

Allometric Scaling of Metabolism, Growth, and Activity in Whole Colonies of the Seed-Harvester Ant *Pogonomyrmex californicus*

James S. Waters,* C. Tate Holbrook, Jennifer H. Fewell, and Jon F. Harrison

School of Life Sciences and Center for Social Dynamics and Complexity, Arizona State University, Tempe, Arizona 85287

Submitted January 16, 2010; Accepted May 29, 2010; Electronically published August 24, 2010

Online enhancement: appendix. Dryad data: <http://hdl.handle.net/10255/dryad.1594>.

ABSTRACT: The negative allometric scaling of metabolic rate with body size is among the most striking patterns in biology. We investigated whether this pattern extends to physically independent eusocial systems by measuring the metabolic rates of whole functioning colonies of the seed-harvester ant *Pogonomyrmex californicus*. These intraspecific scaling data were compared to the predictions of an additive model developed to estimate collective metabolic rates. Contrary to the prediction of the additive model, colony metabolic rate allometry resembled the pattern commonly observed interspecifically for individual organisms, scaling with colony mass^{0.75}. Among the same-aged colonies, net growth rate varied by up to sevenfold, with larger colonies exhibiting higher net growth efficiency than smaller colonies. Isolated worker groups exhibited isometric metabolic rate scaling, suggesting that the social environment of the colony is critical to regulating individual patterns of work output. Within the social environment, individual worker locomotor velocities exhibited power-law distributions that scaled with colony size so that larger colonies exhibited a greater disparity between active and inactive ants than did smaller colonies. These results demonstrate that behavioral organization within colonies may have a major influence on colony-level metabolism and in generating intraspecific variation in growth trajectories.

Keywords: allometry, ants, energetics, metabolic rate, scaling, sociality

Introduction

Colonies of social insects exhibit astonishing patterns of self-organization and emergent complexity (Bonabeau et al. 1997; Camazine et al. 2003; Hölldobler and Wilson 2009). These patterns are generated by the collective action of individual behaviors in the absence of centralized control. Frequently, the emergence of a colony-level phenotype is dependent on colony size. Examples range from the ergonomic optimization of worker castes and task par-

titioning (Wilson 1980; Porter and Tschinkel 1985; Anderson et al. 1999) to the construction of physical nests that passively regulate colony environments (Noirot and Darlington 2000; Korb 2003). Size-dependent homeothermy at the colony level has been observed in honeybee colonies in which individual clustering and thermogenesis regulate the core temperature of overwintering swarms and entire nests (Heinrich 1980; Southwick 1985, 1987). These patterns suggest that colonies may exhibit general patterns of integration similar to those that characterize the scaling of individual organisms.

Among individual organisms, size correlates strongly with rates of metabolism, growth, and locomotion, making it one of the single best predictors of the pace of life (Bonner 2006). The regularity with which metabolic rates scale with mass is a striking relationship that has fueled extensive research and substantial controversy (Glazier 2005). While the null isometric hypothesis is that metabolic rate should scale with mass¹, generally, log-log plots of metabolic rate on mass have slopes significantly lower than predicted (negative allometry). Metabolic rate has been shown to scale with mass^{0.86} for a diverse collection of terrestrial arthropods (Lighton et al. 2001) and with mass^{0.75} among 391 insect species (Chown et al. 2007).

While the majority of studies of the scaling of metabolism have focused on interspecific analyses, intraspecific scaling is important because of the relevance of such patterns to species ecology, life history, and evolution. In general, the intraspecific scaling of metabolism with mass seems more variable than observed in interspecific comparisons (Glazier 2005); however, this may be partially a consequence of the relatively limited body mass ranges available for intraspecific studies. Intraspecifically, metabolic rate frequently exhibits negative allometric scaling within insect species including *Oncopeltus fasciatus* (Niswander 1951), *Tribolium castaneum* (Medrano and Gall 1976), *Manduca sexta* (Alleyne et al. 1997; but see also

* Corresponding author; e-mail: james.waters@asu.edu.

Am. Nat. 2010. Vol. 176, pp. 501–510. © 2010 by The University of Chicago. 0003-0147/2010/17604-51\$15.00. All rights reserved.

DOI: 10.1086/656266

Greenlee and Harrison 2005), *Schistocerca americana* (Greenlee and Harrison 2004), *Atta columbica* (Lighton et al. 1987), and *Pogonomyrmex rugosus* (Lighton et al. 1987). An investigation into intraspecific allometries among individual ants demonstrated that in seven of eight species studied, metabolic rate scaled homogenously with an average scaling exponent of 0.65 (Chown et al. 2007). Furthermore, it has been demonstrated recently that whole colonies of social insects may also exhibit negative allometries on an interspecific basis (Hou et al. 2010), suggesting that a similar pattern may be present on an intraspecific basis between colonies of a single species that vary in size.

Social insect colonies are ideal model systems for investigating how the scaling of metabolic rate and associated parameters extends from individuals to societies. Colonies of social insects range widely in size and diversity of social organization. The nature of colonies as collections of physically independent individuals makes it possible to perform experimental manipulations on aspects such as size and composition that would not be feasible for individual organisms. Compared to other social insect species, non-reproductive ant colonies are particularly convenient model organisms for evaluating scaling relationships, due to the possibility of maintaining functioning colonies in the lab and the absence of flying individuals that would complicate models of whole-colony metabolic rate.

Several prior studies have examined the dependence of metabolic rate on group size in ants. In some of the first attempts at quantifying the metabolic effect of group size, mass-specific metabolic rates of workers did not scale with the number of ants being measured (Brian 1973; Lighton and Bartholomew 1988; Lighton 1989). In other studies, it was shown that mass-specific metabolic rate depended on worker group size in a nonlinear fashion (Galle 1978; Fonck and Jaffe 1996). These investigations focused primarily on pseudocolonies in which groups of individuals were removed from their more natural social milieu and placed into the artificial environment of a respirometry chamber. Such groups of ants likely were unable to engage in normal colony functions, such as foraging, allogrooming, or rearing brood, and consequently were without the potential for maintaining the kind of organizational networks that may be fundamental to regulating the metabolism of integrated social groups.

In this study, we reared colonies of the seed-harvester ant *Pogonomyrmex californicus* in laboratory nests from founding to 10 months old. Colonies ranged in size from 95 to 659 ants, including queens, larvae, pupae, and adult workers, and none had begun to produce sexual castes. The nest design allowed for simple and noninvasive flow-through respirometric measures of the metabolic rate of whole colonies while they carried out normal colony functions, including foraging and brood rearing. To control

for the possibility that larger ant colonies are likely to also have larger workers (Tschenkel 1998), we determined the caste, developmental stage, and mass compositions of each colony. To test for an effect of the colony social environment, metabolic rate measurements were also carried out with worker groups that had been removed from their colonies. To estimate the relative activity differences between colonies, we recorded and analyzed the patterns of locomotion among individual ants within colony nests.

Methods

Collection and Rearing

Queens of *Pogonomyrmex californicus* were collected July 4–6, 2007, following mating flights in Pine Valley, San Diego County, California ($32^{\circ}49'20''\text{N}$, $116^{\circ}31'43''\text{W}$; 1,136-m elevation). Since this population is pleometrotic (cooperative founding), laboratory colonies were initiated with three queens each. Colonies were maintained in an incubator at $28^{\circ}\text{--}32^{\circ}\text{C}$ in plastic artificial nests with cotton-plugged water tubes but no soil or other ground substrate. Each nest provided a total surface area of 242 cm^2 and was partitioned into a brood chamber and foraging arena (fig. A1 in the online edition of the *American Naturalist*). Kentucky bluegrass seeds and dead crickets were provided in excess of consumption to preclude resource limitation. Water tubes were replaced, and debris was removed as necessary. Metabolic rate measurements were conducted after 10 months of rearing. Colony mass data were collected after metabolic rate measurements, at which time colonies ranged in size from 95 to 659 ants and in mass from 311 to 2,223 mg.

