

# Respiration, worker body size, tempo and activity in whole colonies of ants

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**Abstract.** Ants are social, and their metabolism should be measured on at least two levels: (i) the individual workers and brood of which the colony is composed and (ii) the colony in its entirety. Whole colony respiration, tempo (size-free running speed in body lengths per second) and whole colony activity were simultaneously measured for 15 species of ants in four subfamilies, and these data are related to average worker and whole-colony weight, activity, percentage brood and percentage fat. Across all 15 species, whole colony respiration rate ( $\mu\text{L CO}_2 \text{ h}^{-1}$ ) is linearly related to whole colony live weight (log–log slope = 1.0). Colonies composed of large workers respire less than colonies composed of an equal live weight of small workers, and colonies with high tempos respire more than lower tempo colonies of equal weight. The tempos and respiration rates of smaller ants tend to be higher, and a higher tempo exacts a cost in higher respiration independent of the effect of small body size. Individual worker respiration ( $\mu\text{L CO}_2 \text{ h}^{-1}$ ) scales to worker live weight with an exponent of 0.76. Whole colony specific respiration rate ( $\mu\text{L CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) is unrelated to colony live weight. The regressions of respiration rates against colony and worker dry weight, lean weight and metabolic weight have similar slopes to those of live weight but different intercepts. Respiration is not related to worker percentage fat, percentage brood or activity. Ant ecology, tempo, body size, polymorphism and colony size are discussed in relation to respiration.

**Key words.** *Aphaenogaster*, *Brachymyrmex*, *Camponotus*, *Crematogaster*, *Dolichoderus*, *Dorymyrmex*, Formicidae, *Paratrechina*, *Pheidole*, *Pogonomyrmex*, *Pseudomyrmex*, *Solenopsis*.

## Introduction

In social insect societies, selection for colony-level traits such as worker number, labour allocation and energy budgeting shape the characteristics of individual colony members. Interspecific comparisons of individual worker (organism) and whole colony (superorganism) traits are necessary for describing commonalities between colony organization and ecology (Tschinkel, 1991). If species are expected to budget energy in an adaptive way, then comparisons of colony size, worker size and

worker tempo may reveal potential trade-offs in allocation across species.

A common method of inquiry for questions involving colony energetics is to measure respiration rate in response to factors of interest. Previous work on respiration has concentrated on individual ants (Peakin & Josens, 1978), demonstrating that respiration rate and/or metabolic rate per unit body weight decreases with increasing individual body weight (Heusner, 1985; Lighton & Fielden, 1995), increases with temperature (Holm-Jensen *et al.*, 1980; Bartholomew *et al.*, 1981; Nielsen, 1986), increases with running speed (Lighton *et al.*, 1987; Bartholomew *et al.*, 1988; Lighton & Feener, 1989; Fewell *et al.*, 1996), decreases at low humidity (Lighton & Bartholomew, 1988), varies according to metabolic substrate (Boomsma & Isaaks, 1985; Fiedler & Maschwitz, 1988; Passera *et al.*, 1990), differs with growth from increase in cell size versus cell number (Chown *et al.*, 2007) and varies seasonally as well as diurnally (Kneitz, 1967; Jensen, 1978; MacKay & Sassaman, 1984).

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At the colony level, demography and division of labour produce heterogeneity in individual worker activity. For many species, workers move through a complex sequence of specialized roles as they age, and both activity and physiological state vary across adult ontogeny. Therefore, studies of whole colonies or large fragments of colonies offer the best insight into individual worker respiration by considering the average individual's respiration in the context of interacting labour groups, age groups and inactive brood (Cole, 1992; Gillooly *et al.*, 2010; Shik, 2010).

However, few studies of colony energetics actually include whole colonies collected from the field, or collections from multiple time points that represent the changing ratios of brood to workers throughout the annual cycle (Martin, 1991). The reported brood rearing cycles of numerous ant species suggest seasonal variation in respiration rates must be considered in studies spanning more than one season (Hölldobler & Wilson, 1990). Yet, whole colony characteristics such as respiration are frequently estimated from small groups of ants, in the absence of brood, using weight of individual museum specimens,  $Q_{10}$  values of similarly sized organisms, and literature reports of colony size for laboratory colonies (Gallé, 1978; Bartholomew *et al.*, 1988; Lighton & Bartholomew, 1988; Shik, 2008; Hou *et al.*, 2010).

