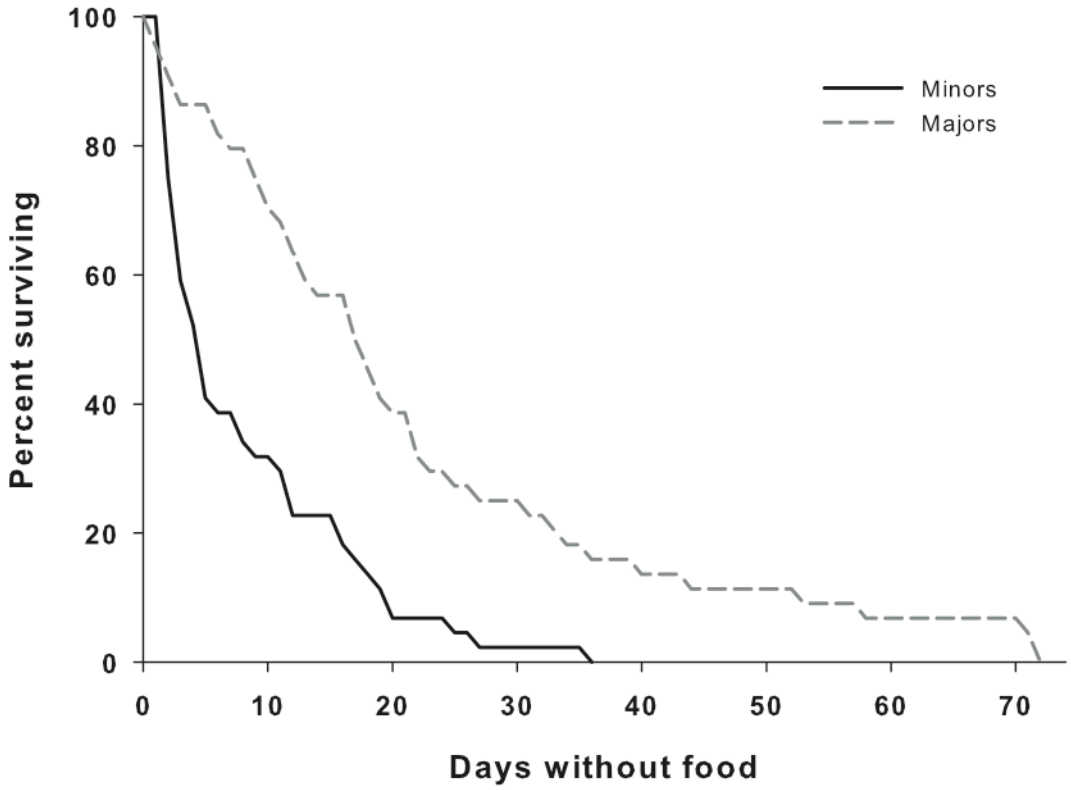

Minors
Majors
Percent surviving
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Days without food

Figure 3

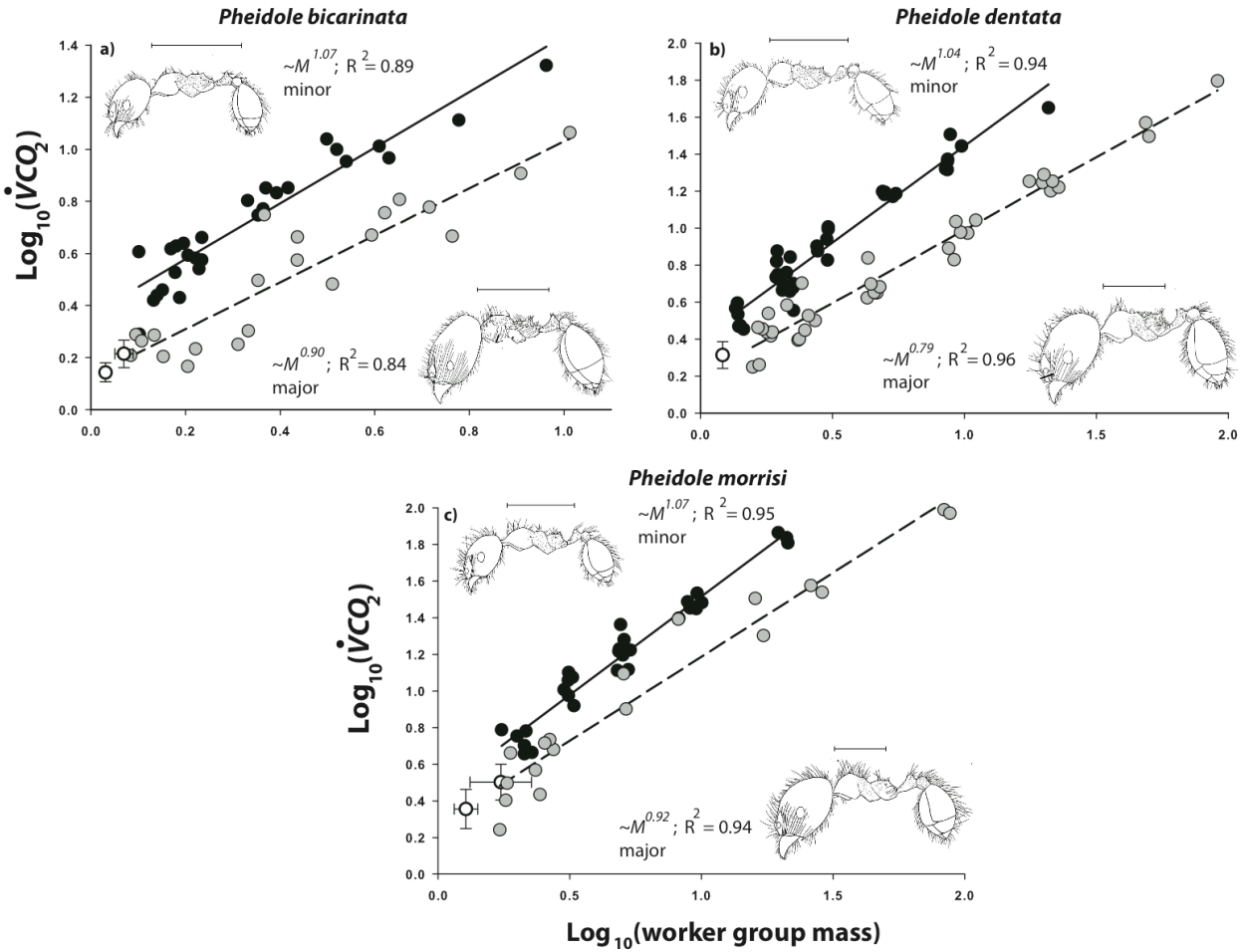
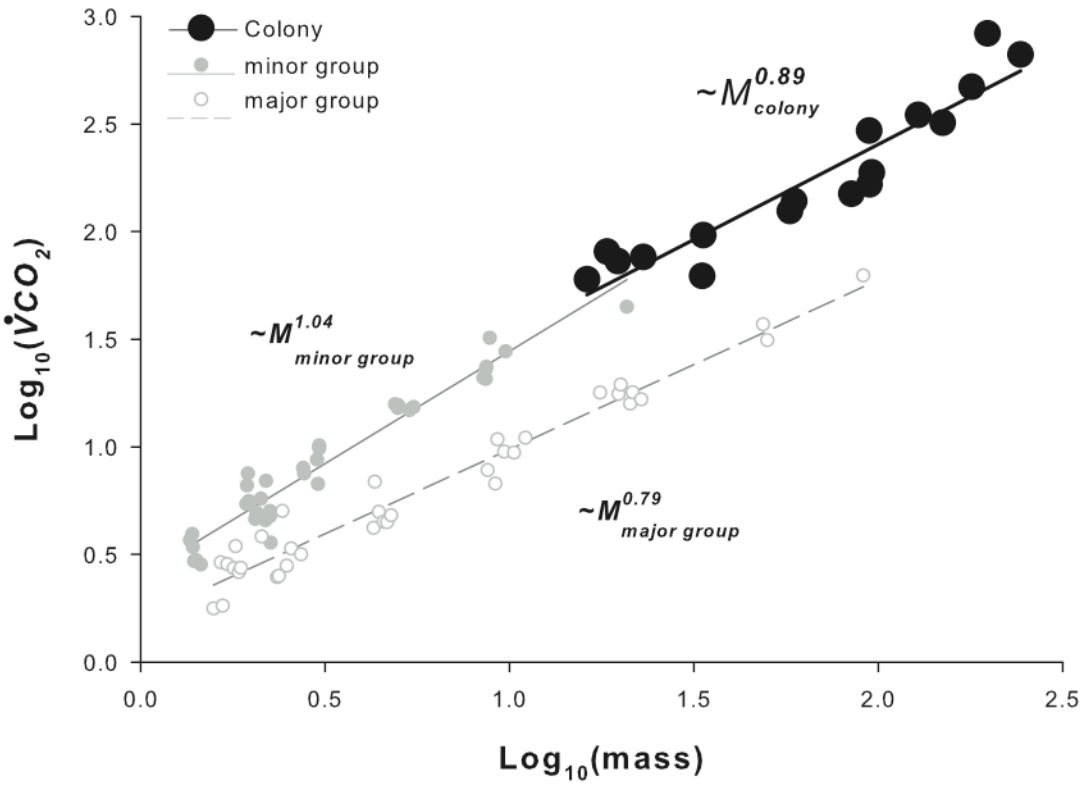

Pheidole bicarinata
a)
~M 1.07; R 2 = 0.89
minor
~M 0.90; R2 = 0.84
major
Pheidole dentata
b)
~M 1.04; R 2 = 0.94
minor
~M 0.79; R2 = 0.96
major
Pheidole morrisi
c)
~M 1.07; R 2 = 0.95
minor
~M 0.92; R 2 = 0.94
major
Log10(V̇CO2)
Log 10(worker group mass)

Figure 4


Colony
minor group
major group
~M^0.89 colony
~M^1.04 minor group
~M^0.79 major group
Log10(V̇CO2)
Log10(mass)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5

Appendix S1 Information about *Pheidole* colonies used to generate group scaling regressions

Species	Collection Date	Collection Code	Specimen Accession Number MEKOU
P. dentata	5/29/08	TSE7	208557
P. dentata	5/28/08	TSE6	208554
P. bicarinata	9/12/08	TSE10	208560
P. bicarinata	9/12/08	TSE11	208561
P. bicarinata	9/14/08	TSE12	208562
P. bicarinata	9/14/08	TSE13	208563
P. bicarinata	9/14/08	TSE14	208564
P. bicarinata	9/20/08	TSE16	208569
P. bicarinata	9/20/08	TSE17	208570
P. morrisi	5/27/09	KR1	208589

TSE: Thirty sixth street extension (N 35.21002, W 97.50235) is a sandy field with scattered pampas grass and juniper trees in Norman, Oklahoma, USA. All colonies of *P dentata*, *P. bicarinata* were harvested here. Nest of *Pheidole* were located by baiting workers with pecan sandies and following them back to the entrance holes, which were typically at the base of grass clumps.