Modeling Whole-Colony Metabolic Rate

To quantitatively evaluate hypotheses on the scaling of metabolic rate among groups or colonies, it was necessary to develop a model to predict the metabolic rate of a group on the basis of its composition. To a first approximation, the metabolic rate (MR) for each individual (with mass, m) within a colony can be predicted by $\text{MR} = a_0 m^b$, where the allometric coefficient, a_0 , and exponent, b , can be estimated by the analysis of standard respirometric measures of individuals. If colonies were simply collections of homogenous individuals, then summing their individually estimated metabolic rates would be predicted to generate an isometric scaling of colony metabolic rates. However, eusocial insect colonies are heterogeneous collections of individuals that vary in a number of factors that can influence their per capita metabolic rates, including mass, caste, and activity level. We propose the following model for estimating whole-colony metabolic rate while taking

into account individual-level variation (e.g., in mass, caste, or activity). In its most general form, the model is defined for a colony with numbers of individuals, N_i , in each of k distinct allometric subgroups by the following equation in which c is an activity coefficient, a_i is the allometric coefficient, m is individual mass, and b_i is the allometric exponent:

$$\text{MR}_{\text{net}} = \sum_{i=1}^k \sum_{n_i=1}^{N_i} c_{n_i} a_i m_{n_i}^{b_i} \quad (1)$$

Applying this model to a social insect colony composed of queen, worker, larva, and pupa allometric subgroups (identified by subscripts q , w , l , and p , respectively) and assuming homogenous activity across all individuals and colonies, colony metabolic rate is predicted by

$$\begin{aligned} \text{MR}_{\text{colony}} = & \sum_{n=1}^{N_q} a_q m_n^{b_q} + \sum_{n=1}^{N_w} a_w m_n^{b_w} \\ & + \sum_{n=1}^{N_l} a_l m_n^{b_l} + \sum_{n=1}^{N_p} a_p m_n^{b_p}. \end{aligned} \quad (2)$$

This model may be simplified by assuming that a single allometric relationship predicts the metabolic rate of all individuals of a given species, depending on their mass and assuming average masses, \bar{m}_p for each allometric subgroup within the colony. We refer to this as the additive model for colony metabolic rate:

$$\text{MR}_{\text{colony}} = N_q a_0 \bar{m}_q^b + N_w a_0 \bar{m}_w^b + N_l a_0 \bar{m}_l^b + N_p a_0 \bar{m}_p^b. \quad (3)$$

This model was computed a series of times using the census data for ants in our laboratory colonies and populated with scaling coefficients based on *Pogonomyrmex rugosus* (Lighton and Bartholomew 1988) and a general arthropod allometry (Lighton et al. 2001). Since data on brood metabolic rates were unavailable for this species, following the results of a study of *Solenopsis invicta* (Vogt and Appel 1999), we also computed the additive model with larvae and pupae estimated to have mass-specific metabolic rates 72% and 56%, respectively, that of individual *P. californicus* workers (Quinlan and Lighton 1999). The additive model (eq. [3]) predicts isometric colony metabolic rate scaling if average individual mass and subgroup composition are both invariant with respect to colony size. However, many factors could lead to either hypermetric or hypometric scaling of colonial metabolic rates, including changes in worker sizes, distribution of types of individuals in the colony (e.g., proportion of the colony that is brood), or variation in activity and metabolic rates among individuals within or across colonies.

Measuring Whole-Colony Metabolic Rate

Whole-colony metabolic rate was measured with flow-through respirometry. Entire colony enclosures (including brood chamber and foraging arena) were placed with minimal disturbance into a 1.0-L airtight acrylic plastic chamber. Dried, CO₂-free air from a compressed air tank flowed through the chamber (38 mL min⁻¹), regulated by Tylan mass flow valves and controller. In this way, the washout characteristics were such that 95% equilibration is estimated to take 79 min (Bartholomew et al. 1981). Using an infrared analyzer (LI-6252, LI-COR, Lincoln, NE), the carbon dioxide concentration of dried, excurrent air was measured (fig. A2 in the online edition of the *American Naturalist*). Air temperature exiting the colony chamber was measured using thermocouples embedded in line with the excurrent airstream. Analog data were digitized (UI-2, Sable Systems International [SSI], Las Vegas, NV) and recorded on a PC (ExpeData, ver. 1.2.6, SSI) at 1-Hz sampling frequency and 10–20 Hz averaging.

Colonies were measured in the respirometry chamber for 24–48 h at 28.4°C ($\pm 0.5^\circ\text{C}$ SD). After this period, colonies were removed from their nest to count the number of individuals present and determine the average per capita mass for each subgroup within the colony (queens, workers, pupae, larvae). Meanwhile, the colony nest was returned to the respirometry chamber to measure the background signal from the water tube, seeds, and debris. Metabolic rates were calculated from baseline- and debris-corrected excurrent carbon dioxide concentrations averaged over the most stable 12-hr recording, standardized to the average temperature of 28.4°C, assuming a Q₁₀ of 2.0, and converted to microwatts, assuming a respiratory quotient of 0.80 (Lighton and Bartholomew 1988).

Net colony growth rates were calculated by dividing wet-tissue biomass by colony age at the time of measurement. Net growth efficiency was calculated as power output divided by power input (calculated from whole-colony metabolic rate, measured as described above). Power output was defined as the product of net rate of dry biomass production (converted from wet biomass data by direct calibration) and tissue caloric density. Caloric equivalents were estimated based on the published value found for *Crematogaster* sp. (4.073 kcal/g; Cummins and Wuycheck 1971).

Worker-Group Metabolic Rate

To control for the effects of density and social environment, we measured the metabolic rates of groups of workers removed from their colonies. For three colonies of *P. californicus* (collected in 2007), workers in groups ranging in size from 1 to 225 ants were removed, and a total of

20 worker groups were measured. Stop-flow respirometry was used to estimate metabolic rate. Worker groups placed in petri dishes with a small water tube were placed in an airtight aluminum and acrylic plastic chamber. Two respirometry chambers were used (30- and 600-mL volumes) with different surface areas (20 and 314 cm²) to accommodate the range of group sizes. Normoxic air from a compressed-gas cylinder was used to both baseline and purge the concentrations of oxygen and carbon dioxide within the chamber. A flow rate of 100 mL min⁻¹ was used with the 30-mL chamber, and 500 mL min⁻¹ was used with the 600-mL chamber. After the purging of ambient air, the chambers were sealed for a period of time estimated to produce a decrease in oxygen concentration within the chamber of not greater than 0.5%. The amount of time chambers were sealed ranged from 0.5 to 15.3 h, and the average drop in oxygen concentration was 0.12% (± 0.08 SD). After the sealed phase, the airstream was redirected into the chamber, and the excurrent air passed through (in order) a drierite column, a carbon dioxide analyzer, an ascarite column, and an oxygen analyzer (FC-2, SSI). Oxygen consumption (mL O₂ min⁻¹) was determined by integrating the baseline-corrected oxygen concentration recording (Lighton 2008) and was converted to microwatts (20.1 J mL O₂⁻¹).

The data on worker-group metabolic rates were analyzed to determine whether there was a correlation between worker group size and mass-specific metabolic rate. Additionally, since the density of ants between measurements varied in this experiment by up to 60-fold (0.05–3.06 ants/cm²), we also tested for a correlation between density and mass-specific metabolic rate. Performing these analyses on a mass-specific basis is justified because the worker body size (2.76 ± 0.28 mg) did not scale with experimental group size (linear regression: $F_{1,18} = 0.12$, $P = .74$) or vary significantly among the three originating colonies (one-way ANOVA: $F_{2,17} = 1.7$, $P = .21$).

Allometric Analysis

Data were analyzed using a variety of regression techniques to validate that results obtained were statistically robust (Packard and Boardman 2008). The allometric scaling coefficient and exponent for whole-colony metabolic rates were estimated using ordinary least squares and reduced major axis algorithms after log transformation in GraphPad Prism, version 5.0 (GraphPad Software, San Diego, CA). Residuals were normally distributed (D'Agostino-Pearson $P = .3$). The scaling coefficient and exponent were similarly estimated on the basis of the predictions generated by additive model 3. Additionally, scaling exponents were also estimated using nonlinear reg-

gression on arithmetic-scaled data (Packard and Boardman 2008).