Although mass specific respiration rate of individual workers can scale isometrically with colony and fragment size in some species, clear indications that colony context can influence activity and respiration exist for others (Lighton, 1989; Fonck & Jaffe, 1996). Waters *et al.* (2010) report that, as colony mass increases in *Pogonomyrmex californicus*, whole colony metabolic rate per unit mass scales non-additively, decreasing as a function of colony mass. This relationship is likely related to the difference in the fraction of active to inactive ants in larger colonies (not individual mass), and emphasizes that the influence of colony size is detectable within species and with small changes in colony size (Holbrook *et al.*, 2011).

Just as individual worker respiration is subject to environmental influence, whole colony respiration may be influenced by a number of factors. Lighton (1989) demonstrated that starvation decreases whole-colony respiration rate in *Camponotus fulvopilosus*, and noted diurnal variation in whole-colony respiration (although this variation may have been caused by diurnal variation of worker activity). Rogers *et al.* (1972), using *Pogonomyrmex occidentalis* (the western harvester ant) report the expected positive relationship between colony respiration rate and temperature. Together, these findings suggest the need to standardize feeding, sample time and temperature in comparative studies and indicate that significant natural variation may exist within wild colonies.

Comparisons of whole colony respiration between colonies also require that colonies contain comparable proportions of workers, larvae and pupae, or that differences in colony composition be accounted for statistically. When the respiratory rates of life stages are measured separately, those of workers tend to be higher than those of similar sized pupae, which in turn are higher than larvae (430, 220 and  $190 \mu\text{L O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , respectively) (Martin, 1991; Takahashi-Del-Bianco *et al.*, 1998). Schmid-Hempel (1990) also suggest that worker

ants are more active in the presence of brood, indicating that realistic measurements of individual and whole colony metabolic rates may vary seasonally as brood and worker ratios change.

Tempo, defined as the species specific rate of individual movement, is a little studied, highly variable component of the behaviour of social insects. Previous observations suggest an association between tempo and body size, colony size, labour saturation and type of resources exploited. Oster & Wilson (1978) rate tempo as ranging from hot/nervous to cool/calm, and observe that high tempo ants tend to have large, very active colonies, as well as differentiated worker castes, and depend on unpredictable yet rich food resources. As a result of large colony size, high tempo species are termed 'labour saturated' in that many individuals within a colony perform the same set of tasks; for example, crazy ants (*Paratrechina longicornis*) and fire ants (*Solenopsis invicta*). At the other extreme, low tempo ants are typified by slow, deliberate movements, small colonies, little caste differentiation and the use of predictable or specialized resources. For example, *Aphaenogaster* spp. or *Odontomachus brunneus* (trap jaw ants) appear to waste little movement or energy in task performance. Such apparent differences in 'wastefulness' and speed of movement suggest that ants that operate at high tempo may have higher respiration rates than low tempo ants. Although clear differences in worker tempo (the rate of individual movement) and body size exist among ant species, the relationship of tempo to colony size, labour saturation, type of resources exploited and colony energetics remains undescribed for most species.

Reliability theory illustrates the importance of tempo during foraging (Oster & Wilson, 1978; Herbers, 1981). As the rate of movement of individuals (i.e. tempo) increases, the probability of correctly completing tasks decreases. It follows that more energy may be required by high tempo species to complete a given task compared with that for low tempo species. This relationship may explain the apparent link between colony size and individual activity level (Waters *et al.*, 2010; Holbrook *et al.*, 2011).

Given the variation in predictability and abundance of resources used by ant species, tempo may differ between generalist and specialist species. Davidson & Patrell-Kim (1996) suggest that ant species using predictable carbohydrate resources, such as honeydew from homopterans or nectar from flowers, gain excess energy and use it to fuel high tempo movement, high activity, alertness, carbohydrate-based chemical defences and aggressiveness, again suggesting a positive relationship between tempo, respiration and resource use.