KR1: Grassland in Norman, Oklahoma (N35.24302, W. 97.17092).

CHAPTER 4

Agricultural innovation and the metabolic rates of fungus-growing ant societies

(formatted for *Proceedings of the Royal Society B*)

ABSTRACT

Ants belonging to the tribe Attini practice agriculture—they harvest resources that they use to produce fungal gardens. While the benefit of these gardens are clear—providing food—their costs to the colony remain unmeasured. Here, we use respirometry to measure the fraction of colony metabolism contributed by either fungi or the ant farmers in 12 species spanning the attine phylogeny. Because fungal gardens averaged 90% of each colony's total mass, they determined the overall metabolism of the ant-fungus symbiosis, although fungal metabolism averaged only 31% that of ants on a mass-specific basis. Metabolic allometries, however, reduced the relative costs of maintaining larger gardens across species of higher attines with increasingly domesticated fungal cultivars. Compared with 26 non-agricultural ant species, attine colonies used less energy, gram for gram. Such economies of scale, also found in human agriculture, may contribute to the evolution of larger colony sizes in the attine clade, from basal species with less than 100 workers to the derived superorganisms of the genus *Atta* with several million workers.

1. INTRODUCTION

The rise of agriculture by humans 10,000 years ago was preceded by ants of the tribe Attini which began cultivating fungi *ca.* 50 million years ago, and have since evolved into >230 species common across the New World tropics (Weber 1972; Mueller *et al*. 2005; Schultz & Brady 2008). An attine agricultural system (*hereafter* colony-farm) consists of ants and their fungi within a nest, and a foraging territory from which ants collect the plant tissue and detritus they use as substrate to manure their fungal gardens (Martin & Weber 1969; Leal & Oliveira 2000; Wirth *et al*. 2003). Fungi allow their farmers to exploit resources unavailable to most other ants, but agriculture also has costs, many of which are analogous to those faced by human farmers (*e.g.*, losses due to crop pests; Currie *et al*. 2003; Mueller *et al*. 2005; Fernandez-Marin *et al*. 2009). Cultivars also divert resources from their hosts through both maintenance respiration (*e.g.* Kleineidam & Roces 2000) and potentially, through selfish investment in reproductive effort (Mueller 2002). Many of these costs and benefits reduce to a currency of energy and thus metabolism, which governs the rate at which fungi process resources and use them to fuel agricultural production.

Fungicultural energetics is likely mediated by a variety of factors that vary across species including cultivar physiology (Gomes de Siqueira *et al.* 1998; Abril & Bucher 2002; De Fine Licht *et al*. 2010) and complex microbial interactions (Pinto-Tomas *et al*. 2009; Scott *et al*. 2010). Cultivar metabolism also likely reflects the performance of ant farmers whose colony size and organization have increased during the course of attine evolution, from basal genera with <100 monomorphic workers, to the largest and most complex of all ant societies—the superorganisms of the leaf cutter

genus *Atta* that can have millions of workers with specialized castes of different sizes (Hölldobler & Wilson 2008, 2010). Here, we use a metabolic scaling approach to unify the study of these diverse agricultural systems around a shared metabolic currency. Biologists have long known that larger organisms tend to have lower mass-specific energy demands because of allometric scaling ($Metabolic\ rate = Mass^{b\approx0.75}$) (Kleiber 1932; Peters 1983; Brown *et al*. 2004), and recent findings suggest this allometry extends to superorganism ant colonies (Hou *et al*. 2010; Waters *et al*. 2010). We test for similar metabolic allometry in the extended phenotype of colony-farms and use the residual variation to explore the costs and benefits of agricultural innovation in the tribe Attini.

(a) ***The metabolism of colony-farms***

To persist, colony-farms must meet the combined maintenance demands of both ant workers and fungal cultivars. The metabolism of the workforce is likely governed by the classic metabolic allometry reflecting lower mass-specific metabolic demands with increasing size ($\sim M^{0.69}$; Chown *et al.* 2007; Shik 2010). Given the 200-fold variation in worker dry mass across the attine phylogeny (*Cyphomyrmex costatus* 0.091-mg to *Atta sexdens* 19.25-mg) that, in come cases, also exists within attine colonies (0.11 to 19.25-mg *A. sexdens* worker castes) (Shik *unpublished*, Wilson 1980), colony-farm energetics likely depend upon worker morphology. Likewise, fungal gardens, which lack energetically costly traits like brains (Laughlin *et al*. 1998) and muscle tissue of animals (Roces & Lighton 1995), should be cheaper to maintain than ants, gram for gram. However, gardens may exceed the mass of ants in colonies by >1 order of magnitude (Seal & Tschinkel 2008). To date, however, the basic

energetics of colony-farms, and how they scale with colony size and across the diverse attine lineage, remain unknown. Here, we use metabolic scaling to compare the maintenance costs of attine workers and their gardens within colonies and across species.

(b) ***Metabolism and agricultural innovation across attine societies***

Key agricultural innovations map onto the attine phylogeny (*e.g.*, yeast agriculture in the genus *Cyphomyrmex* and coral fungus (Pterulaceae) farming in the genus *Apterostigma*; Munkacsi *et al.* 2004; Schultz & Brady 2008), but an evolutionary trend towards increasingly derived cultivars (*i.e.*, greater domestication) more generally separates the *lower* from the *higher* attines (Mueller 2002). The seven genera of lower attines have continually acquired novel strains of leucocoprinaceous fungi (Basidiomycota: Agaricales: Agariaceae) during their evolution (Mueller 2002, Vo *et al.* 2009), yielding cultivars retaining suites of enzymes that degrade cell walls in a manner similar to free-living fungi (De Fine Licht *et al.* 2010). In contrast, the four genera of higher attines (*Trachymyrmex*, *Sericomyrmex*, *Acromyrmex*, and *Atta*) domesticated a single fungal symbiont that has since diversified and evolved traits enhancing its production value—namely swollen, nutrient-rich hyphal tips called gonglydia (Mueller 2002; Mikheyev *et al.* 2010) and enzyme profiles suited for extracting protein and starch from manured substrate (De Fine Licht *et al.* 2010). Here, we test whether selection under domestication for this single symbiont yields a higher effectiveness in the degradation of substrate, and with it, higher metabolic activity.

(c) ***The metabolism of agricultural and non-agricultural ant societies***

Attine colony-farms, especially those in tropical forests, inhabit species-rich communities of other ants, many of which scavenge the same substrate for direct consumption. Attine ants often exceed these ants in terms of abundance and ecological impacts (Wirth *et al.* 2003) and they may have a competitive advantage stemming from the superior processing capacity of their fungal gardens. However, attine ants also face energetic constraints resulting from harvesting energy from low trophic levels, through detritivory and herbivory (Kaspari 2001), while expending energy to maintain not only ant biomass, but also fungal biomass and nest environments favourable for fungal production (Bollazzi *et al.* 2008). We used metabolic scaling to examine whether the rise of attine agriculture yielded energetic efficiencies over ancestral hunter-gatherer-type ant societies.

2. MATERIALS AND METHODS

(a) *Harvesting and culturing ants*

Ant colonies were harvested from habitats across the southern USA and from Barro Colorado Island (9° 09′ N, 79° 51′ W), a lowland tropical forest managed by the Smithsonian Tropical Research Institute in Panama (electronic supplementary table S1). Colonies were established in respirometry chambers designed for long-term ant habitation and connected by plastic tubing to foraging arenas (electronic supplementary appendix S1) and maintained until they transformed fungal mycelia into a homogenized mass of fungus (*ca.* 3 weeks to 2 months). Leaf cutter ants (colonies of *Atta* and *Acromyrmex*) were fed *ad libitum* the minced pith from orange peels, occasional leaves from rose plants (only for *Atta* species), and a mixture of

ground rolled oats and polenta, replaced weekly. All other colonies were provided with only the oat-polenta mixture. Non-agricultural species (electronic supplementary table S1), harvested as part of a larger project on colony energetics (Shik *et al.* In Prep), were generally provided water until they were used for respirometry within 2 days of capture.

(b) ***Recording individual worker*** $\dot{V}CO_2$

Equipment from Sable Systems International (SSI; Las Vegas, Nevada, USA) was used to perform constant volume respirometry and record metabolic rate (μL CO_2 hr^{-1}, hereafter, $\dot{V}CO_2$). Before all trials, a CA-10 CO_2 analyzer (accuracy of 1%, resolution of 0.00001%) was zeroed with N_2 gas and then spanned with a gas of known CO_2 concentration (1,000 p.p.m. CO_2 in N_2 ± 1%). When possible, 6 workers were chosen for each species to reflect the range of body sizes found in colonies. For each trial, individual workers were placed in chambers attached to an RM8 multiplexer—six contained single ants; one remained empty as a control. Hourly CO_2 values from the empty chamber were subtracted from all experimental chambers to correct for extrinsic CO_2. Respirometry chambers for individual ants were 10-ml syringe barrels fitted with rubber stoppers and were cleaned with 95% EtOH between trials. To baseline measures of ant respiration, air scrubbed of CO_2 was passed through tubing affixed to the eighth position on the multiplexer between each experimental trial.