Activity Analysis

Patterns of locomotory activity were assayed by digitizing the movements of ants in recorded video of colony behavior. Video recordings were made for 1 h of the nest region of eight colonies. For each colony, a 60-s segment of video was subsampled at 1 frame s⁻¹ and exported as a TIFF (tagged image file format) image stack. The subsampling rate of 1 frame s⁻¹ was chosen to minimize the number of frames to be analyzed without excessive loss of spatial resolution due to ants moving more than one body length between frames. Image stacks were loaded into

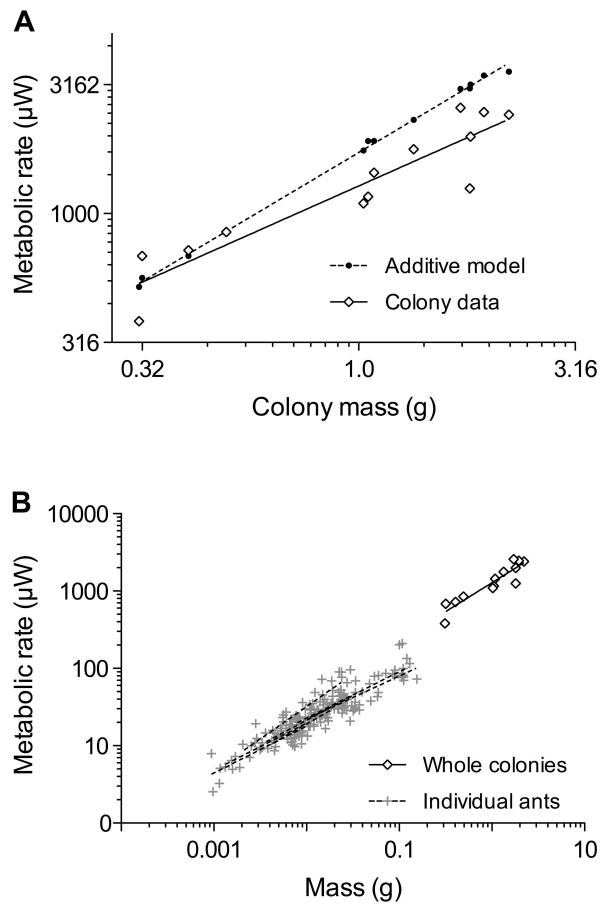


Figure 1: *A*, Whole-colony metabolic rate allometry on double-log axes, with a hypometric slope (0.75) statistically less than the isometric slope (1.0) predicted by the additive model. *B*, Intraspecific whole-colony metabolic rates scale in essentially the same way as observed for intraspecific scaling of metabolic rate for individual ants, as seen with seven intra-specific negative metabolic rate allometries (data adapted from Chown et al. 2007) with slopes ranging from 0.56 to 0.84.

Table 1: Whole-colony metabolic rate allometries

Regression	Intercept (\pm SE)	Slope (\pm SE)	r^2	N	Range (g)
Additive model:					
1	3.237 \pm .004	1.000 \pm .014	.99	13	.311–2.222
2	3.347 \pm .001	1.001 \pm .006	.99	13	.311–2.222
3	3.268 \pm .005	.980 \pm .015	.99	13	.311–2.222
Colony measurement	3.107 \pm .028	.747 \pm .093	.85	13	.311–2.222
Worker group	3.468 \pm .050	1.008 \pm .033	.98	20	.003–.641

Note: Ordinary linear regression results for metabolic rate allometries based on \log_{10} -transformed data for the additive model predictions, whole-colony metabolic rate measurements, and the worker group metabolic rate data, all standardized to 25°C. Three additive models were computed with scaling coefficients based on *Pogonomyrmex rugosus* (1), general nontick nonscorpion arthropods (2), and estimated *Pogonomyrmex californicus* mass-specific metabolic rates (3).

NIH Image, and the coordinates of individual ants in each frame were manually digitized with QuickImage (Walker 2001). These coordinate data ($N = 73,444$) were analyzed to determine an average velocity for each ant in each of the colony nests. For each colony, we determined the frequency distribution of individual average velocities. Data on the relative speeds of individuals in each colony were also used to generate predictions for colony metabolic rate allometry using the compositional additive model (eq. [1]) by incorporating an activity coefficient. Since metabolic rate increases linearly with the speed of a running ant (Lighton et al. 1987; Lipp et al. 2005), the activity coefficient was used to scale the predictions for individual metabolic rates proportional to their measured speed. Values are reported as means \pm SE throughout.

Results

Whole-Colony Metabolic Rate Allometry

Whole-colony metabolic rate scaled with colony mass with negative allometry, with a scaling exponent of 0.75 ± 0.09 (fig. 1A; table 1; table A1 in the online edition of the *American Naturalist*). The empirically determined exponent was significantly less than the isometry predicted by equation (3), using the measured masses of individual queens, workers, larvae, and pupae for each colony ($F_{1,22} = 7.20, P = .01$). All additive models generated isometric scaling (exponents not significantly different from 1), regardless of whether we used coefficients based on the general arthropod (Lighton et al. 2001) or *Pogonomyrmex rugosus* (Lighton and Bartholomew 1988) allometries or included the data estimated for *Pogonomyrmex californicus* workers and brood (table 1; $F_{1,33} = 0.86, P = .43$). The scaling exponent estimated through ordinary least squares regression of log-transformed data did not significantly differ from the estimate generated by reduced major axis linear regression ($t_{24} = 1.25, P = .2$) or the estimate generated by nonlinear regression using the arithmetic-scaled data ($t_{24} = 0.11, P = .9$). Additionally, the scaling ex-

ponent did not depend on whether the minimum, average, or maximum colony metabolic rates were used in the analysis ($F_{2,33} = 0.12, P = .9$). The negative allometric scaling of whole-colony metabolic rate is consistent with the general intraspecific scaling pattern observed for individual ants (fig. 1B). Colonies of *P. californicus* exhibited an intraspecific scaling exponent not significantly different (one-way ANOVA: $F_{7,200} = 1.2, P = .3$) from seven of the eight other species for which intraspecific individual ant metabolic rate scaling data were available (fig. A3 in the online edition of the *American Naturalist*).

Colony Composition

Negative allometric scaling in *P. californicus* could not be explained by trends in the scaling of individual ant size across colonies or by changes in colony composition (table A2 in the online edition of the *American Naturalist*). Average worker mass (mean 3.2 ± 0.1 mg) did not show a significant regression with colony size ($F_{1,11} = 0.96, P = .35$). There was also no significant scaling of average queen mass (12.0 ± 0.2 mg; $F_{1,11} = 2.77, P = .12$) or average larva mass (1.9 ± 0.3 mg; $F_{1,11} = 4.39, P = .06$). The fractions of colony mass composed of workers (0.83 ± 0.03), larvae (0.09 ± 0.01), and pupae (0.05 ± 0.02) all exhibited nonsignificant regression with colony size ($F_{1,11} = 0.07–1.29, P = .28–.78$). Fractional composition of the colonies by queens did scale with colony size ($F_{1,11} = 28.03, P = .0003$), from 11.5% in the smallest colony to 0.6% in one of the largest, but overall, queen mass was a small proportion of colony size (0.04 ± 0.01).

Since all colonies were the same age, the sevenfold range in colony mass means that net growth rate increased linearly with colony size and was sevenfold greater in the largest compared to the smallest colonies. Net growth efficiency increased more than three times with colony size ($F_{1,11} = 5.96, P = .03, r^2 = 0.35$), due to larger colonies exhibiting sevenfold higher net growth rates and 30% lower mass-specific metabolic rates.

Isometric Scaling of Worker Groups

Worker groups, removed from the social environment of the colony, exhibited isometric metabolic rate scaling (fig. 2A; table 1; table A3 in the online edition of the *American Naturalist*), consistent with some of the results of previous studies on the effects of group size on the mass-specific metabolic rate of groups of ants placed in a respirometer outside of their colony. Metabolic rate scaled with group mass raised to the exponent 1.01 ± 0.03 (linear regression on log-transformed data: $F_{1,18} = 913.7$, $P < .0001$, $r^2 = 0.98$). There was no effect of source colony on the estimated scaling exponent (one-way ANOVA: $F_{2,17} = 0.98$, $P = .39$). Furthermore, despite a 60-fold range in the density of worker groups within the respirometry chambers in this study, there was no significant linear regression correlating mass-specific metabolic rate with worker density (fig. 2B; $F_{1,18} = 0.55$, $P = .47$). Due to the use of two different-sized respirometry chambers in the worker-group experiment, experimental group size did not correlate with worker density (Pearson's $r = 0.08$, $P = .73$).