Inactivity is the rule for the average ant worker, which is not moving 70–75% of the time (Hölldobler & Wilson, 1990). Across ant species, average work load (the proportion of time an individual is active) increases with increasing colony size (Schmid-Hempel, 1990), suggesting that high tempo ants (with large colonies) may not only move faster, but also may be active a greater proportion of time relative to low tempo ants.

However, worker activity level is likely influenced by developmental age and the availability of tasks related to nest maintenance, brood care and feeding. It is therefore critical

that laboratory measurements of colony respiration include all demographic groups. The present study describes the inter-relationships among simultaneously measured respiration, tempo, activity and body size in whole colonies of multiple species of ants. The study investigates how whole-colony respiration and whole-colony weight are related, as well as whether activity and tempo contribute to respiration rate independently of other factors affecting respiration.

## Materials and methods

### Species selection

Mature colonies of common ants were collected within the Apalachicola National Forest near Tallahassee, Florida, U.S.A. Most species are in the diverse and abundant subfamilies Formicinae, Myrmicinae, and Dolichoderinae, and included, *Camponotus floridanus* Buckley, *Camponotus nearcticus* Emery, *Brachymyrmex obscurior* Forel, *Paratrechina longicornis* Latreille, *Pheidole dentata* Mayr, *Pogonomyrmex badius* Latreille, *Crematogaster ashmeadi* Mayr, *Crematogaster minutissima* Mayr, *Aphaenogaster ashmeadi* Emery, *Aphaenogaster treatae* Forel, *Solenopsis invicta* Buren, *Solenopsis geminata* Fabricius, *Dolichoderus mariae* Forel, *Dorymyrmex (Conomyrma) bureni* Trager and *Pseudomyrmex gracilis* Fabricius. The subfamilies, number of colonies and number of queenright colonies sampled per species are presented in Table 1.

### Colony maintenance

All colonies were maintained in the laboratory at constant temperature ( $30 \pm 2^\circ\text{C}$ ), relative humidity ( $50 \pm 10\%$ ) and illumination (continuous overhead fluorescent room lighting) for at least 2 weeks before use. Colonies were housed in plaster nests placed in photography trays, the sides of which were coated with fluon to prevent escape. Colonies were fed frozen crickets (*Acheta domesticus*) or tenebrionid larvae (*Zophobus atratus*) every other day, and had access to sugar water and water *ad libitum*. To assure experimental uniformity, colonies were fed 24 h before experimentation.

### Colony composition

Worker density has been demonstrated to influence activity and respiration rate in social insect colonies, (Cao & Dornhaus, 2008). To minimize the effects of density, all experimental colonies covered less than 50% of the floor of their observation nests. Colony composition was standardized, immediately prior to placing the colony in a Plexiglas® respiration chamber (see below) by removing workers or brood to a target of approximately 4:1 ratio of workers to brood (by weight); brood comprised half larvae and half pupae (by weight). Actual percentage brood at the time of colony collection averaged 17% (SD 12%) but ranged from 0% to 60%. Except for some

**Table 1.** The species used in the present study, the nesting habits and the number of colonies studied. All except *Pseudomyrmex gracilis* were collected in the Tallahassee area.

| Species                          | Subfamily        | Number of colonies | Number queenright |
|----------------------------------|------------------|--------------------|-------------------|
| <i>Camponotus floridanus</i>     | Formicinae       | 6                  | 2                 |
| <i>Camponotus nearcticus</i>     | Formicinae       | 3                  | 2                 |
| <i>Brachymyrmex obscurior</i>    | Formicinae       | 3                  | 1                 |
| <i>Paratrechina longicornis</i>  | Formicinae       | 3                  | 3                 |
| <i>Pheidole dentata</i>          | Myrmicinae       | 3                  | 3                 |
| <i>Pogonomyrmex badius</i>       | Myrmicinae       | 3                  | 2                 |
| <i>Crematogaster ashmeadi</i>    | Myrmicinae       | 4                  | 1                 |
| <i>Crematogaster minutissima</i> | Myrmicinae       | 3                  | 3                 |
| <i>Aphaenogaster ashmeadi</i>    | Myrmicinae       | 7                  | 2                 |
| <i>Aphaenogaster treatae</i>     | Myrmicinae       | 3                  | 1                 |
| <i>Solenopsis invicta</i>        | Myrmicinae       | 9                  | 3                 |
| <i>Solenopsis geminata</i>       | Myrmicinae       | 3                  | 1                 |
| <i>Pseudomyrmex gracilis</i>     | Pseudomyrmecinae | 1                  | 0                 |
| <i>Dolichoderus mariae</i>       | Dolichoderinae   | 4                  | 4                 |
| <i>Dorymyrmex bureni</i>         | Dolichoderinae   | 1                  | 1                 |