Incurrent air was first scrubbed of H_2O and CO_2 using a drierite/ascarite/drierite column (Lighton 2008). Flow rate was set to 50 ml min^{-1} using

an SS-3 subsampler pump, and regulated by a 200 ml min^{-1} Sierra Valve set by an MFC unit. This air was scrubbed by a second drierite/ascarite/drierite column and then sent to the multiplexer, programmed to switch between chambers using SSI Expedata software. Ambient temperature was continuously recorded adjacent to respirometry chambers using a Thermistor cable. All equipment was interfaced with a computer using a SSI UI-2.

In this way, ants in chambers were placed in air free of CO_2. After 1 hr, this air was flushed out for 200 s at 50 ml min^{-1}, passed through a 10-cc column of magnesium perchlorate (Cl_2MgO_8) to remove any remaining moisture, and then to a CO_2 analyzer. Trials were run for 6 hr, yielding six measurements per chamber. The first hour of data included extrinsic CO_2 and was never used. Thereafter, ants were generally inactive and CO_2 readings stabilized at a lower value (Lighton 2008). Each data point was the mean of five hourly measurements of single ant respiration taken during this time. After experimental measurements, ants were frozen, dried at 60°C for 24 hr, and weighed to the nearest 10^{-3} mg. Individual ants were always used for only a single experimental trial.

(c) ***Recording colony $\dot{V}CO_2$***

Colony-level $\dot{V}CO_2$ was generally recorded as described above. An exception was that colony respirometry chambers were designed for long-term ant habitation, and flow rates were determined to adequately flush the chamber volumes (electronic supplementary appendix S1). For trials with large chambers, Peltier Effect Air Driers (Sable Systems PC-3) were used to reduce the dew point of incurrent and excurrent air

to 1ºC. At the completion of each trial, desired colony components (including cultivated fungi) were dried and weighed as above. To quantify worker size variation within colonies, all workers were individually weighed and the coefficient of variation (CV) calculated for each colony. To partition $\dot{V}CO_2$ between ants and cultivars, fungal gardens were removed following colony-farm trials, and measurements taken for only ants. The queen was then separated and placed in a 10-ml chamber and her $\dot{V}CO_2$ determined as described for individual ants. Because colony chambers were not axenic, empty chamber $\dot{V}CO_2$ was recorded following all experimental trials and subtracted from their respective colony-level values.

(d) ***Data analysis***

SSI ExpeData software was used to subtract the empty chamber CO_2 from each experimental measurement and correct for small variations in flow rate (±0.1ml min^{-1}). This software was then used to generate the variable $\dot{V}CO_2$ by transforming CO_2 measurements from p.p.m. to μl hr^{-1} and integrating these values for trial intervals. $\dot{V}CO_2$ measurements were standardized to 25°C assuming $Q_{10} = 2$ (Lighton 2008). Minimal temperature corrections were necessary, however, because the mean of 348 hourly temperature measurements was 23.6 ± 0.4°C. Ordinary least square regression was used to estimate a and b in the scaling equation $\log_{10}y = \log_{10}a + b\log_{10}M$. Scaling characterized the dependence of $\dot{V}CO_2$ ($\mu l\ CO_2\ hr^{-1}$; y) on worker or colony-farm size (*dry biomass mg*; M). To detect allometry ($b \neq 1$), an F-statistic was calculated to test the null hypothesis of isometry (H_0: $b = 1$). Where necessary,

ANCOVAs were used to compare regressions, and were rerun to examine main effects after removing non-significant interactions. For each scaling relationship, a standard measure of the range of mass was calculated as $pWR = \log_{10}(M_{max}/M_{min})$ (Prothero 1986).

(e) ***Chitin assays to estimate fungal biomass***

Attine gardens are a complex matrix of cultivated fungi and associated microbes as well as undigested substrate that adds mass but not metabolic activity. An allometry of fungal biomass with colony-farm size, or systematic differences between higher and lower attines in percent fungal content would suggest differences in the metabolically active portion of gardens that could influence metabolic scaling results. We tested these assumptions using chitin assays to estimate fungal biomass in gardens (*as per* Seal & Tschinkel 2007) (electronic supplementary appendix S1).

3. RESULTS

(a) ***The metabolism of colony-farms***

Across 12 ant species, $\dot{V}CO_2$ scaled as $worker\ mass^{0.81\pm0.11}$ (figure 1), from the smallest (*A. colombica*; 0.098 mg) to the largest sampled worker (*A. texana*; 2.430 mg) (table 1), with a slope that was significantly less than isometry (table 2). Colonies of *Atta* and *Acromyrmex*, higher attines with the most derived caste systems, had the greatest worker size variation (*i.e.* highest CV; table 3) and the widest range of worker maintenance costs (table 1; figure 2).

Gardens averaged, gram for gram, 31% the energetic maintenance costs of their ant farmers (figure 2). However, gardens made up on average 90.0% (± 7.7%) of

each colony's total mass and 72.1% (± 13.1%) of the colony-farm's energy demands (table 3). Furthermore, colony-farms grew in mass mostly by adding garden mass (~*colony mass*$^{1.04}$; $R^2 = 0.99$), not worker mass (~*colony mass*$^{0.68}$; $R^2 = 0.67$) (electronic supplementary figure S1A). Thus, gardens generally respired more than ants, regardless of whether colony-farms contained <10 or >100 workers (table 3).

(b) ***Metabolism and agricultural innovation across attine societies***

When all 12 attine species were plotted together (from 25.95-mg *Apterostigma dentigerum* to 3,819.45-mg *Atta cephalotes*), metabolic rate increased linearly (table 2) with colony-farm mass (regression line not plotted, figure 3). Variation around this relationship appeared to be correlated with the level of fungal domestication, with lower attines from the genera *Mycocepurus* and *Cyphomyrmex* and the basal coral fungus-farming *Apt. dentigerum* falling below the regression line (figure 3). This pattern was driven by the lower mass-specific metabolic rates of lower attine gardens (mean ± 1SD: 0.207 ± 0.08 µl CO_2 mg^{-1} hr^{-1}; n = 4 colonies) relative to higher attine gardens (mean ± 1SD: 0.637 ± 0.33 µl CO_2 mg^{-1} hr^{-1}; n = 18 colonies) (electronic supplementary table S2). The mass-specific $\dot{V}CO_2$ for higher attine gardens was significantly higher than the mean value (0.207) for lower attines (one-sample t-test: $t_{16,0.05} = 5.35$; $p = 0.001$), and chitin assays suggest this resulted because higher attine gardens were composed of more fungal biomass than lower attine gardens (electronic supplementary figure S2).

When colony-farms of higher attines (*i.e. Trachymyrmex*, *Acromyrmex*, and *Atta*) were studied in isolation $\dot{V}CO_2$ scaled allometrically ($b = 0.87$; $R^2 = 0.93$) (figure

3), with a slope significantly less than isometry (table 2). Chitin mass scaled isometrically with garden mass (b = 1.07; R^2 = 0.80; electronic supplementary figure S1B) suggesting that the higher attine allometry was not due to systematic changes in garden composition with colony-farm size.

(c) ***The metabolism of agricultural and non-agricultural ant societies***

Across colonies of 26 non-agricultural species, $\dot{V}CO_2$ scaled as $colony\ mass^{0.86}$ (R^2 = 0.97; figure 3, table 2). This slope was less than the slope for all attine colony-farms combined (ANCOVA: $F_{1,85}$ = 4.46; p = 0.04), but did not differ from the slope for only the higher attines (ANCOVA: $F_{1,78}$ = 0.06; p = 0.81). Both the regression with only higher attines (ANCOVA: $F_{1,79}$ = 11.14; p = 0.001) and the regression combining all attine colonies ((ANCOVA: $F_{1,85}$ = 16.30; p = 0.0001) had significantly lower intercepts than the regression for non-agricultural species, indicating lower mass-specific metabolic rates in colony-farms.

4. DISCUSSION

Metabolic rates of attine colony-farms vary predictably with the size and organization of their agricultural systems. First, fungi have low mass-specific metabolic rates, but garden mass and thus total energy demands far exceed that of the corresponding ant farmers. Second, the domesticated cultivars of derived higher attines appear to do more metabolic work, gram for gram, than those of lower attine fungi, and their elevated energy demands may reflect their enhanced production value (*e.g.*, greater enzyme activity and nutrient-rich gonglydia). Third, metabolic allometry

suggests that large colonies of higher attines net greater agricultural production from relatively less metabolic work. These results support a hypothesis that an energetic economy of scale favours the evolution of larger agricultural systems, laying the groundwork for the evolution of superorganisms in the genus *Atta*. However, this allometry also means that attine colonies face metabolic inefficiencies during the first months of existence. This inefficiency is because each attine colony-farm begins small—a foundress queen disperses from her natal nest with a bit of cultivar to start her own garden and colony.

We propose the following hypotheses linking the traits of workers and cultivars with metabolic allometry across higher attine colony-farms. First, a positive correlation between colony size and division of labour across higher attines, from *Trachymyrmex* with <1,000 workers (Beshers and Traniello 1996) to *Atta* with >1 million (Hölldobler & Wilson 2010) is predicted to yield energetic savings through task specialization (Wilson 1980; Lighton *et al*. 1987; Roces & Lighton 1995). Second, larger colony-farms would use relatively less energy if they experience relatively fewer losses through cultivar-host conflict. However, widespread horizontal transfer of fungi decouples the evolutionary interests of host and symbiont in even the largest *Atta* colonies (Mikheyev *et al.* 2007) yielding potentially energetically costly conflicts (Mueller 2002). Determining the mechanisms generating metabolic allometry will be critical for understanding the ecological factors shaping the evolution of ever-larger colony-farms.

Respirometry experiments require caveats. First, it was not possible for us to separate ant brood from gardens and they likely added some $\dot{V}CO_2$ to fungal

measurements. However, brood may also be necessary for proper fungal metabolism, providing key digestive enzymes and serving as a 'digestive caste' (D'Ettorre *et al.* 2002; Erthal *et al.* 2007). Second, maximizing phylogenetic scope involved comparing young colonies of some species (*e.g.*, *Atta* and *Acromyrmex*) with reproductively mature colonies of other species (*e.g.*, *T. cornetzi* and *M. smithii*). Further study will be needed to test how the metabolic scalars reported here govern energy flow in mature colonies found in the field. Third, chitin assays represent a first step to examining the metabolic composition of fungal gardens. Gardens are complex biotic structures and further study will be needed to partition the energy demands among all microbial components (*e.g.*, Scott *et al.* 2010; Pinto-Thomas *et al.* 2009), and how microbial traits vary across the attine phylogeny as well as with factors such as garden age, health, and diet.