Velocity Distributions

We recorded the trajectories and determined the average locomotor velocities for more than 1,200 ants across eight colonies (fig. A4 in the online edition of the *American Naturalist*). Average ant velocity did not show a significant regression with colony mass (fig. 3A; $F_{1,6} = 2.75$, $P = .09$, $r^2 = 0.41$), although there was a trend toward slower speeds in larger colonies. Ant speed was positively correlated with colony mass-specific metabolic rate ($F_{1,5} = 6.40$, $P = .05$, $r^2 = 0.56$).

Velocity distributions within colonies were strikingly nonnormal (fig. 3B) and more accurately represented by a power law of the form $y = ax^b$, where a frequency, y , of individuals moving at a certain speed, x , scales with the exponent b . The exponent of this scaling equation was estimated following the method of log transformation and linear regression. The linear regressions (fig. 3C) on double-log-transformed axes were highly significant (for all eight colonies, $P < .0001$, and mean $r^2 = 0.75$). The slopes of the regressions, equivalent to the exponents of the power laws describing the velocity distributions, decreased with colony size, from -0.39 in the smallest colony to -1.32 in the largest colony. The degree (or exponent) of the velocity distributions correlated significantly with colony size (fig. 3D; Pearson's $r = -0.93$, $P = .0009$). Larger colonies exhibit a greater disparity between fractions of active and inactive ants, with a few key individuals moving the most and the majority moving far less.

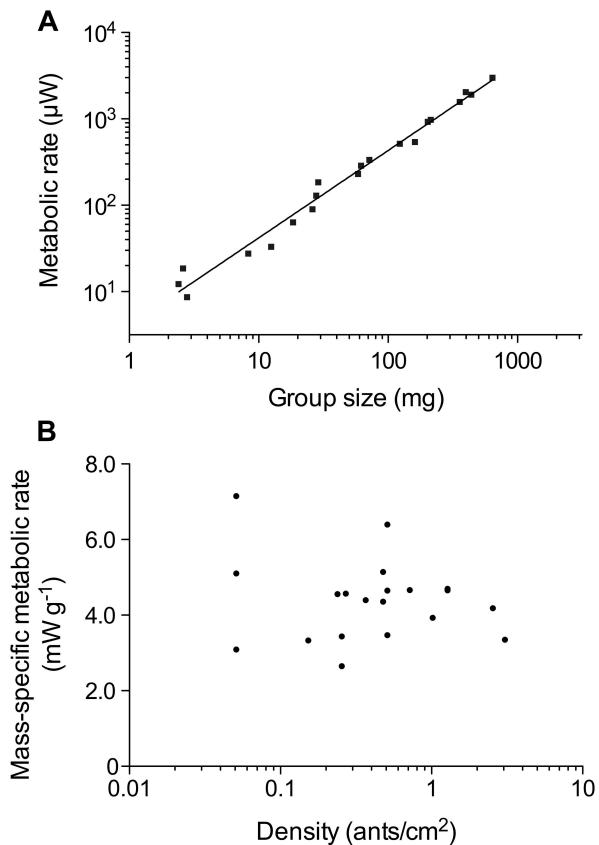


Figure 2: *A*, Isometric scaling of metabolic rate among worker groups removed from the social environment of their colonies. *B*, Worker-group study ant densities, which ranged greater than 60 times but showed no correlation with the mass-specific metabolic rates of the workers.

Discussion

Colonies of *Pogonomyrmex californicus* exhibited striking patterns of metabolic rate, growth, and activity scaling. Unlike the isometric scaling predicted for and empirically measured among groups of individual ants, functioning whole colonies exhibited hypometric metabolic rate scaling. Since net growth rate exhibited isometric scaling, net growth efficiency was much higher in larger colonies than in smaller colonies. The patterns of individual speeds within each colony were well represented by power-law distributions in which the majority of ants were relatively inactive compared to the relatively few highly active individuals. Furthermore, the extent of this disparity among individual speeds within colonies increased with colony size.

As predicted given that these colonies were actively foraging and rearing brood, colonial metabolic rates were higher than measured for individual ants measured under standard conditions (fig. 1B), which usually means that

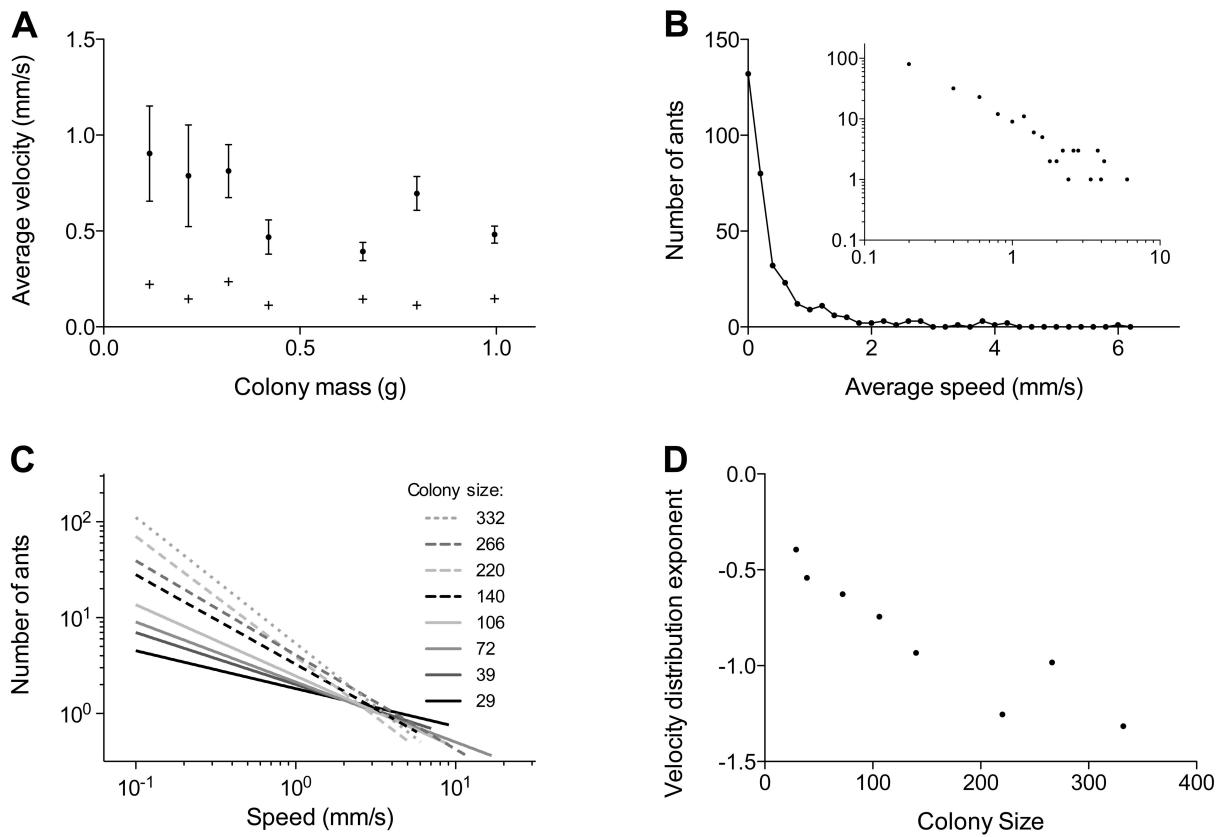


Figure 3: *A*, Average ant velocity within a colony as a function of colony size; *bars*, standard errors; *plus signs*, median velocities for each colony. *B*, Frequency distribution of ant velocities within a colony having 332 workers; *inset*, same data on double-logarithm axes. *C*, Linear regressions of log-transformed velocity distributions for a range of colony sizes. *D*, Slopes of the regressions in *C* as the scaling exponents for the velocity distributions as a function of colony size.

ants are alone, without food, in the dark, and often not moving. While the scaling slopes are homogenous, the difference in the metabolic elevation, or intercept, of our whole-colony lines and the average intercept for intraspecific scaling of ant individuals in Chown et al.'s (2007) study was 845 μW , representing about a threefold elevation of metabolic rate over that predicted for an average inactive "standard" ant. The data we collected are more analogous to the scaling of field metabolic rates for individuals, and the scaling of such field metabolic rates has the potential to differ from that occurring for resting animals for many reasons. However, the data that do exist suggest that field metabolic rates of individual vertebrates tend to scale with negative allometry (Nagy 2005).