colony fragments, all colonies were queenright or fragments of queenright colonies and all species except *P. longicornis* were monogyne. Alates were excluded but, in some cases, sexual larvae or pupae were included and queens were included in some colonies (Table 1). To facilitate weighing, colony composition adjustment and eventual transfer into a respirometry chamber, colonies were cooled, weighed and composed in a refrigerated room ( $4^\circ\text{C}$ ) for 15–45 min before handling. In some cases, it was impossible to alter the colony composition because of the small size or limited availability of some life stages.

For all species, only colonies that consistently produced brood under laboratory conditions were used for respiration, activity and tempo measurement. For three species with large mature colony size (*C. floridanus*, *S. invicta* and *C. ashmeadi*), colonies were broken into fragments, so that a single source colony yielded two or three fragments that were measured separately as independent replicates.

### Respirometry

To allow simultaneous recording of respiration and videotaping of activity and tempo, a standardized colony was placed in a clear Plexiglas® respirometry nest chamber. Chamber bottoms were constructed by routing a connected pair of either 0.25 or 0.5 cm depressions in a square piece of 13 mm thick Plexiglas®. Chambers were covered with a piece of 6.5 mm Plexiglas® screwed to the bottom. Window stripping was placed along the perimeter of the chamber between the cover and bottom to provide an air-tight seal. Teflon nozzles were inserted on opposite sides of the chamber to permit flow through the chamber. The internal opening of each nozzle was covered with fine wire mesh to prevent ant escapes. An appropriate sized chamber was chosen depending on the size and number of individuals in the colony to be measured, so that 50% of the floor space was occupied by ants. Chambers ranged from 66 mL

( $16.25 \times 16.25 \times 0.25 \text{ cm}^3$ ) to 500 mL ( $31.6 \times 31.6 \times 0.5 \text{ cm}^3$ ) in volume.

Once in the chamber, a colony was maintained at constant illumination (fluorescent room lighting), with a slow flow of charcoal filtered humidified air passing through the chamber for a 12-h acclimation period prior to measurement. Colonies were maintained at a constant temperature ( $30 \pm 1^\circ\text{C}$ ) using an air bath constructed from a Nichrome heating wire controlled by a Yellow Springs Thermostat (model 63RC; Yellow Springs Inc., Yellow Springs, Ohio), a photography tray ( $35 \times 45 \text{ cm}^2$ ) with a Plexiglas® cover and a battery powered fan. Temperature inside the respirometry chamber was monitored using a constantan-copper thermocouple and an Omega Instruments digital thermometer (Omega Engineering, Inc., Stamford, Connecticut). Food in the form of a 10% (by weight) aqueous solution of casamino acids (Difco, Becton Dickinson, Inc. Franklin Lakes, New Jersey) and a 20% (by volume) aqueous sucrose solution were deposited on filter paper disks and placed in the nest chamber just prior to the acclimation period. Ants were sometimes observed feeding. The relative humidity inside the nest chamber remained at 60–80%, thereby eliminating the effects of low humidity on respiration rate.