Attine colony-farms are unique in using harvested resources to sustain fungal gardens, rather than directly feeding larvae. Despite the energetic costs of fungus cultivation (Wilson 1980; Lighton *et al.* 1987; Roces & Lighton 1995), agricultural ant societies use energy at lower rates, gram for gram, than their non-agricultural ancestors (figure 3). However, the metabolic rates of both types of ant societies are constrained by the same allometries (figure 3), which may reflect highly conserved mechanisms thought to govern metabolic rates across all living things (Brown *et al.* 2004), or the intricacies of colony life (*e.g.*, allometries of worker behaviour; Waters *et al.* 2010). Further exploration of these mechanisms will provide important insights into the costs and benefits of attine agriculture, and more generally, into the costs and benefits of eusocial colony life.

ACKNOWLEDGEMENTS

D. Donoso, O. Fincke, P. Jeyasingh, A. Kay, and R. Knapp provided excellent suggestions for improving this manuscript. We thank R. Adams, S. Van Bael, and D. Donoso for help collecting and cultivating attine colonies. Technical support from B. Joos and J.R.B Lighton at Sable Systems International made this research possible. This project was funded by the National Science Foundation under the grants DEB-0842038 to A. Kay & M. Kaspari, DEB-0639879 to U. Mueller, IOS-0920138 to U. Mueller & J. Seal, and by an Alumni Fellowship from the University of Oklahoma to J. Shik.

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Tables

Table 1 Summary of worker traits for attine species used in respirometry experiments. Size is dry mass (mg) of individual ants averaged for n individuals. The value pWR = $\log_{10}$ (maximum mass / minimum mass) is a standard measure of the range of mass.

Species		Worker size				Worker maintenance costs		
	n	Mean mass (mg)	SD	pWR	Range	Mean $\dot{V}CO_2$ (µl CO_2 hr^{-1})	SD	pWR
Mycocepurus smithii	1	0.130	n/a	n/a	n/a	0.232	n/a	n/a
Apterostigma dentigerum	3	1.163	0.135	0.10	1.036 to 1.304	1.151	0.552	0.47
Cyphomyrmex wheeleri	14	0.205	0.016	0.12	0.187 to 0.248	0.563	0.148	0.38
C. longiscapis	3	0.250	0.005	0.02	0.245 to 0.254	0.255	0.035	0.12
Trachymyrmex septentrionalis	4	0.544	0.053	0.10	0.496 to 0.620	1.005	0.303	0.22
T. cornetzi	6	0.457	0.027	0.07	0.420 to 0.493	0.846	0.114	0.17
T. turrifex	6	0.524	0.056	0.12	0.454 to 0.596	0.892	0.270	0.35
T. zeteki	7	0.741	0.190	0.29	0.541 to 1.052	1.094	0.290	0.35
Acromyrmex versicolor	5	0.667	0.292	0.40	0.470 to 1.183	0.746	0.263	0.11
Atta colombica	10	0.468	0.360	1.03	0.098 to 1.045	1.408	0.857	0.86
Atta cephalotes	9	0.710	0.576	0.95	0.178 to 1.580	2.608	2.088	1.09
Atta texana	30	0.927	0.657	1.21	0.151 to 2.430	1.906	1.670	1.38

Table 2 Results from log-log least squares regression scaling: Worker $\dot{V}CO_2$, Attine colony-farm $\dot{V}CO_2$ (attine queen, worker, brood, fungal garden for all 12 attine species), lower attines, higher attine colony-farm $\dot{V}CO_2$ (species of *Trachymyrmex*, *Acromyrmex*, and *Atta*), non-agricultural $\dot{V}CO_2$ (non-attines). For each regression, deviation of slope from isometry (b = 1.0) denoted by *(P < 0.05), **(P < 0.01), ***(P < 0.001), ****(P < 0.0001). N denotes number of worker or colonies in analysis.

Dependent Variable	N	MS model	MS error	F	r^2	*a*	SE of *a*	*b*	SE of *b*	± 95% CI of *b*
Worker $\dot{V}CO_2$	98	7.47	0.04	212.80	0.69	0.25	0.03	0.81***	0.06	0.11
Attine colony-farm $\dot{V}CO_2$	25	9.71	0.07	140.17	0.86	-0.26	0.21	1.00	0.08	0.17
Lower attine colony farm $\dot{V}CO_2$	7	0.52	0.01	59.48	0.92	0.23	0.16	0.60**	0.08	0.20
Higher attine colony-farm $\dot{V}CO_2$	18	4.80	0.02	213.19	0.93	0.19	0.16	0.87*	0.06	0.13
Non-agricultural $\dot{V}CO_2$	64	45.70	0.02	1837.4	0.97	0.40	0.03	0.86****	0.02	0.04

Table 3 Composition of colony-farms used in respirometry experiments. All masses are dry (mg). Worker CV is the coefficient of variation of worker mass. ‡ Percent fungus $\dot{V}CO_2$ unavailable because one whole whole colony-farm metabolic recording was made.

Species	ID	Worker		% of colony that is:		
		Number	CV	Fungal garden mass	Chitin mass	Fungal $\dot{V}CO_2$
Lower Attines						
M. smithii	UGM30A	34	0.11	93	n/a	70
M. smithii	UGM30C	37	0.08	86	1.7	63
Apt. dentigerum	Gb1	5	0.16	82	1.1	n/a‡
C. wheeleri	UGM02A	26	0.10	99	0.4	87
C. wheeleri	UGM02C	24	0.08	99	0.7	90
C. longiscapus	Gb2	16	0.09	94	1.0	n/a‡
C. longiscapus	RMMA1	12	0.16	96	1.5	n/a‡
Higher Attines						
T. septentrionalis	TSE1	11	0.13	83	0.4	67
T. septentrionalis	TSE20	67	0.20	93	0.6	87
T. cornetzi	ColMR1	76	0.19	96	1.8	72
T. turrifex	BFL1	36	0.13	77	0.3	71
T. zeteki	NHC21	4	0.12	98	4.3	93
T. zeteki	S61	65	0.14	86	1.6	81
T. zeteki	TZ100	90	0.25	88	3.2	70
T. zeteki	TZ101	42	0.26	87	2.1	58
Acr. versicolor	VE096	11	0.34	86	3.4	88
At. versicolor	VE099	9	0.47	66	1.2	61
At. texana	Atex2	164	0.75	97	3.8	86
At. texana	Atex3	104	0.49	94	2.0	70
At. texana	Atex4	110	0.63	96	3.6	80
At. texana	Atex5	87	0.58	96	5.9	73
At. colombica	Sun17	329	0.98	91	1.4	57
At. colombica	Sun31	230	0.94	91	0.7	64
At. colombica	Sun43	258	0.87	86	1.4	45
At. cephalotes	Sun3	486	0.97	91	0.6	53

Figure Captions

Figure 1 Allometric scaling of $\dot{V}CO_2$ with worker mass for 12 attine species.

Figure 2 Mass-specific maintenance costs for workers and fungus gardens. Statistics are one-sample t-tests testing H_0: worker mass-specific metabolic rate = mean fungus mass-specific metabolic rate. Fungus data are means (± 1 SD). All masses are dry (mg).

Figure 3 Scaling of $\dot{V}CO_2$ with colony mass across higher attines and non-agricultural colonies. Lower attines *Mycocepurus smithii*, *Apterostigma dentigerum*, *Cyphomyrmex longiscapis*, and *C. wheeleri* fell below the higher attine regression.

Figure 1

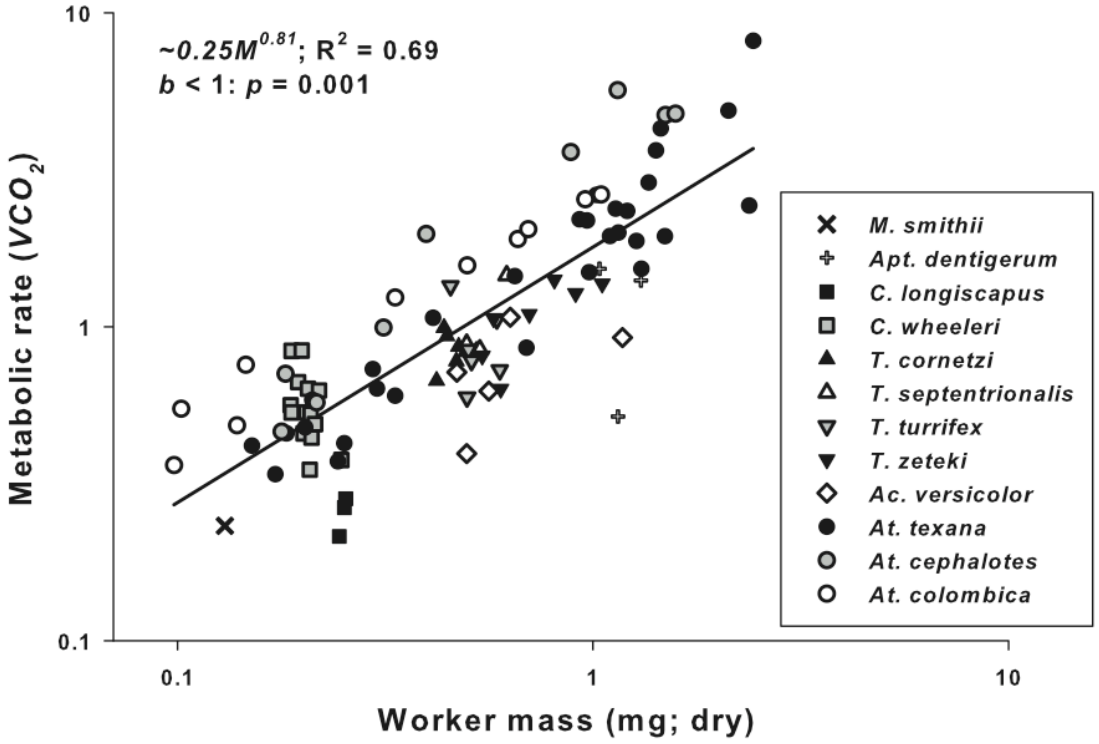

~0.25M^0.81; R^2 = 0.69
b < 1: p = 0.001
Metabolic rate (VCO2)
Worker mass (mg; dry)
10
1
0.1
0.1
1
10
M. smithii
Apt. dentigerum
C. longiscapus
C. wheeleri
T. cornetzi
T. septentrionalis
T. turrifex
T. zeteki
Ac. versicolor
At. texana
At. cephalotes
At. colombica