We investigated a number of possible explanations for the observed negative allometry of metabolic rate. Larger colonies of *P. californicus* consumed up to one-third less oxygen on a mass-specific basis than did smaller colonies, and this result could not be explained by changes in colony

demography, scaling of the size of individual ants, or changes in density. Two recent studies have demonstrated that density can influence population-level metabolic rates (Cao and Dornhaus 2008; DeLong and Hanson 2009); however, we did not see such an effect. Density manipulations on colonies of *Temnothorax rugatulus* showed a positive relationship between crowding and mass-specific metabolic rate (Cao and Dornhaus 2008); in our case, the opposite trend was seen since larger colonies in the same-size boxes had lower mass-specific metabolic rates. When groups of individuals were removed from their colonies in our study, density did not correlate with mass-specific metabolic rate. Although this pattern of increasing density correlating with reduced mass-specific metabolic rates was also observed among aquatic unicells (DeLong and Hanson 2009), it is not immediately clear how a similar mechanism relating to resource constraint, as proposed in that study, could function among our colonies, which all were fed with excess food always available.

Changes in the patterns of locomotory activity may contribute to the observed negative allometry of metabolic rate. The velocity distributions of ants within our colonies scaled with colony size so that larger colonies exhibited a greater disparity of active and inactive ants than did smaller colonies. A general way to estimate the contribution of locomotory patterns to estimates of whole-colony metabolic rate data is to modify the additive model so that individual metabolic rates are predicted as a function of their velocity. The average increase in metabolic rate for ants running at peak speeds relative to “resting ants” is about sixfold (Lighton et al. 1987, 1993; Fewell 1988; Weier et al. 1995; Lipp et al. 2005). Thus, the activity coefficient in the additive model (eq. [1]) can be used to scale each ant’s metabolic rate to be linearly proportional to its speed, with the fastest ants being modeled with six-times-greater metabolic rates than the least active ants. In this way, we estimated that the fraction of whole-colony metabolic rate attributable to locomotory movement decreases with colony size (fig. 4A). We also used the additive model to predict how much of an effect velocity would have to have on individual metabolic rates, to fully account for the observed three-quarter-power metabolic rate scaling. On the basis of the measured velocity distributions, the additive model predicts three-quarter-power metabolic allometry among our colonies, if instead of a sixfold elevation, running at peak velocity elevates metabolic rate above those of inactive ants by 25-fold (fig. 4B). This magnitude of an effect seems unlikely, given that the scope of metabolic rate associated with running is about six times in ants, suggesting that variation in locomotory performance is only part of the explanation for the observed negative allometry of metabolic rate.

A number of other hypotheses can be proposed to explain the negative allometry of metabolic rates observed for the whole ant colonies in this study. One possibility is that certain colonies grew faster due to relatively higher growth efficiency and relatively lower metabolic rates. In this way, colony size, per se, is not hypothesized to be the factor that determines metabolic rate so much as the reverse; that is, variation in colony-size-independent metabolic physiology is hypothesized to result in different growth rates and effective colony sizes. A second possibility is that maintenance costs associated with colony growth and function scale with negative allometry (Jeanson et al. 2007). This hypothesis predicts that as colony size increases, the number of ants necessarily engaging in particular activities decreases so that a surplus of individuals with low metabolic rates may accumulate and thus produce negative metabolic allometry. However, our evidence for higher efficiency in larger colonies should be considered preliminary, as we have not shown that these colonies

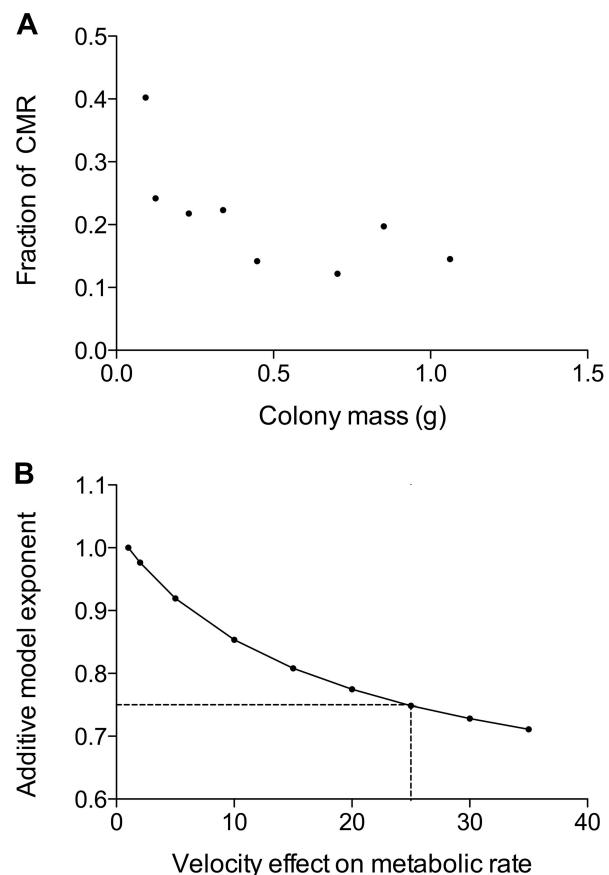


Figure 4: *A*, Fraction of whole-colony metabolic rate (CMR) estimated to be due to movement patterns decreases with increasing colony size. *B*, Estimated allometric exponent generated by the additive model modified to take into account both the effect of velocity on metabolic rate and the colony velocity distributions. If maximum running speed elevates individual metabolic rates by 25 times, then the additive model predicts metabolic rate scaling with mass^{0.75}.

have similar egg production rates or worker survival or that metabolic rates are consistent during ontogeny.

Another nonalternative type of explanation for negative metabolic allometry in *P. californicus* colonies is that eusocial insect colonies may be metabolically and behaviorally integrated in ways that are more commonly thought of as restricted to the physiology of physically connected individual organisms. Marine colonial ascidians, for example, have been shown to exhibit near three-quarter-power metabolic scaling but only when individuals are physically connected by a vascular network (Nakaya et al. 2003). In the same way that the geometry of vascular networks is proposed to be the emergent result of shear forces and material properties on the local scale (LaBarbera 1990), conceivably the behavioral interactions in a net-

worked social group may lead to emergent patterns of nutrient or information transfer that influence the scaling of metabolism. Studies of the network interactions of food and behavior within ant colonies will help assess whether this is possible. The ability to perform direct manipulations on colony size and composition while observing patterns of activity makes social insect colonies particularly useful models for evaluating general hypotheses of metabolic integration. It should be possible to directly observe the scaling properties of distribution networks between foragers and inside-nest ants and individual activity rates and to directly manipulate superorganism size, an experimental approach not easily performed with individual organisms. In this way, data can be collected to empirically test the predictions of hypotheses that aim to explain the mechanistic basis of metabolic allometry.