Each colony's  $\text{CO}_2$  gas production was recorded at the same time that the colony's activity was videotaped. To quantify whole colony respiration rate, the volume of  $\text{CO}_2$  (STP-corrected using ambient barometric pressure and temperature) produced by a colony in a respirometry nest chamber was measured by flow-through respirometry methods similar to those of Bartholomew *et al.* (1981) and Lighton (1991). Air was humidified using an aquarium air stone in a 1-L flask of water before entering the chamber, and dried before entering the infrared gas analyzer (IRGA, Horiba Instruments, Inc. Ann Arbor, Michigan). Rotameters (variable area flowmeters, 150 mm; Omega Engineering, Inc.) measured the flow rate through the chamber and IRGA. The output of the IRGA ( $\text{CO}_2$  concentration in p.p.m.) of a colony was continuously recorded on a chart recorder for at least 40 min.  $\text{CO}_2$  production was sampled in 1-min increments every 5 min. The volume of  $\text{CO}_2$  gas produced during 1 min was averaged, corrected to STP and expressed as  $\mu\text{L CO}_2 \text{ h}^{-1}$ . For some analyses, these volumes were converted to mass-specific respiration rates ( $\mu\text{L g}^{-1} \text{ h}^{-1}$ ) by dividing by the total live, dry, lean, cuticle-free weight of the entire colony or fragment or of the workers.

To assess the resolution of the IRGA, before and after each run, a bypass circuit routed the same activated charcoal purified,  $\text{CO}_2$  scrubbed, dried air supply that was used for respirometry directly to the IRGA and used this to zero the IRGA. Compressed air with 502 p.p.m.  $\text{CO}_2$  from a 23-kg cylinder was then attached to the IRGA input and the IRGA output was adjusted to read 502 p.p.m. Before setting the machine to 0 and 502, the readings on the IRGA were recorded. The difference between the readings from 0 and 502 were recorded and averaged to give a measure of drift (noise), and this drift was assumed to be linear and  $\text{CO}_2$  concentration was adjusted accordingly. The range of measured drift was  $0\text{--}0.94 \text{ ppm min}^{-1}$  with an average of  $0.26 \pm 0.04 \text{ p.p.m. min}^{-1}$ . Across all species,  $\text{CO}_2$  concentrations during 1-min measurements ranged from 6.2 to 550.6 p.p.m.

The flow rate through the system was varied according to chamber (colony) size so that the volume of air in the chamber was replaced approximately once per minute. Chambers of 66, 120, 240, 337 and 500 mL were used to measure respiration of different size colonies. The delay in the system was measured by first running  $\text{CO}_2$  free air through the bypass circuit and then switching the path of the flow to run through a chamber filled with 502 p.p.m.  $\text{CO}_2$  air and then recording the time it took for the air to pass through the tubing and reach the IRGA. This was also compared with the time it took to clear out the tubing leading from the respirometry chamber to the IRGA. Delays were 2.5, 2.0, 1.0, 0.75 and 0.5 min for the small to large chambers respectively, and these values varied by approximately 15–30 s depending on actual flow rate. The delay was then used to align the timing of the IRGA output to the correct period on the videotape of the colony in the chamber.

Twenty-nine colonies were intentionally disturbed after 30 and 38 min of recording by lifting the respirometry chamber 10 cm and dropping it on the table. This was carried out to increase the range of activity, tempo and respiration rate. The relationship between respiration rate, activity and tempo in disturbed colonies was recorded as above but analyzed separately for comparison with undisturbed colonies.

### Tempo

Tempo has most often been studied as running speed (distance/time) during foraging (Leonard & Herbers, 1986; Herbers & Choiniere, 1996). However, running speed depends strongly on body size (which is also related to leg length) and, in a comparative study, it is desirable that tempo be independent of body size. For example, a 1-mm ant running at  $10 \text{ mm s}^{-1}$  should have a higher tempo than a 10 mm ant running at the same speed. Typically, one adjusts for differences in body size by dividing running speed by body length. However, this removes the total effect of body size only if the regression of running speed against body length passes through the origin (Packard & Boardman, 1999) and, in the present study, it did not do so (running speed =  $13.7 + 0.72 \text{ body length}$ ). Subtracting the value of the running speed at the origin from each measured running speed and dividing this by body length produced an unbiased, although sometimes negative, estimate of tempo that was truly proportional to running speed. Appendix S1 shows that the difference between the biased and unbiased tempo increases as body size decreases, so that tempos for small ants are greatly inflated. Therefore, the unbiased computation of tempo was used for all analyses in the present study.

