ORIGINAL PAPER

The metabolic costs of building ant colonies from variably sized subunits

Jonathan Z. Shik

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, and 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 *Pheidole 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.

Communicated by J. Traniello

J. Z. Shik (✉)
Graduate Program in Ecology and Evolutionary Biology,
Department of Zoology, University of Oklahoma,
730 Van Vleet Oval,
Norman, OK 73019, USA
e-mail: jshik@ou.edu

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, 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.

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*, Calabi and Porter 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.

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.

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; $b_{\text{major mass}}>b_{\text{minor mass}}$), 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 *Pheidole dentata* ranging from incipient to reproductive and examined energetic costs of ontogenetic changes in caste structure.

Methods

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 (Table A1). Ants were housed in plastic nest boxes lined with Fluon for at least 48 h prior to

Table 1 Summary of individual and group traits for the *Pheidole* species used in this study

Species	Caste	Number	Individual worker traits		Group traits		
			Worker mass (mg; ±1SE)	$\dot{V} CO_2$ ($\mu l CO_2 h^{-1}$) (±1SE)	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

Dry mass of individual ants averaged for *n* individuals. The value pWR=$\log_{10}$ (maximum mass/minimum mass) describes the range of mass analyzed

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 *P. 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.

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 (in microliter CO_2 per hour; 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 ppm 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.

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)

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

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)

Incurrent air was first scrubbed of H_2O and CO_2 using a Drierite/Ascarite/Drierite column (sensu 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 h, this air was flushed out for 200 s at 50 ml min^{-1}, passed through a 10-cm^3 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 h, yielding six measurements per chamber. The first-hour data included extrinsic CO_2 and were 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 *Pheidole bicarinata* minors (0.07 mg; Table 1), 2 h were necessary to generate stable CO_2 measurements. For this species, data points were the average of four hourly measurements (hours 3–6).

Respirometry chambers for individual and group trials were 10-cm^3 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 h, and weighed to the nearest 10^{-3} mg. Individual ants were always used for only one experimental trial. Although each worker group consisted of only nestmates, the results of trials from multiple colonies were pooled to generate regressions.

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—10×2.75-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 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 1°C. 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.

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±0.1 ml min^{-1}). I then used ExpeData software to generate a new variable $\dot{V}CO_2$ by transforming CO_2 measurements from parts per million to microliter per hour 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 (±1SD).

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 $\dot{V}CO_2$ (in microliter CO_2 per hour; y) on group or colony size (dry biomass in milligrams; M). To detect allometry ($b\neq1$), 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).

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 25°C 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 third 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.

Results

Body size and maintenance costs

Majors of *P. dentata*, *P. bicarinata*, and *Pheidole 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 ± 1SD, 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 $U=2,456.5$; $n=44$, 44; $p=0.0001$; Fig. 2).

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
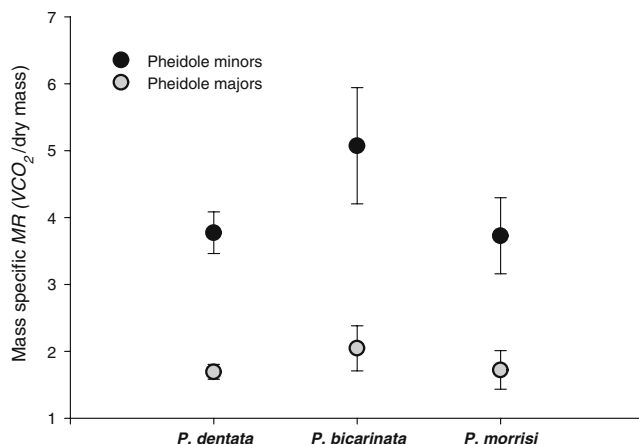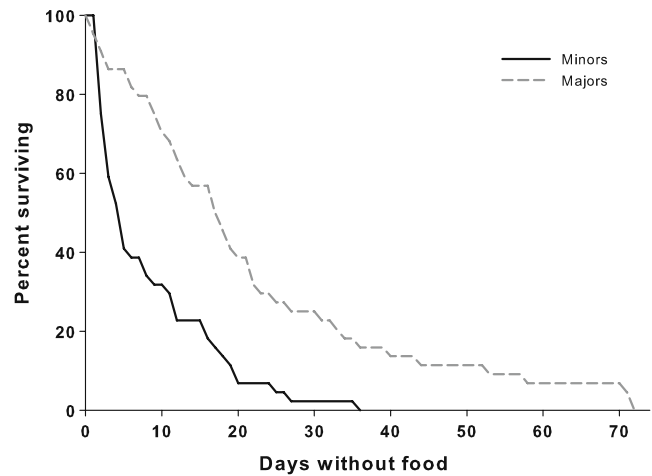