Acknowledgments

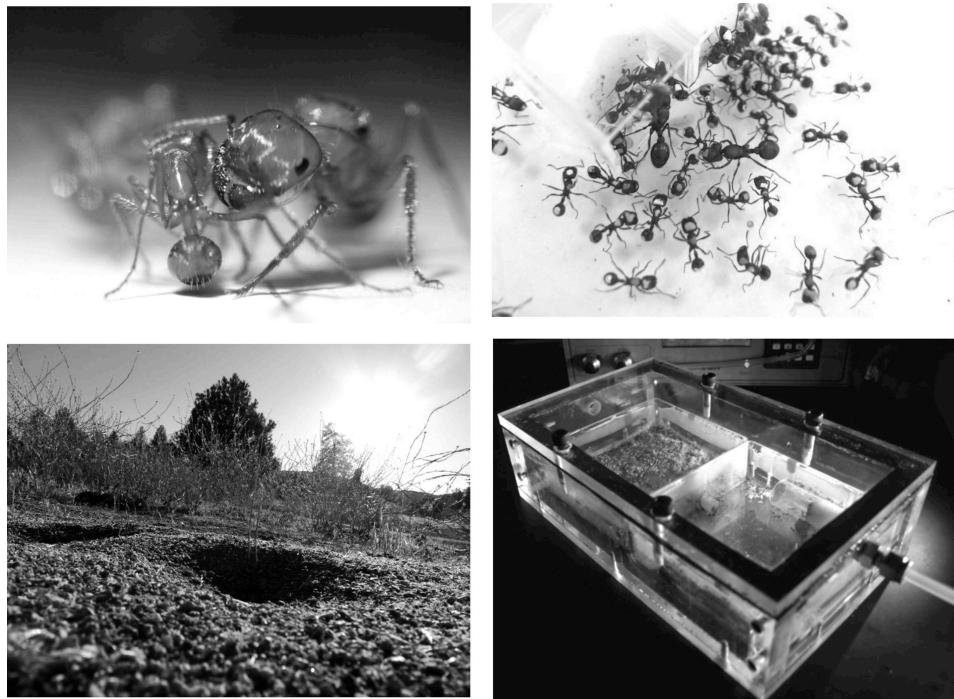
We thank P. Barden, R. Clark, D. Grayson, D. Jindrich, A. Kaiser, C. J. Klok, S. Pratt, M. Quinlan, and P. Rajyaguru for assistance. We also thank two anonymous reviewers for substantial feedback. This work was supported by National Science Foundation (NSF) Integrative Biology and Neuroscience 0419704 and NSF Earth Sciences 0746352 to J.F.H., NSF 0446415 to J.H.F., NSF graduate research fellowships to J.S.W. and C.T.H., and a Sigma Xi grant in aid of research to J.S.W.

Literature Cited

- Alleyne, M., M. A. Chappell, D. B. Gelman, and N. E. Beckage. 1997. Effects of parasitism by the braconid wasp *Cotesia congregata* on metabolic rate in host larvae of the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* 43:143–154.
- Anderson, C., F. Ratnieks, and D. Feener Jr. 1999. Task partitioning in insect societies. I. Effect of colony size on queueing delay and colony ergonomic efficiency. *American Naturalist* 154:521–535.
- Bartholomew, G. A., D. Vleck, and C. Vleck. 1981. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *Journal of Experimental Biology* 90:17–32.
- Bonabeau, E., G. Theraulaz, J. Deneubourg, S. Aron, and S. Camazine. 1997. Self-organization in social insects. *Trends in Ecology & Evolution* 12:188–193.
- Bonner, J. T. 2006. Why size matters: from bacteria to blue whales. Princeton University Press, Princeton, NJ.
- Brian, M. V. 1973. Feeding and growth in the ant *Myrmica*. *Journal of Animal Ecology* 42:37–53.
- Camazine, S., J. Deneubourg, N. Franks, J. Sneyd, G. Theraula, and E. Bonabeau. 2003. Self-organization in biological systems. Princeton University Press, Princeton, NJ.
- Cao, T., and A. Dornhaus. 2008. Ants under crowded conditions consume more energy. *Biology Letters* 4:613.
- Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. R. B. Lighton, and T. M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Functional Ecology* 21:282–290.
- Cummins, K. W., and J. C. Wuycheck. 1971. Caloric equivalents for investigations in ecological energetics. Schweizerbart, Stuttgart.
- DeLong, J. P., and D. T. Hanson. 2009. Density-dependent individual and population-level metabolic rates in a suite of single-celled eukaryotes. *Open Biology Journal* 2:32–37.
- Fewell, J. H. 1988. Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. *Behavioral Ecology and Sociobiology* 22:401–408.
- Fonck, C., and K. Jaffe. 1996. On the energetic cost of sociality. *Physiology and Behavior* 59:713–719.
- Galle, L. 1978. Respiration as one of the manifestations of the group effect in ants. *Acta Biologica Szeged* 24:111–114.
- Glazier, D. S. 2005. Beyond the “ $3/4$ -power law”: variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews of the Cambridge Philosophical Society* 80:611–662.
- Greenlee, K. J., and J. F. Harrison. 2004. Development of respiratory function in the American locust *Schistocerca americana*. I. Across-instar effects. *Journal of Experimental Biology* 207:497–508.
- . 2005. Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta*. *Journal of Experimental Biology* 208:1385–1392.
- Heinrich, B. 1980. Mechanisms of body temperature regulation in honeybees, *Apis mellifera*. I. Regulation of head temperature. *Journal of Experimental Biology* 85:61–72.
- Hölldobler, B., and E. O. Wilson. 2009. The superorganism: the beauty, elegance, and strangeness of insect societies. Norton, New York.
- Hou, C., M. Kaspari, H. Vander Zanden, and J. Gillooly. 2010. Enthalpic basis of colonial living in social insects. *Proceedings of the National Academy of Sciences of the USA* 107:3634–3638.
- Jeanson, R., J. Fewell, R. Gorelick, and S. Bertram. 2007. Emergence of increased division of labor as a function of group size. *Behavioral Ecology and Sociobiology* 62:289–298.
- Korb, J. 2003. Thermoregulation and ventilation of termite mounds. *Naturwissenschaften* 90:212–219.
- LaBarbera, M. 1990. Principles of design of fluid transport systems in zoology. *Science* 249:992–1000.
- Lighton, J., G. Bartholomew, and D. Feener. 1987. Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guer. *Physiological Zoology* 60:524–537.
- Lighton, J., J. Weier, and D. Feener Jr. 1993. The energetics of locomotion and load carriage in the desert harvester ant *Pogonomyrmex rugosus*. *Journal of Experimental Biology* 181:49.
- Lighton, J. R., and G. A. Bartholomew. 1988. Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: effects of temperature, body mass, group size, and humidity. *Proceedings of the National Academy of Sciences of the USA* 85:4765–4769.
- Lighton, J. R. B. 1989. Individual and whole-colony respiration in an African formicine ant. *Functional Ecology* 3:523–530.
- . 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, Oxford.
- Lighton, J. R. B., P. H. Brownell, B. Joos, and R. J. Turner. 2001. Low metabolic rate in scorpions: implication for population biomass and cannibalism. *Journal of Experimental Biology* 204:607–613.
- Lipp, A., H. Wolf, and F.-O. Lehmann. 2005. Walking on inclines:

- energetics of locomotion in the ant *Camponotus*. *Journal of Experimental Biology* 208:707–719.
- Medrano, J. F., and G. A. E. Gall. 1976. Food consumption, feed efficiency, metabolic rate and utilization of glucose in lines of *Tribolium castaneum* selected for 21-day pupa weight. *Genetics* 83: 393–407.
- Nagy, K. A. 2005. Field metabolic rate and body size. *Journal of Experimental Biology* 208:1621–1625.
- Nakaya, F., Y. Saito, and T. Motokawa. 2003. Switching of metabolic-rate scaling between allometry and isometry in colonial ascidians. *Proceedings of the Royal Society B: Biological Sciences* 270:1105–1113.
- Niswander, R. E. 1951. Life history and respiration of the milkweed bug *Oncopeltus fasciatus* (Dallas). *Ohio Journal of Science* 51:27–33.
- Noirot, C., and J. Darlington. 2000. Termite nests: architecture, regulation and defence. Pages 121–140 in T. Abe, D. E. Bignell, and M. Higashi, eds. *Termites: evolution, sociality, symbioses, ecology*. Kluwer, Dordrecht.
- Packard, G. C., and T. J. Boardman. 2008. Model selection and logarithmic transformation in allometric analysis. *Physiological and Biochemical Zoology* 81:496–507.
- Porter, S., and W. Tschinkel. 1985. Fire ant polymorphism: the ergonomics of brood production. *Behavioral Ecology and Sociobiology* 16:323–336.
- Quinlan, M. C., and J. R. B. Lighton. 1999. Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Physiological Entomology* 24: 293–302.
- Southwick, E. E. 1985. Allometric relations, metabolism and heat conductance in clusters of honeybees at cool temperatures. *Journal of Comparative Physiology* 156:143–149.
- . 1987. Cooperative metabolism in honey-bees: an alternative to antifreeze and hibernation. *Journal of Thermal Biology* 12:155–158.
- Tschinkel, W. R. 1998. Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insectes Sociaux* 45:385–410.
- Vogt, J. T., and A. G. Appel. 1999. Standard metabolic rate of the fire ant, *Solenopsis invicta* Buren: effects of temperature, mass, and caste. *Journal of Insect Physiology* 45:655–666.
- Walker, J. A. 2001. QuickImage: a modification of NIH Image with enhanced digitizing tools. Department of Biology, University of Southern Maine. <http://www.usm.maine.edu/~walker/software.html>.
- Weier, J., D. Feener, and J. Lighton. 1995. Inter-individual variation in energy cost of running and loading in the seed-harvester ant, *Pogonomyrmex maricopa*. *Journal of Insect Physiology* 41:321–327.
- Wilson, E. O. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). II. The ergonomic optimization of leaf-cutting. *Behavioral Ecology and Sociobiology* 7:157–165.