Figure 2

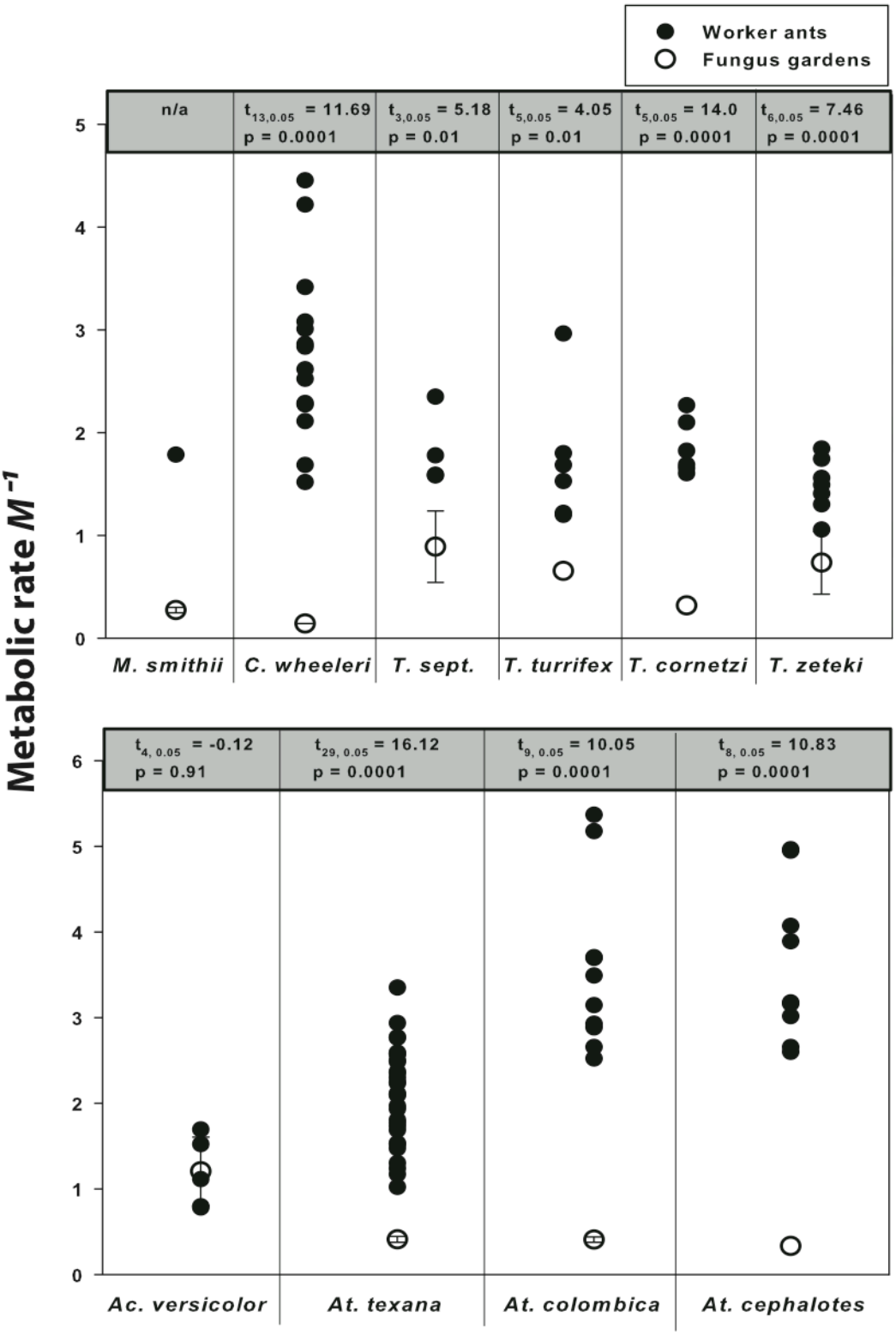
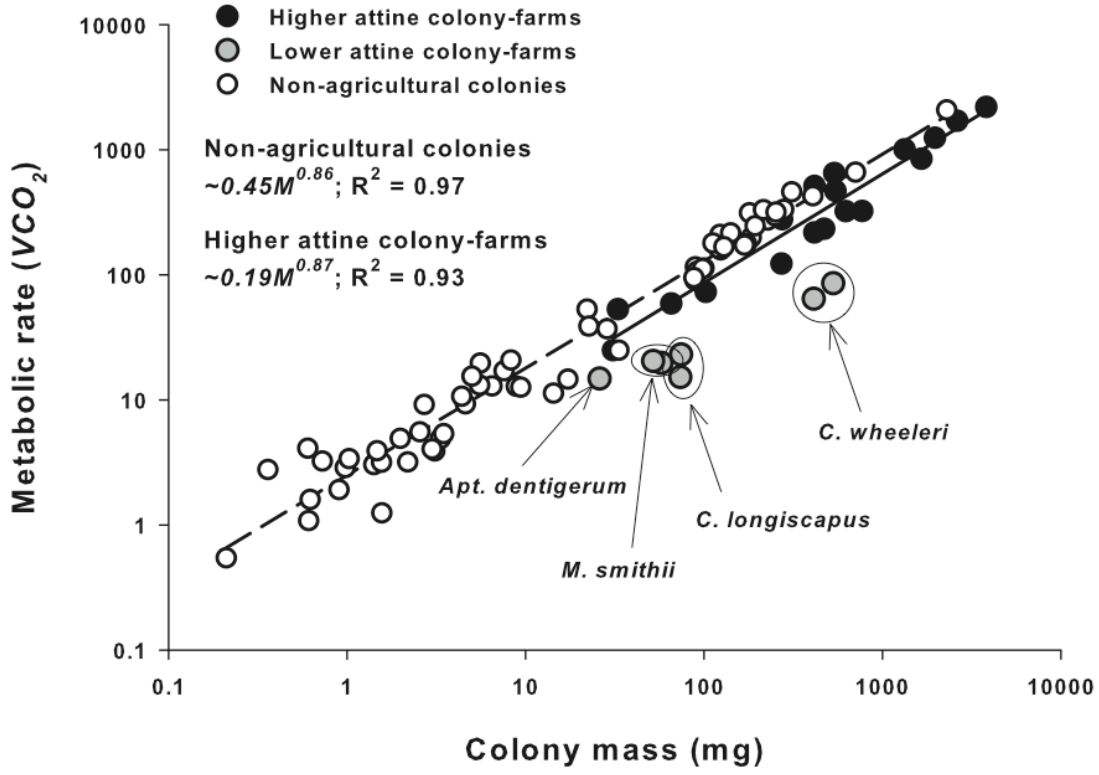
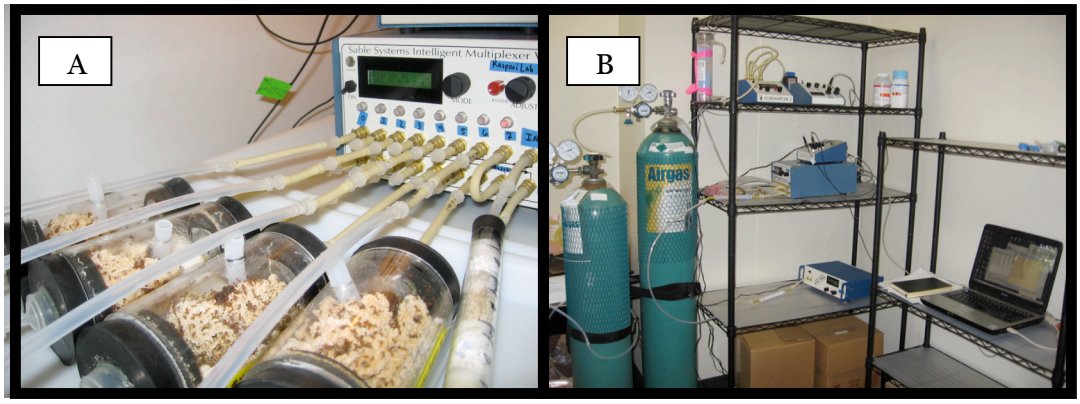

Worker ants
Fungus gardens
Metabolic rate M^{-1}
n/a
$t_{13,0.05}$ = 11.69
p = 0.0001
$t_{3,0.05}$ = 5.18
p = 0.01
$t_{5,0.05}$ = 4.05
p = 0.01
$t_{5,0.05}$ = 14.0
p = 0.0001
$t_{6,0.05}$ = 7.46
p = 0.0001
M. smithii
C. wheeleri
T. sept.
T. turrifex
T. cornetzi
T. zeteki
$t_{4,0.05}$ = -0.12
p = 0.91
$t_{29,0.05}$ = 16.12
p = 0.0001
$t_{9,0.05}$ = 10.05
p = 0.0001
$t_{8,0.05}$ = 10.83
p = 0.0001
Ac. versicolor
At. texana
At. colombica
At. cephalotes

Figure 3


Higher attine colony-farms
Lower attine colony-farms
Non-agricultural colonies
Non-agricultural colonies
~0.45M^0.86; R^2 = 0.97
Higher attine colony-farms
~0.19M^0.87; R^2 = 0.93
Apt. dentigerum
M. smithii
C. longiscapus
C. wheeleri
Metabolic rate (VCO2)
Colony mass (mg)
10000
1000
100
10
1
0.1
0.1
1
10
100
1000
10000

Appendix S1 The respirometry system used to record colony-farm metabolic rate: a) Colonies of *A. colombica* cultured in nest chambers and hooked up to Sable Systems multiplexer. b) The Sable Systems respirometry system used at the University of Oklahoma and on Barro Colorado Island.