Fig. 2 Majors of *P. dentata* lived significantly longer than conspecific minors when starved

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. 3b); for *P. bicarinata* ($M^{1.07}$; Fig. 3a) 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 one to two ants and two 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.

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

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

Table 3 Results of ANCOVA 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) × 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) × 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) × caste	1	0.11	14.73	0.0005
	Error	34			
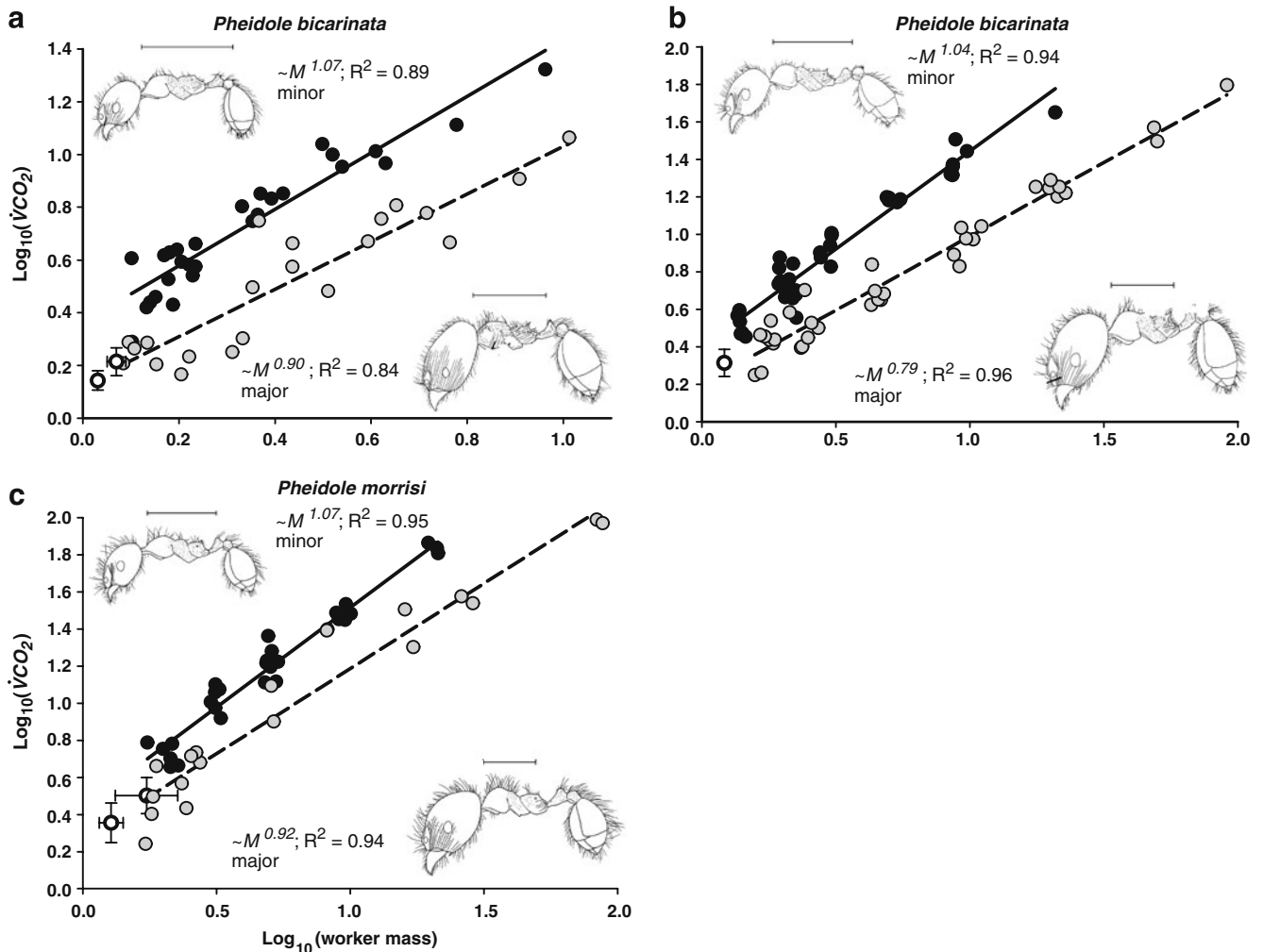
Fig. 3 Scaling of metabolic rate on worker group mass for castes of *P. bicarinata* (**a**), *P. dentata* (**b**), and *P. morrisi* (**c**). Minors denoted by *black circles*. Majors denoted by *gray circles*. Major slopes significantly lower than minor slopes for each species. *Open circles* are the average of one or two minor worker data (bidirectional error bars ± 1 SD). Pheidole images reprinted by permission of the publisher from Pheidole in the new world: a dominant, hyperdiverse ant genus by Edward O. Wilson, pp. 282, 325, 561, Cambridge, Mass.: Harvard University Press, Copyright © 2003 by the President and Fellows of Harvard College. *Scale bars* above images=1 mm