Associate Editor: Kevin J. Gaston
Editor: Donald L. DeAngelis



Top left, California seed harvester queen (*Pogonomyrmex californicus*) and her daughter; *top right*, queens, workers, and brood painted with unique color combinations; *bottom left*, nest entrance in Pine Valley, California, where queens were collected; *bottom right*, laboratory nest enclosures within respirometry chamber. Photographs by James Waters.

**Appendix from J. S. Waters et al., “Allometric Scaling of Metabolism,
Growth, and Activity in Whole Colonies of the Seed-Harvester Ant
Pogonomyrmex californicus”
(Am. Nat., vol. 176, no. 4, p. 501)**

Supplemental Data

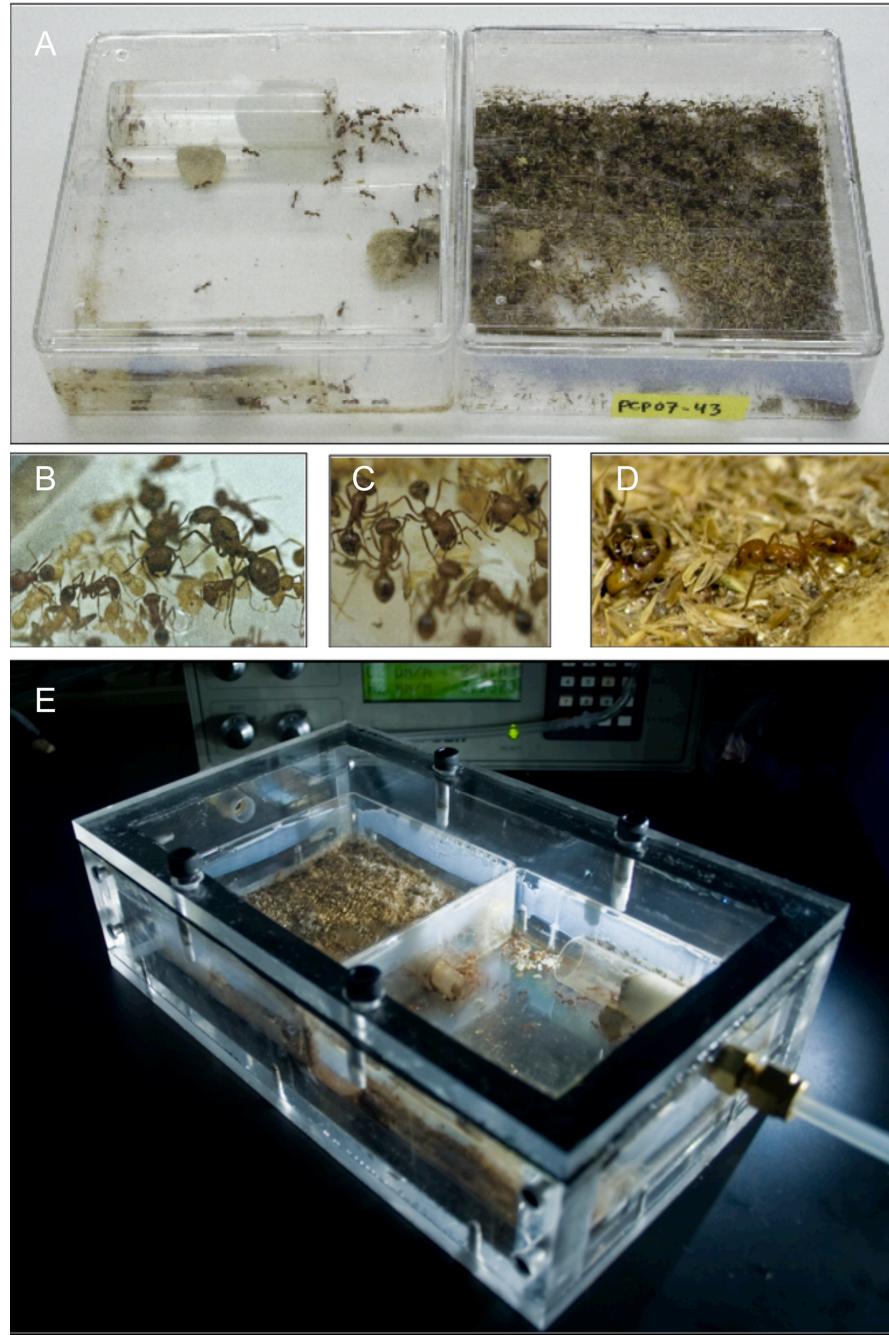


Figure A1: A, Colony of *Pogonomyrmex californicus* in its artificial nest enclosure; B, queens; C, workers tending to brood; D, workers foraging for seeds; and E, colony nest enclosure within the flow-through respirometry chamber.

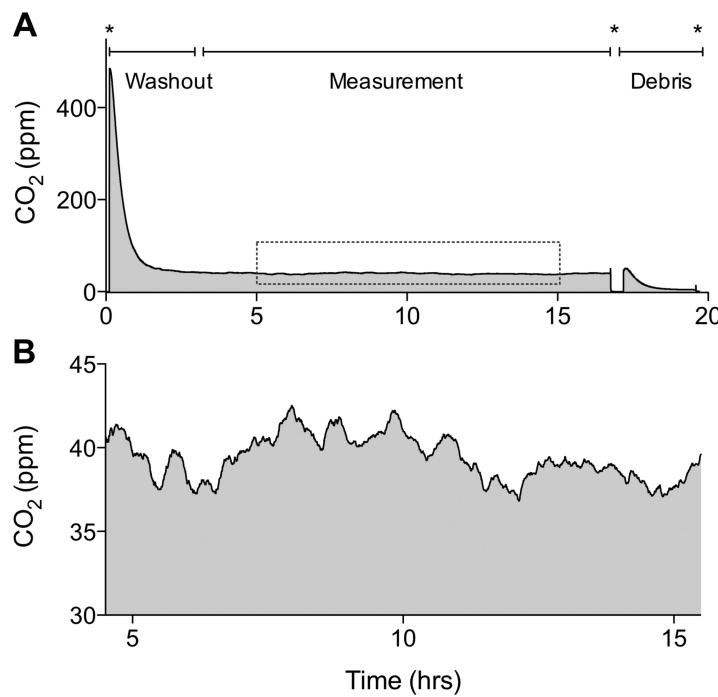


Figure A2: Representative trace of CO₂ recording during whole-colony measurements. *A*, Entire recording, which includes three baseline measurements (asterisks) and shows the excurrent airstream CO₂ concentration both for the whole-colony measurement as well as after the ants had been removed so that the background CO₂ emission from debris could be measured. *B*, Increased resolution of CO₂ emission from the colony (dotted box in *A*).