Colony demography

The demographies of most colonies used in this study were the product of each colony's own development. Exceptions were monogynous colonies of *M. smithii* (N = 40 workers) and *Cyphomyrmex wheeleri* (N = 40 workers) that were established from larger polygynous colonies based on field estimates of colony size. In addition, four queenless subcolonies of *Atta texana* were established from a larger colony by removing *ca.* 5-cm^3 fragments of fungi and all ants contained within.

Respirometry chambers for whole colonies

Both colony-farms and non-agricultural colonies were cultured in chambers designed for long term ant habitation: 10 cm x 2.75 cm clear cylindrical acrylic tubes (*ca.* 238 ml) set lengthwise with plaster filling 1/3 of the volume (*Supplementary Figure S1a*). All edges where plaster met the acrylic tube were lined with small

amounts of aquarium sealant. For species with smaller colonies (*i.e.*, *C. wheeleri* and *M. smithii*), nests were built in the same way, but with 30-ml syringe barrels as nest chambers. At bi-weekly intervals, the plaster nest floor of all nests was hydrated with 0.025 ml dH_2O inserted through plastic tubes that were set into the plaster and extended through the chamber ceilings.

Both ends of colony tubes were plugged with large rubber stoppers (#12 Cole-Parmer) into which a hole was drilled and the end of a 35-ml syringe affixed, sealed, and screened from the inside by fine wire mesh. The syringe tips could be locked into the multiplexer, and air pushed through diffusely to ensure complete mixing. Flow rates were set to 320 ml min^{-1}, with data recorded hourly over 400 s for each colony chamber attached to the multiplexer. For 30-ml nests, smaller rubber stoppers were used (# 4 Cole-Parmer) and flow rates were set to 70 ml min^{-1} and recorded over 300 s.

Chitin extractions for estimating fungal biomass in gardens

We tested whether systematic changes in garden composition with garden mass or between higher and lower attine colony-farms affected scaling results. Specifically, we examined whether a negative allometry of metabolic rate (Fig. 3) was due to increases in the relative abundance of metabolically inactive undigested substrate. To do this, we determined the amount of chitin in attine gardens using the acid hydrolysis test for free aldehydes recently employed to estimate the amount of fungal biomass in attine fungus gardens (Seal & Tschinkel 2007). Chitin is the main

component of fungal cells walls, and is often used to estimate total (living and dead) fungal biomass in fungal substrates (Plassard *et al.* 1982).

The method of chitin determination we employed is a non-specific test for free aldehydes that result from the hydrolysis of 30-50 mg garden samples by 6N HCl for 18h at 80ºC and subsequent deamination of the glucosamine residues by nitrous acid (HNO_2) (Plassard *et al.* 1982; Vignon *et al.* 1986; Seal and Tschinkel 2007). Free aldehydes form a complex with MBTH (3-Methyl-2-Benzothiazolone hydrazone hydrochloride), which turns blue in the presence of ferric chloride ($FeCl_3$). Absorbances were read by a NanoDrop spectrophotometer at 650 nm. The chitin content of garden samples was estimated using a standard curve obtained by performing the same procedures on 5 dilutions of known amounts of chitin from crab shells (Sigma-Aldrich) (range 0.088 to 1.025 mg mL^{-1}) (Seal and Tschinkel 2007).

REFERENCES

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

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

Vignon, C., Plassard, C., Mousain, D. & Salsac, L. 1986 Assay of fungal chitin by estimation of mycorrhizal infection. *Physiol. Veget.* **24**, 201-207.

Figure S1A The scaling of fungus garden and ant biomass with colony-farm mass. Increases in garden mass drove increases in colony-farm mass (see methods for details about drying and weighing fungi and ants).

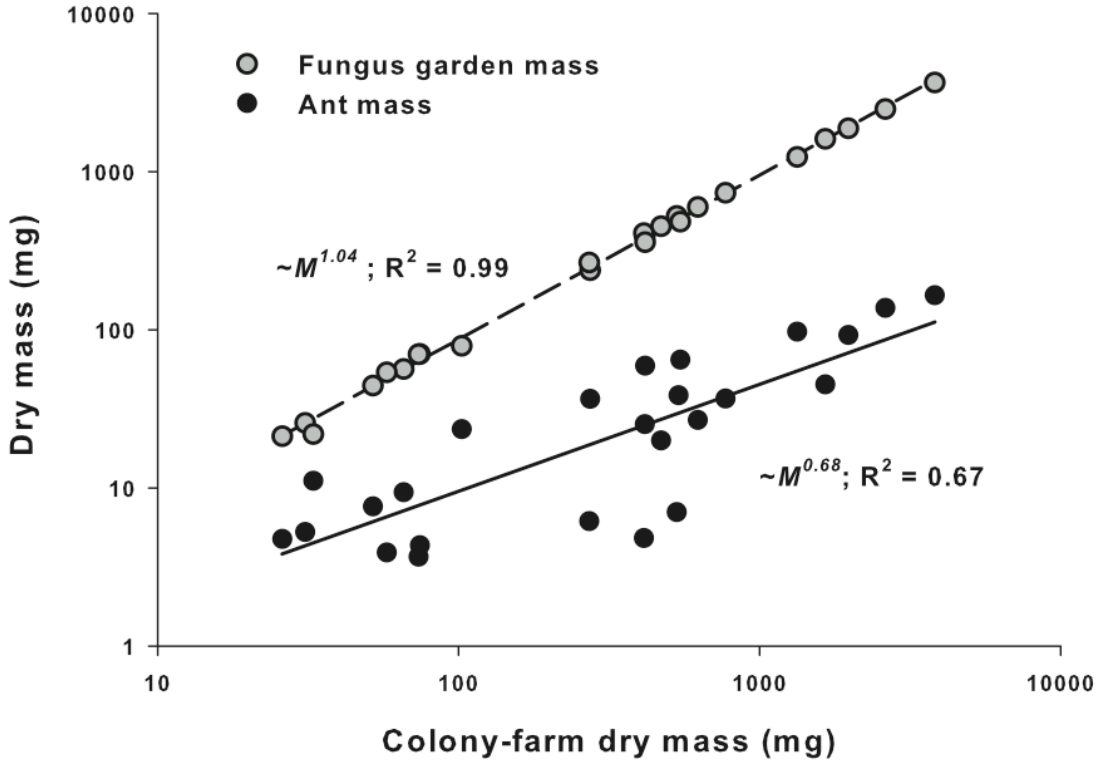


Figure S1B Chitin biomass (used as an index of fungal biomass) scaled isometrically with garden mass (F-test for $b = 1$: $F_{1,22} = 0.41$; $p = 0.53$).

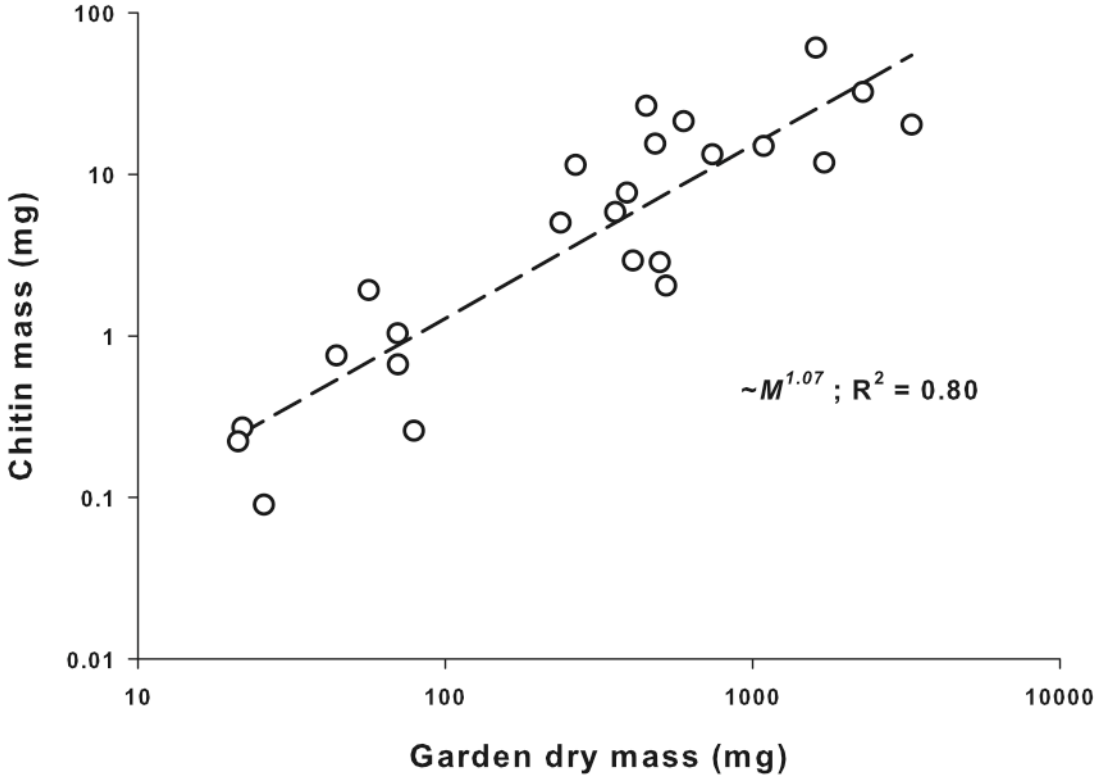


Figure S2 Average percent (chitin ± 1 SE) for gardens of colonies used in respirometry experiments, with raw % chitin values listed in Table 3. A one sample t-test was used to test H_0: higher attine percent chitin = mean lower attine % chitin (0.1; N = 6 colonies). All data were arcsine transformed prior to the analysis. Higher attines had significantly higher chitin content ($t_{17,0.05} = 2.94$; $p = 0.009$).