Immediately after respiration and activity measurement, the video image was magnified approximately four times to allow measurement of the rate of movement of randomly selected individual ants as an estimate of tempo. Only ants that moved in one direction for 3–5 s were used. Distance moved was measured by pausing the video playback, choosing an ant, and marking the position of the head on the video screen. The video player was then advanced 10 frames (approximately 330 ms of recording time) and the position of the head was marked again. This was repeated for 3–5 s of recording time and the total



on-screen distance moved, as well as ant body length, were measured from the video image. From these measurements, the number of body lengths per second was calculated. Lines of known length drawn on the floor of the respirometry chamber were used to calculate actual running speed and actual body length. The mean tempo of 24 ants from all four nest quadrants was used as colony tempo in the data analysis.

### Colony activity

Colony activity was defined as the proportion of workers in a colony that were active at a particular time. To quantify activity, the Multiple Animal Movement Analyzer (MAMA) (Hoy *et al.*, 1997) was used to analyze videotapes of colony activity. This software was used to compare successive video images separated by 0.25 s, and tabulate the number of video screen pixels that change value between images. Colony activity was estimated from the number of ants in the respirometry chamber, the average size of an ant in pixels, and the average number of pixel changes within each of 20 pairs of video images. This was converted to the proportion of ants that are active by dividing the average number of pixel changes by the total area (in pixels) of ants in the chamber. Ant size (in pixels) was estimated directly from video screen images by measuring body area (head width  $\times$  body length) for a random sample of 20 ants and converting this to pixels using:

$$\text{Number of pixels per ant} = \text{body area (mm}^2\text{)} 0.64 \text{ pixels mm}^{-2} \quad (1)$$

Total area (in pixels) of ants in a chamber was estimated by multiplying the number of pixels per ant by the total number of ants in the chamber. Activity and respiration measurements were linked in time as described above.

### Body composition

After respiration, activity and tempo measurement, each colony was killed by freezing, and was separated into queen, workers, larvae and pupae, and these were then counted and weighed. For large colonies, the number of workers was estimated from average weights and counts of five 50-mg random samples. The mean live body weight (mg) of the workers was computed by dividing the weight of workers in a colony by the number of workers.

The colony components were then dried (60 °C for 2–5 days) to constant weight. Fat was extracted from dried constituent parts in refluxing ethyl ether for approximately 72 h (to constant weight). The proportion of worker body fat (on a live and dry basis) was calculated from the lean weight, live weight and dry weights.

The proportion of worker body weight composed of exoskeleton was determined by hydrolyzing all noncuticular tissue from dry lean workers in a hot 10% (w/w) sodium hydroxide solution for 10 min, followed by exhaustive rinsing in distilled water, drying and re-weighing. The proportion of exoskeleton was determined by dividing the weight of the dried exoskeletons by the

live sample weight. Total worker metabolic weight (dry, lean, cuticle-free weight) of a colony was determined by subtracting the weight of fat, and cuticle from total dry worker weight. Mean worker metabolic weight was determined for each colony by dividing the total metabolic weight of all workers by the number of workers. Hydrolysis of brood often resulted in unmeasurable samples because brood contain so little cuticle. Whole colony metabolic weight was therefore estimated as the sum of total worker metabolic weight plus brood lean weight. Any contributed error is likely to be very small.

### Statistical analysis

Respiration, activity and tempo data are colony means and species means taken from undisturbed colonies or colony means taken from disturbed colonies. Colony averages, rather than multiple measures, were used to avoid pseudoreplication (Hurlbert, 1984). The weight of individual workers was not correlated to whole colony weight (see Supporting information, Appendix S3, correlation = 0.13; not significant) or whole colony CO<sub>2</sub> production (see Supporting information, Appendix S3, correlation = -0.09; not significant). Many of the variables were correlated to one another as well as to the dependent variable, complicating interpretations of primary and subsidiary relationships (see Supporting information, Appendix S3).

Pearson correlation coefficients were used to determine which variables were related to respiration rate, and thus to determine which variables should be included in subsequent regression analysis. STATISTICA (StatSoft, Inc., Tulsa, Oklahoma) was used in all the data analyses.