Table A1
Mass and metabolic rate data for whole colonies of *Pogonomyrmex californicus*

Colony	Mass (g)	Volume CO ₂ (mL/min)	Temperature (°C)	Metabolic rate (μW at 25°C)
1	2.2228	.0091611	28.45	2,416.22
2	1.0851	.0054600	28.45	1,440.07
3	.3110	.0013845	27.80	381.99
4	1.0518	.0044850	28.70	1,162.59
5	1.9470	.0096603	28.88	2,473.07
6	1.3390	.0064740	27.87	1,777.55
7	1.7183	.0100230	28.85	2,571.26
8	1.8048	.0047190	28.37	1,251.55
9	1.8124	.0075660	28.50	1,988.61
10	.3163	.0027300	29.18	684.51
11	.4948	.0031590	28.20	847.74
12	1.0259	.00249591	27.56	1,094.03
13	.4040	.0027222	28.40	720.47

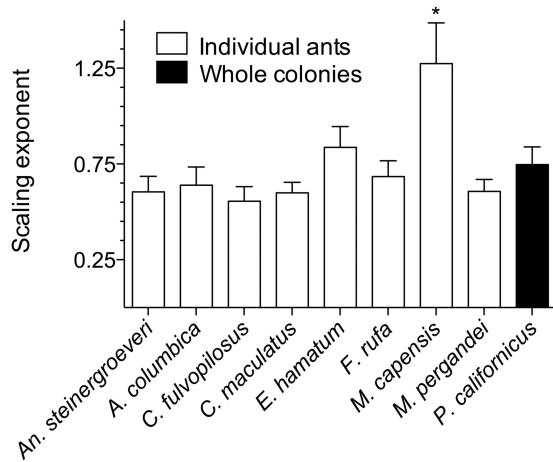


Figure A3: Intraspecific scaling exponent by species. With the exception of *Messor capensis*, the homogenously hypometric intraspecific metabolic rate scaling allometries of individual ants (data from Chown et al. 2007) are not significantly different from the hypometric scaling allometry for whole colonies of *Pogonomyrmex californicus*.

Table A2
Colony census data at time of measurement

Colony	Queens		Workers		Pupae		Larvae	
	No.	Mass (mg)	No.	Mass (mg)	No.	Mass (mg)	No.	Mass (mg)
1	1	12.8	405	1,530	70	320	75	360
2	3	37.2	251	730	33	116.4	80	201.5
3	3	35.7	80	265.7	0	0	12	9.6
4	3	32.4	317	862	17	58.3	55	99.1
5	3	37.4	532	1,613.4	36	136.2	88	160
6	3	36.4	400	1,273.1	0	0	18	29.5
7	3	34.2	413	1,247.8	57	224.4	124	211.9
8	2	24.5	503	1,724.7	0	0	36	55.6
9	2	26.7	514	1,654.7	0	0	82	131
10	2	24.6	92	260.4	0	0	19	31.3
11	2	24.8	120	391	19	65.5	9	13.5
12	1	11.6	260	888.8	0	0	55	125.5
13	3	30.8	105	350.6	0	0	16	23

Note: Ten-month-old whole colonies of *Pogonomyrmex californicus*. Measured masses of the total number of ants within a category, not average per capita values, are shown.

Table A3
Mass and metabolic rate data for *Pogonomyrmex californicus* worker groups

Workers	Mass (g)	Temperature (°C)	Volume O ₂ (mL O ₂ /min)	Metabolic rate (μW at 25°C)
1	.0028	31.86	2.44E-05	5.38
1	.0024	30.36	3.45E-05	8.45
1	.0026	30.35	5.24E-05	12.83
3	.0083	30.94	7.79E-05	18.32
5	.0184	30.11	1.78E-04	44.39
5	.0125	30.30	9.33E-05	22.94
10	.0278	31.83	3.64E-04	80.47
10	.0289	29.55	5.21E-04	134.85

Table A3 (Continued)

Workers	Mass (g)	Temperature (°C)	Volume O ₂ (mL O ₂ /min)	Metabolic rate (μW at 25°C)
10	.0260	30.06	2.54E-04	63.53
20	.0585	31.83	6.48E-04	143.29
25	.0717	29.91	9.48E-04	239.48
25	.0618	31.55	8.10E-04	182.64
50	.1232	29.81	1.45E-03	369.26
60	.1612	30.30	1.52E-03	373.94
75	.2024	31.86	2.60E-03	573.00
85	.2139	30.13	2.75E-03	685.24
115	.3584	30.11	4.44E-03	1,104.81
150	.4398	30.94	5.40E-03	1,269.23
150	.3985	31.34	5.77E-03	1,320.01
225	.6405	31.83	8.42E-03	1,860.50

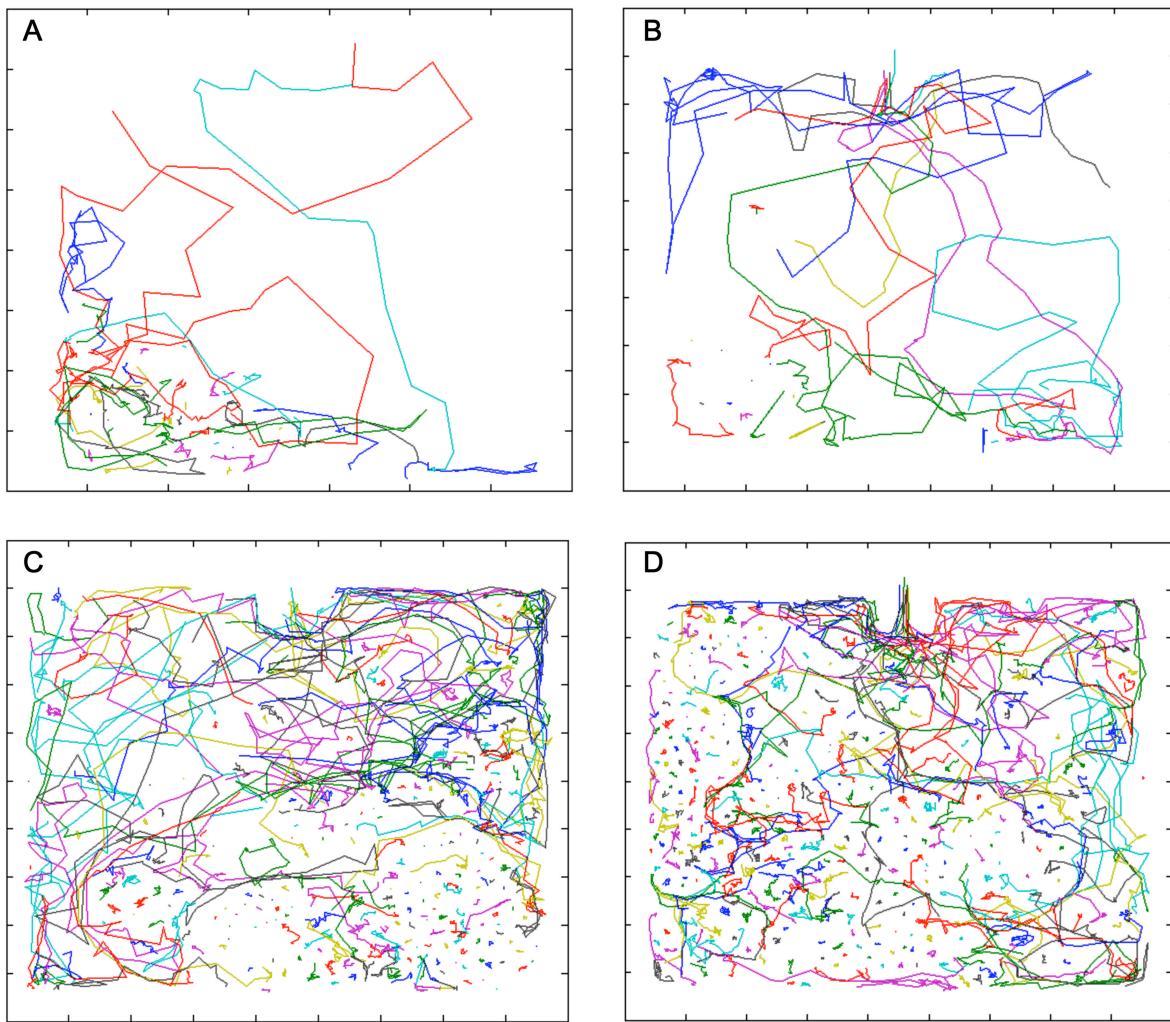


Figure A4: Digitized trajectories of ants in colonies with 32 (A), 39 (B), 267 (C), and 332 (D) workers. Each frame shows the trajectories of ants over 60 s within the colony nest of 11-cm width and length. Colors indicate different ants.