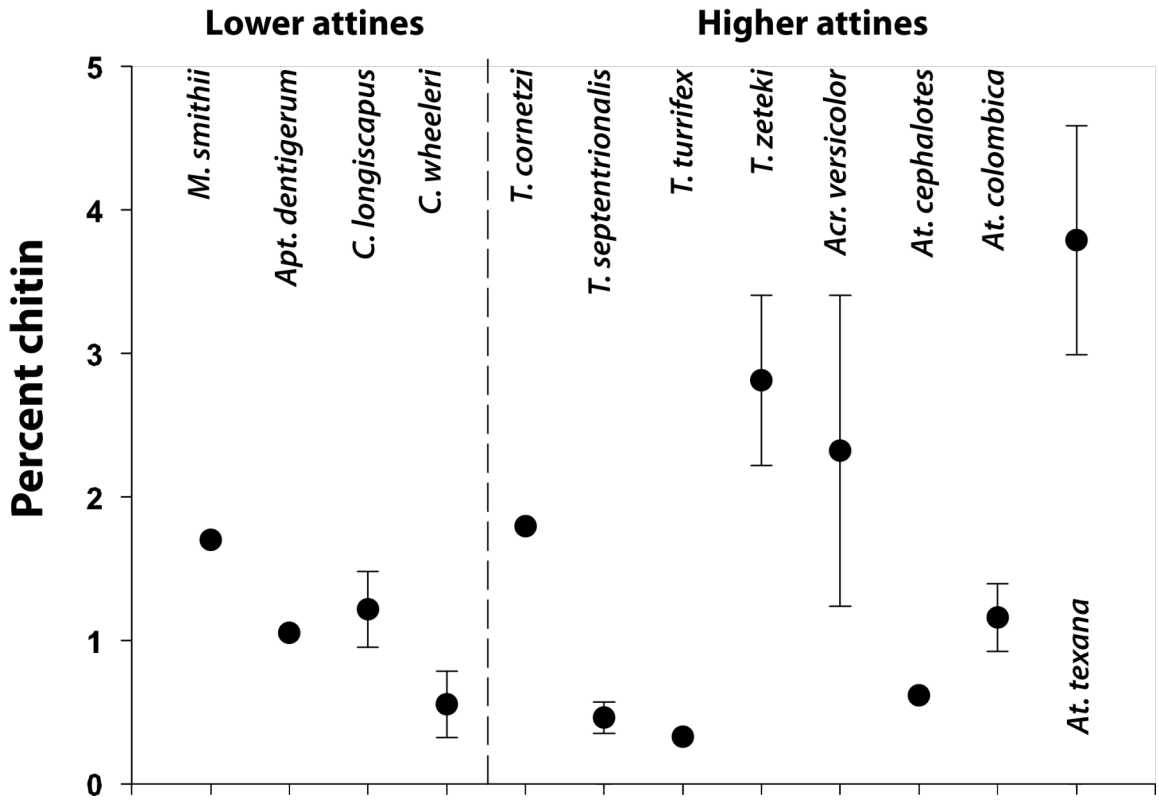


Table S1 Collection locality information for colonies used in metabolic experiments. Unless otherwise indicated, colonies from Barro Colorado Island were harvested from twigs in leaf litter.

Species	ID	Harvest Date	Locality details			Notes
				Lat.	Long.	
M. smithii	UGM 010329-30					2 monogynous colonies taken from this larger polygynous colony
Apt. dentigerum	gb/06/04/2010-14	6/4/10	Parque National Soberania, panama, forest in Gamboa, Panama along Pipeline road (hereafter: 'Pipeline')			
C. wheeleri	UGM 061001-02		Bull Creek Park, Austin, TX			2 monogynous colonies taken from this larger polygynous colony
C. longiscapus	gb/06/04/290-11	6/29/10	Pipeline			
C. longiscapus	RMMA1006-08-11	6/8/10	Pipeline			
T. septentrionalis	TSE1	5/23/08	36 Street Extension	35.21002	97.50235	Sandy flood plain Norman, Oklahoma
T. septentrionalis	TSE20	6/8/09	36 Street Extension	35.21002	97.50235	
T. turrifex	BFL1	3/17/10	Brackenridge Field Lab, Austin, TX	30.28458	97.78268	Austin, Texas MEKOU 102531
T. cornetzi	colMR1	7/7/10	Barro Colorado	9.15844	79.83475	

			Island, Panama: Thomas Barbour 10			
T. zeteki	NHC21		Pipeline Road			RMMA 050812-09 & 050818-04
T. zeteki	TZ100					
T. zeteki	TZ101					
T. zeteki	S6-070510-2	7/5/10	Gamboa			
A. versicolor	VE096		Tuscon, AZ			
A. versicolor	VE099		Tuscon, AZ			
A. texana	UGM0705 19-03B		Graham, Young County, TX			Colony used for some of the individual worker metabolic data
A. texana	Atex18		Austin, Travis County, TX			4 queenless colony fragments from this larger colony with label: At18
A. colombica	Sunshine17		Gamboa			Colonies grown from captured alates by Sunshine, kept by David for about 6 months
A. colombica	Sunshine31		Gamboa			
A. colombica	Sunshine43		Gamboa			
A. cephalotes	Sunshine3		Gamboa			
Non-agricultural species from Barro Colorado Island						
Brachymrmyex sp. 1	WMR109	9/18/10	Donato 4	9.16175	79.83716	
Crematogaster cf. *sumichrasti*	WMR135	9/23/10	Thomas Barbour 9	9.15844	79.83475	
Crematogaster sp1	MR0024	7/13/10	Fausto 1	9.16350	79.83871	
Ectatomma ruidum	LabECT1		Thomas Barbour			All colonies of *Ectatomma ruidum* dug up from the general

						area near Thomas Barbour trail (N: 9.15844, W: 79.83475) during May to July 2010 and used for an additional feeding experiment in the lab prior to metabolic measurements.
Ectatomma ruidum	LabECT1		Thomas Barbour			
Ectatomma ruidum	LabECT2		Thomas Barbour			
Ectatomma ruidum	LabECT3		Thomas Barbour			
Ectatomma ruidum	LabECT4		Thomas Barbour			
Ectatomma ruidum	LabECT5		Thomas Barbour			
Ectatomma ruidum	LabECT6		Thomas Barbour			
Ectatomma ruidum	LabECT7		Thomas Barbour			
Ectatomma ruidum	LabECT8		Thomas Barbour			
Ectatomma ruidum	LabECT9		Thomas Barbour			
Ectatomma ruidum	LabECT10		Thomas Barbour			
Ectatomma ruidum	LabECT11		Thomas Barbour			
Ectatomma ruidum	LabECT12		Thomas Barbour			
Ectatomma ruidum	LabECT14		Thomas Barbour			
Ectatomma ruidum	LabECT15		Thomas Barbour			
Ectatomma ruidum	LabECT16		Thomas Barbour			
Ectatomma ruidum	LabECT17		Thomas Barbour			
Ectatomma ruidum	LabECT18		Thomas Barbour			
Ectatomma ruidum	LabECT19		Thomas Barbour			
Ectatomma ruidum	LabECT20		Thomas Barbour			
Ectatomma ruidum	LabECT21		Thomas Barbour			
Ectatomma ruidum	LabECT22		Thomas Barbour			
Ectatomma ruidum	LabECT23		Thomas Barbour			
Ectatomma ruidum	LabECT24		Thomas Barbour			
Ectatomma ruidum	LabECT25		Thomas Barbour			

Ectatomma ruidum	LabECT26		Thomas Barbour			
Gnamptogenys horni	WMR67	9/7/10	Zetek 10	9.15364	79.85680	
Gnamptogenys horni	WMR75	9/7/10	Zetek 10	9.15364	79.85680	
Hypoponera opacior	MR0028	7/13/10	Snyder Molino 1	9.16350	79.83871	
Odontomachus bauri	WMR113a	9/22/10	Standley 1	9.15408	79.85802	foundress
Odontomachus sp.1	WMR152	9/27/10	Standley 9.5	9.16115	79.86132	foundress
Pachycondyla harpax	WMR117	9/22/10	Standley 1	9.15408	79.85802	foundress
Pachycondyla cf. *villosa*	ColMR40	7/28/10	Lab clearing	9.16595	79.83621	Colony overtook a Melliponine nestbox of Meg Eckles
Paratrechina guatemalensis	WMR123	7/21/10	Standley 1	9.15408	79.85802	
Pheidole LASH8	MR0026	7/13/10	Fausto 1	9.16350	79.83871	
Pheidole cf.LASH9	WMR77	9/7/10	Zetek 10	9.15364	79.85680	
Pheidole radozkowskii	WMR113b	7/22/10	Standley 1	9.15408	79.85802	
Pheidole radozkowskii	MR0010	7/11/10	Barbour Lathrop 4			
Pheidole rugiceps	MR0020	7/11/10	Barbour Lathrop 4			
Pheidole rugiceps	WMR87	9/14/10	Balboa 4	9.16059	79.84335	
Pheidole cf. *rugiceps*	WMR147	9/27/10	Standley 15	9.16115	79.86132	
Pheidole ruida	MR0011	7/11/10	Barbour Lathrop 4			
Pheidole ruida	MR0015	7/11/10	Barbour Lathrop 4			
Pheidole ruida	MR0018	7/11/10	Barbour Lathrop 4			
Pheidole ruida	MR0021	7/11/10	Barbour Lathrop 4			

Pheidole ruida	WMR141	9/27/10	Standley 9.5	9.16115	79.86132	
Pheidole cf. ruida	WMR148	9/27/10	Standley 15	9.16115	79.86132	
Pheidole multispina	WMR72	9/7/10				
Probolomyrmex bolivensis	WMR114b	9/21/10	Standley 1	9.15408	79.85802	
Pyramica brevicornis	WMR58	9/7/10	Zetek 10	9.15364	79.85680	
Pyramica brevicornis	WMR91	9/14/10	Balboa 4	9.16059	79.84335	
Pyramica cf. *brevicornis*	WMR134	9/23/10	Thomas Barbour 9	9.15844	79.83475	
Pyramica gundlachi	WMR106	9/18/10	Donato 4	9.16175	79.83716	
Pyramica subedentata	WMR98	7/14/10	Balboa 4	9.16059	79.84335	
Solenopsis sp.1	MR0025	7/13/10	Snyder Molino 1	9.16350	79.83871	
Solenopsis diploSP1	WMR56	9/7/10	Zetek 10	9.15364	79.85680	
Solenopsis diploSP2	WMR103	7/18/10	Donato 4	9.16175	79.83716	
Solenopsis diploSP4	WMR149	9/27/10	Standley 9.5	9.16115	79.86132	
Solenopsis diploSP4	WMR144	9/27/10	Standley 9.5	9.16115	79.86132	
Wasmannia auropunctata	WMR97A	9/14/10	Balboa 4	9.16059	79.84335	
Wasmannia auropunctata	WMR97B	9/14/10	Balboa 4	9.16059	79.84335	

Table S2 Mass and $\dot{V}CO_2$ composition of colony-farms. All mass measurements are dry (mg) All colonies are monogynous unless otherwise indicated.