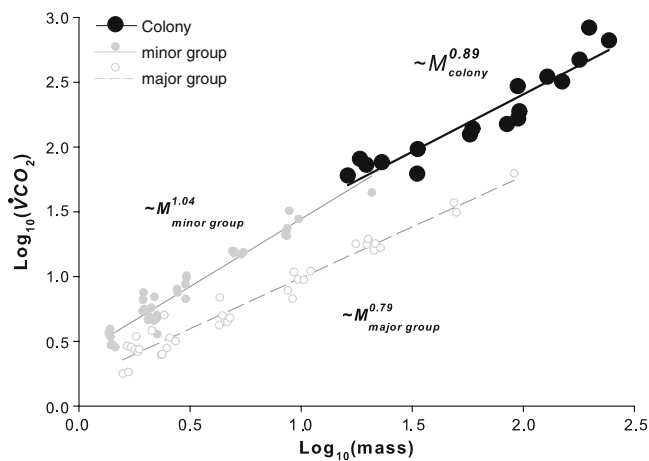
Fig. 4 Intraspecific scaling of whole colony $\dot{V}CO_2$. The colony slope for *P. dentata* is between major and minor groups and may reflect offsetting developmental shifts in caste mass and number

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).

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 et al. 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-Mrozowska 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.

References

Beckers SR, Goss S, Deneubourg JL, Pasteels JM (1989) Colony size, communication and ant foraging strategy. Psyche 96:239–256

Beshers SN, Fewell JH (2001) Models of division of labor in social insects. Annu Rev Entomol 46:413–440

Bhatkar A, Whitcomb WH (1971) Artificial diet for rearing various species of ants. Florid Entomol 53:229–232

Brian MV (1978) Production ecology of ants and termites. Cambridge University Press, Cambridge

Brian MV, Carr CAH (1960) The influence of the queen on brood rearing in ants of the genus *Myrmica*. J Insect Physiol 5:81–94