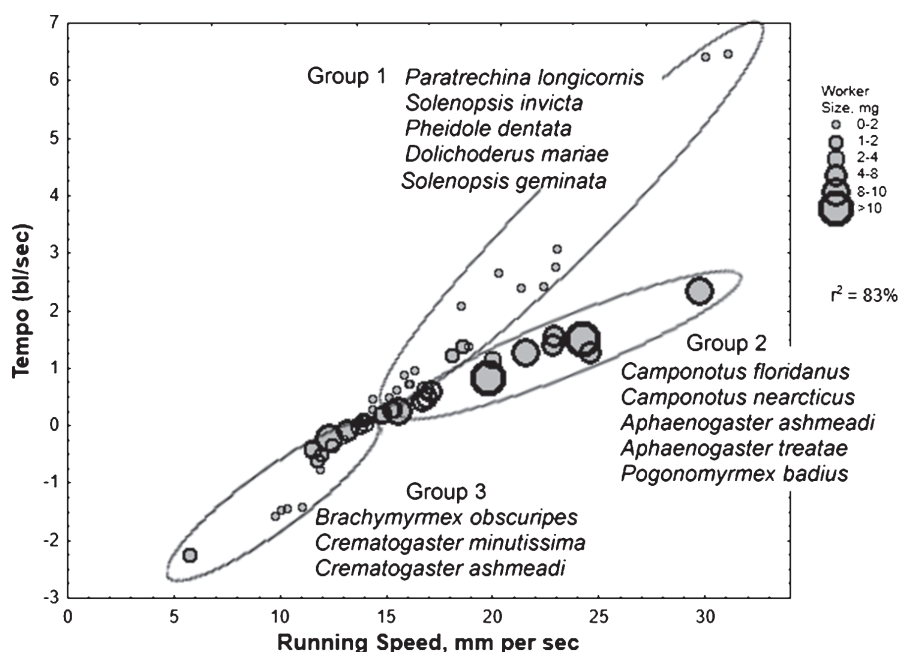
Whole colony respiration was analyzed by multiple regression. Assumptions of normality were tested with residual and normal-score plots, and most data were log transformed to stabilize the variance and to linearize power relationships. Non-significant variables were removed from the regression one by one to determine the combination of variables that maximized  $r^2$  (Myers, 1990). Partial correlations were used to determine the unique contribution of each significant variable to the variance in the dependent variable.

The within-species means of dependent and independent variables can be found in the Supporting information (Appendix S2), where the within-species variability is expressed as the coefficient of variation (COV; SD/mean).

## Results

### Tempo, running speed and body length

Running speed across all colonies varied from 5.7 to 31 mm s<sup>-1</sup>, and varied significantly among species (one-way analysis of variance:  $F_{14,41} = 5.05$ ;  $P < 0.0003$ ). Within species, variation was moderate with COV values averaging 18% and ranging from 1% to 40% (see Supporting information, Appendix S2). Tempo in body-lengths per second (bl s<sup>-1</sup>) is a size-free measure that allows comparison among ants of greatly different sizes. For ants of a given size, tempo increased with



**Fig. 1.** The relationship among running speed, tempo and body length. Each point represents the mean of a colony of one of the species. The species are allocated into groups: those in whom tempo increases rapidly with running speed (slope = 0.39), those in whom it increases more slowly (slope = 0.13), and those whose running speed is usually low (slope = 0.25).

running speed (obviously) and, for ants of a given running speed, tempo decreased with body length. Tempo as a function of running speed is shown for all colonies in Fig. 1. Tempo ranged from  $-2.25$  to  $6.49 \text{ bl s}^{-1}$ . Averaged by species run more than once, mean tempo spanned an almost five-fold range from  $-1.3 \text{ bl s}^{-1}$  (*C. minutissima*) to  $5.0 \text{ bl s}^{-1}$  (*P. longicornis*). Tempo of large ants increased more slowly with running speed (group 2) than that of small ones (group 1) so that, at higher running speeds, the tempo of large ants was distinctly lower than that of small ones (Fig. 1). This suggests that greater body size bears a cost in relative running speed. An alternate view is that a large ant must run faster than a small one to achieve the same higher tempo. Some species never exhibited high tempos (group 3).