Species	ID	Colony demography (mg)			Colony $\dot{V}CO_2$ ($\mu l\ CO_2\ hr^{-1}$)		
		queen	worker	fungus	queen	worker	fungus
Lower Attines							
M. smithii	UGM30A	0.65	3.25	53.88	0.53	5.48	53.88
M. smithii	UGM30C	0.65	6.96	44.34	n/a	7.54[∫]	12.91
A. dentigerum[‡]	Gb1	n/a	4.75	21.20	n/a	n/a	n/a
C. wheeleri	UGM02A	0.42	6.60	523.36	0.57	10.75	74.09
C. wheeleri	UGM02C	n/a	4.39	407.85	n/a	6.43[∫]	57.75
C. longiscapus[‡]	Gb2	0.43	3.90	70.20	n/a	n/a	n/a
C. longiscapus[‡]	RMMA1	0.84*	2.82	70.00	n/a	n/a	n/a
Higher Attines							
T. septentrionalis	TSE1	1.28	3.98	25.70	1.23	7.03	16.56
T. septentrionalis	TSE20	1.11	37.45	499.15	2.68	80.09	566.96
T. cornetzi	ColMR1	n/a	30.78[§]	740.14	n/a	89.58	233.62
T. turrifex	BFL1	1.29	20.92	72.98	0.74	19.28	51.82
T. zeteki	NHC21	2.26	3.89	265.51	1.91	7.02	113.78
T. zeteki	S61	n/a	59.23	357.40	n/a	99.05	412.85
T. zeteki	TZ100	2.43	62.05	482.00	n/a	141.83	324.45
T. zeteki	TZ101	2.16	34.37	237.10	n/a	116.25	161.65
A. versicolor	VE096	5.51	3.87	56.36	2.42	4.59	52.02
A. versicolor	VE099	5.10	5.98	21.86	2.38	18.02	32.50
A. texana	Atex2	n/a	44.94	1609.80	n/a	116.78	726.08
A. texana	Atex3	n/a	25.22	390.39	n/a	64.90	152.95
A. texana	Atex4	n/a	26.85	596.57	n/a	65.51	256.30
A. texana	Atex5	n/a	19.94	451.29	n/a	63.26	170.39
Atta colombica	Sun17	97.00	137.45	2287.30	25.09	707.97	965.71
A. colombica	Sun31	83.70	92.63	1712.20	41.88	989.37	1159.25
A. colombica	Sun43	74.50	96.98	1087.00	40.91	511.11	452.93
A. cephalotes	Sun3	179.30	165.05	3295.80	34	413.48	798.00

* 2 queens, whose individual masses were 0.44 and 0.40 mg

[§] worker mass includes 16 males (5.84 mg, 19% of total mass)

[‡] Percent fungus $\dot{V}CO_2$ unavailable because only one whole colony-farm metabolic recording was made.

[∫] Separate measures of queen and worker $\dot{V}CO_2$ were not recorded, and these values were combined in the worker column.

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Education

The University of Oklahoma, Norman, OK
2004 - present PhD, Ecology and Evolutionary Biology
Dissertation: *The ecological importance of ant colony size*
Advisor: Dr. Mike Kaspari

McGill University, Montreal, Cananda
2003 B.Sc., Biology
Thesis: *The effects of human activity on the ants of a biosphere reserve*
Advisor: Dr. Martin Lechowicz

Publications

Shik, J.Z., Yanoviak, S.P., Kaspari, M. (*In Prep*) A nematode parasite adds mass but not energy demands to its paratenic host, the tropical ant *Cephalotes atratus*.

Shik, J.Z., Seal, J.N., Mueller, U.G., Kaspari, M. (*In Review*) Agricultural innovation and the metabolic rates of fungus growing ant socieites.

Shik, J.Z. (2010) The metabolic costs of building ant colonies from variably sized subunits. *Behavioral Ecology and Sociobiology*, 64:1981-1990.

Bisel, R., **Shik, J.Z.** (*In Press*, Journal of Communication) Book Review: The Superorganism.

Kaspari, M., Stevenson, B., **Shik, J.Z.**, Kerekes, J. (2010) Scaling biodiversity: how bacteria, fungi, and ant communities respond to the same tropical landscape. *Ecology*, 91:2221-2226.

Shik, J.Z., Kaspari, M. (2010) More food, less habitat: how necromass and leaf litter decomposition combine to regulate a litter ant community. *Ecological Entomology*, 35:158-165.

Shik, J.Z., Kaspari, M. (2009) Male lifespan in ants linked to mating systems. *Insectes Sociaux*, 52:131-134.

Shik, J.Z. (2008) Ant colony size and the scaling of reproductive effort. *Functional Ecology*, 22: 674-681.

Shik, J.Z., Francoeur, A. & Buddle, C.M. (2005) The effect of human activity on ant species (Hymenoptera: Formicidae) richness at the Mont St. Hilaire Biosphere Reserve, Quebec. *The Canadian Field Naturalist*, 118:38-42.

Conference Presentations (* indicates speaker)

***Shik, J.Z.** Metabolic scaling links the traits of individual ants to their colonies. Ecological Society of America, Albuquerque, NM. 2009

***Shik, J.Z.** Ant colony size and the scaling of reproductive effort. Ecological Society of America, Milwaukee, WI. 2008

***Shik, J.Z.** The metabolic implications of ant colony size. Gordon Research Seminar: Metabolic Basis of Ecology, Biddeford, ME. 2008

Published Abstracts from Professional Meetings

Shik, J.Z. Using metabolic scaling to examine how ant colonies work: The case of *Pheidole* majors. The International Society for the Study of Social Insects, Copenhagen, Denmark. 2010

Teaching Experience

Fall 2005
Spring 2005
Fall 2006

Principles of Ecology
The University of Oklahoma

Spring 2006
Fall 2007

Introductory Zoology Laboratory
The University of Oklahoma

Spring 2007
Fall 2008
Spring 2010

Concepts in Biology (non-majors course)
The University of Oklahoma

Guest lecturer in:

Fall 2005
Spring 2006
Fall 2007

General Entomology
The University of Oklahoma

Fall 2008
Fall 2009

Concepts in Biology
The University of Oklahoma

Fall 2009

Introductory Zoology Lecture
The University of Oklahoma

Undergraduate Zoological Society

Spring 2010 *The University of Oklahoma*

Undergraduate Careers Day Panel
Spring 2010 *The University of Oklahoma*

Gamboa Field Course: Community Ecology Field Methods
Fall 2010 *The Smithsonian Tropical Research Institute*

Undergraduate advising:

NSF REU Undergraduate Deana Flatt
Summer 2010 *The University of Oklahoma*, field work on Barro Colorado Island, Panama

Fellowships and Awards

The University of Oklahoma Biostation
2009 *Summer Research Fellowship*

The University of Oklahoma Graduate Program
2008 *Grant to redesign Introductory Zoology Lab*
Assistant to Dr. Phil Gibson

The University of Oklahoma Department of Zoology
2004 - 2008 *Adams Summer Research Fellowship*

Gordon Research Seminar: Metabolic Basis of Ecology
2008 *Invited seminar at the Graduate Research Conference*

The Graduate College of the University of Oklahoma
2004 - 2009 *Alumni Fellowship*

The University of Oklahoma Department of Zoology
2004 - 2009 *Graduate Research Assistance Supplemental Stipend*

Smithsonian Tropical Research Institute
2005 *Short term fellowship*

2005 **Honorable mention: NSF Graduate Research Fellowship**

Invited Lectures

University of Oklahoma
2010 *Departmental of Zoology Seminar*
Smithsonian Tropical Research Institute
Bambi seminar
Behavior Discussion Group

2009 **Kansas State University**
Seminars in Ecology and Evolutionary Biology Series

2007 **Smithsonian Tropical Research Institute**
Bambi seminar

Specialized Training

Sable Systems International, *Las Vegas, NV*
2008 *The Respirometry Course*

The Ohio State University Acarology Laboratory, *Colombus, OH*
2006 *The Soil Acarology Course*

California Academy of Sciences & Harvard University's MCZ, *Portal, AZ*
2005 *The Ant Course*

Field and Laboratory Experience

Barro Colorado Island, Panama
2010 The stoichiometry of brown food webs

Oklahoma, sites across the state
2008 - 2009 *Metabolic scaling and ant colony size, Dissertation fieldwork*

Barro Colorado Island, Panama
2004 - 2007 *Summers of fieldwork: Resource limitation in a tropical ant community*

2004 **University of Oklahoma**
Graduate Research Assistant, Army ant impact study, P.I. was Dr. M. Kaspari

2003 **Gaspe, Quebec, Canada**
Dead wood arthropod communities, Asst. to graduate student H. Varady-Szabo

Mt. St. Hilaire, Quebec, Canada
2002 – 2003 *Summer 2003: Ant survey with Dr. A. Francoeur*
Summer 2002: Plant community ecology, Asst. to graduate student B. Gilbert
Summers 2002 – 03: Senior thesis on ant ecology

San Gerardo de Dota, Costa Rica

2000 *Dipteran phylogenetics, Asst. to Dr. M. Condon*

Cornell College

1999 - 2000 *Undergraduate Research Assistant, in the lab of Dr. M. Condon*

Referee for peer-reviewed journals

Functional Ecology, *Acta Ethologica*, *Rangeland Ecology and Management*, *Oecologia*, *Insectes Sociaux, Journal of Insect Behavior, Journal of Insect Science*

Professional Service

The University of Oklahoma

2008 *Departmental representative in graduate student senate*

2007 *Graduate student faculty representative*

2006 *Graduate student representative on admissions committee*