Brown JJ, Traniello JFA (1998) Regulation of brood-care behavior in the dimorphic castes of the ant *Pheidole morrisi* (Hymenoptera: Formicidae): effects of caste ratio, colony size, and colony needs. J Ins Behav 11:209–219

Brown JH, Gillooly JF, Allen P, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789

Buhl J, Gautrais J, Deneubourg JL, Theraulaz G (2004) Nest excavation in ants: group size effects on the size and structure of tunneling networks. Naturwiss 91:602–606

Burkhardt JF (1998) Individual flexibility and tempo in the ant, *Pheidole dentata*, the influence of group size. J Insect Behav 11:493–504

Calabi P, Porter SD (1989) Worker longevity in the fire ant *Solenopsis invicta*—ergonomic considerations of correlations between temperature, size and metabolic rates. J Insect Physiol 35:643–649

Cao TT, Dornhaus A (2008) Ants under crowded conditions consume more energy. Biol Lett 4:613–615

Chen SC (1937) Social modification of the activity of ants in nest building. Physiol Zool 10:420–436

Chown SL, Marais E, Terblanche JS, Klok C, Lighton JRB, Blackburn TM (2007) Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Funct Ecol 21:282–290

Darveau LA, Suarez RK, Andrews RD, Hochachka PW (2002) Allometric cascade as a unifying principle of body mass effects on metabolism. Nature 417:166–170

Davidson J (1956) An analysis of cell growth and metabolism in the crayfish (Procambrus alleni). Biol Bull 110:264–273

Detrain C, Pasteels JM (1991) Caste differences in behavioural thresholds as a basis for polyethism during food recruitment in the ant, Pheidole pallidula (Nyl) (Hymenoptera, Myrmicinae). J Insect Behav 4:157–176

Fewell J, Schmidt SK, Taylor T (2009) Division of labor in the context of complexity. In: Gadau J, Fewell J (eds) Organization of insect societies: from genome to sociocomplexity. Harvard University Press, Cambridge, pp 483–502

Fonck C, Jaffe K (1996) On the energetic cost of sociality. Physiol Behav 59:713–719

Foster KR, Wenseleers J, Ratnieks FLW (2006) Kin selection is the key to altruism. Trends Ecol Evol 21:57–60

Gadau J, Fewell J (eds) (2009) Organization of insect societies: from genome to sociocomplexity. Harvard University Press, Cambridge

Gallé L Jr (1978) Respiration as one of the manifestations of the group effect in ants. Act Biol Szeg 24:111–114

Glazier DS (2005) Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biol Rev 80:611–662

Gobin B, Heinze J, Stratz M, Roces F (2003) The energetic cost of reproductive conflicts in the ant Pachycondyla obscuricornis. J Insect Physiol 49:747–752

Golley FB, Gentry JB (1964) Bioenergetics of the southern harvester ant Pogonomyrmex badius. Ecology 45:217–225

Gordon DM (1993) What is the function of encounter patterns in ant colonies? Anim Behav 45:1083–1100

Gordon DM (1995) The development of an ant colony's foraging range. Anim Behav 49:649–659

Gordon DM (2002) The regulation of foraging activity in red harvester ant colonies. Am Nat 159:509–518

Hebling-Beraldo MJA, Mendes EG (1981) The respiratory metabolism of the castes of two leaf cutting ants, Atta laevigata (F. Smith, 1858) and Atta sexdens rubropilosa (Forel 1908). Comp Biochem Physiol 68A:241–247

Heinze J, Hölldobler B, Peeters C (1994) Conflict and cooperation in ant societies. Naturwiss 81:489–497

Hochachka PW, Somero GN (2002) Biochemical adaptation: mechanism and process in physiological evolution. Academic Press, New York

Hölldobler B, Wilson EO (2008) The superorganism: the beauty, elegance, and strangeness of insect societies. Norton, New York

Horn-Mrozowska E (1976) Energy Budget elements of an experimental nest of Formica pratensis Retzius 1783 (Hymenoptera: Formicidae). Pol Ecol Stud 23:55–98

Hou C, Kaspari M, Vander Zanden HB, Gillooly JF (2010) Energetic basis of colonial living in social insects. Proc Natl Acad Sci USA 107:3634–3638

Howard DF, Tschinkel WR (1980) The effect of colony size and starvation on food flow in the fire ant, Solenopsis invicta (Hymenoptera: Formicidae). Behav Ecol Sociobiol 7:293–300

Jensen TF (1978) An energy budget for a field population of Formica pratensis Retz. (Hymenoptera: Formicidae). Nat Jutland 20:203–226

Kaspari M (2005) Global energy gradients and size in colonial organisms: worker mass and worker number in ant colonies. Proc Natl Acad Sci USA 102:5079–5083

Kaspari M, Byrne MM (1995) Caste allocation in litter Pheidole—lessons from plant defense theory. Behav Ecol Sociobiol 37:255–263

Kaspari M, Vargo EL (1995) Colony size as a buffer against seasonality: Bergmann's rule in social insects. Am Nat 145:610–632

Kay A, Rissing SW (2005) Division of labor in ants can mediate demands for food and safety. Behav Ecol Sociobiol 58:165–174

Kleiber M (1932) Body size and metabolism. Hilgardia 6:315–353

Kozlowski J, Konarzewski M, Gawelczyk AT (2003) Cell size as a link between noncoding DNA and metabolic rate scaling. Proc Natl Acad Sci USA 100:14080–14085

Lighton JRB (1989) Individual and whole-colony respiration in an African formicine ant. Funct Ecol 3:523–530

Lighton JRB (2008) Measuring metabolic rates: a manual for scientists. Oxford University Press, London

Lighton JRB, Bartholomew GA, Feener DH (1987) Energetics of locomotion and load carriage and a model of the energy costs of foraging in the leaf cutting ant Atta colombica Guer. Physiol Zool 60:524–537

MacKay WP (1985) A comparison of the energy budgets of 3 species of Pogonomyrmex harvester ants (Hymenoptera, Formicidae). Oecologia 66:484–494

Martin PJ (1991) Respiration of the ant Leptothorax unifasciatus (Hymenoptera: Formicidae) at individual and society levels. J Insect Physiol 37:311–318

McGlynn TR, Owen JP (2002) Food supplementation alters caste allocation in a natural population of Pheidole flavens, a dimorphic leaf-litter dwelling ant. Insect Soc 49:8–14

Mehdiabadi NJ, Reeve HK, Mueller UG (2003) Queen versus workers: sex-ratio conflict in eusocial Hymenoptera. Trends Ecol Evol 18:88–93

Mertl AL, Traniello JFA (2009) Behavioral evolution in the major worker subcaste of twig-nesting Pheidole (Hymenoptera: Formicidae): does morphological specialization influence task plasticity? Behav Ecol Sociobiol 63:1411–1426

Moreau CS (2008) Unraveling the evolutionary history of the hyperdiverse ant genus Pheidole (Hymenoptera: Formicidae). Molec Phylogen Evol 48:224–239

Moses ME, Hou C, Woodruff WH, West GB, Nekola JC, Zuo W, Brown JH (2008) Revisiting a model of ontogenetic growth: estimating model parameters from theory and data. Am Nat 171:632–645

Nielsen MG (1972) An attempt to estimate energy flow through a population of workers of Lasius alienus (Forst) (Hymenoptera: Formicidae). Nat Jutland 16:98–107

Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton

Partridge L, Barrie B, Fowler K, French V (1994) Evolution and development of body size and cell size in Drosophila melanogaster in response to temperature. Evolution 48:1269–1276

Passera L, Roncin E, Kaufmann B, Keller L (1996) Increased soldier production in ant colonies exposed to intraspecific competition. Nature 379:630–631

Petal J (1972) Methods of investigating of the productivity of ants. Ekol Polsk 20:9–22

Peters RH (1983) The ecological implications of body size. Cambridge University Press, Cambridge

Pie MR, Traniello JFA (2007) Morphological evolution in a hyper-diverse clade: the ant genus Pheidole. J Zool 271:99–109

Porter SD (1986) Revised respiration rates for the southern harvester ant, Pogonomyrmex badius. Comp Biochem Physiol 83A:197–198

Powell S (2009) How ecology shapes caste evolution: linking resource use, morphology, performance and fitness in a superorganism. J Evol Biol 22:1004–1013

Powell S, Franks NR (2005) Caste evolution and ecology: a special worker for novel prey. Proc Roy Soc Lond B 272:2173–2180

Prothero J (1986) Methodological aspects of scaling in biology. J Theor Biol 118:259–286

Reeve HK, Hölldobler B (2007) The emergence of a superorganism through intergroup competition. Proc Natl Acad Sci USA 104:9736–9740

Ricklefs RE (2003) Is the rate of ontogenetic growth constrained by resource supply or tissue growth potential? a comment on West et al.'s model. Funct Ecol 17:384–393

Robinson GE, Page REJ (1989) Genetic basis for division of labor in an insect society. In: Breed MD, Page REJ (eds) The genetics of social evolution. Westview, Boulder, pp 61–80

Savage VM, Allen AP, Brown JH, Gillooly JF, Herman AB, Woodruff WH, West GB (2007) Scaling of number, size, and metabolic rate of cells with body size in mammals. Proc Natl Acad Sci USA 104:4718–4723

Seal JN, Tschinkel WR (2006) Colony productivity of the fungus-gardening ant Trachymyrmex septentrionalis (Hymenoptera: Formicidae) in a Florida pine forest. Ann Entomol Soc Am 99:673–682

Seid MA (2006) Age-related repertoire expansion and division of labor in Pheidole dentata (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants. Behav Ecol Sociobiol 60:631–644

Shik JZ (2008) Ant colony size and the scaling of reproductive effort. Funct Ecol 22:674–681

Starostova Z, Kubicka L, Konarzewski M, Kozlowski J, Kratochvil L (2009) Cell size but not genome size affects scaling of metabolic rate in eyelid geckos. Am Nat 174:E100–E105

Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insect. Science 191:249–263

Tschinkel WR (1991) Insect sociometry, a field in search of data. Insect Soc 38:77–82

Tschinkel WR (1993) Sociometry and sociogenesis of colonies of the fire ant Solenopsis invicta during one annual cycle. Ecol Monog 63:425–457

Walker J, Stamps J (1986) A test of optimal caste ratio theory using the ant Camponotus (Colobopsis) impressus. Ecology 67:1052–1062

Wetterer JK (1999) The ecology and evolution of worker size-distribution in leaf cutting ants (Hymenoptera: Formicidae). Sociobiol 34:119–144

Wilson EO (1968) The ergonomics of caste in the social insects. Am Nat 102:41–66

Wilson EO (1976) The organization of colony defence in the ant Pheidole dentata Mayr (Hymenoptera: Formicidae). Behav Ecol Sociobiol 1:63–81

Wilson EO (1980) Caste and division of labour in leaf-cutter ants (Hymenoptera: Formicidae: Atta). II. The ergonomic optimization of leaf cutting. Behav Ecol Sociobiol 7:157–165

Wilson EO (1984) The relation between caste ratios and division of labour in the ant genus Pheidole (Hymenoptera: Formicidae). Behav Ecol Sociobiol 16:89–98

Wilson EO (1985) The sociogenesis of insect colonies. Science 228:1489–1495

Wilson EO (2003) Pheidole in the new world: a dominant hyper-diverse ant genus. Harvard University Press, Cambridge

Yang AS (2006) Seasonality, division of labor, and dynamics of colony-level nutrient storage in the ant Pheidole morrisi. Insect Soc 53:456–462

Yang AS, Martin CH, Nijhout HF (2004) Geographic variation of caste structure among ant populations. Curr Biol 14:514–519