#### Colony respiration, weight, tempo and worker size

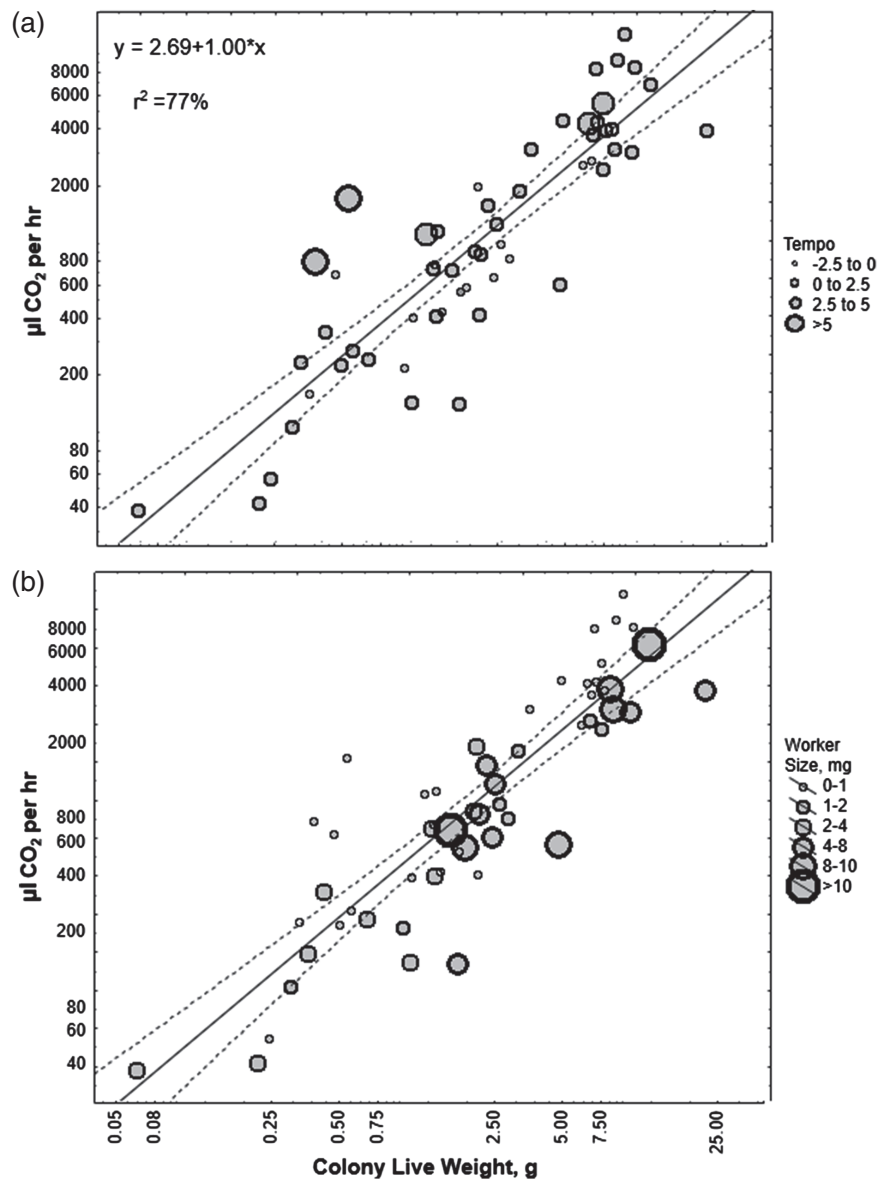
At the most basic level, log whole colony  $\text{CO}_2$  production ( $\mu\text{L h}^{-1}$ ) was exactly isometric with log whole colony live weight (Fig. 2 and Table 2, regression 1;  $r^2 = 77\%$ ). Thus,  $\text{CO}_2$  production rate was a simple multiple of the colony live weight across the entire range of colony live weight, and the  $\text{CO}_2$  production per gramme of live colony did not change across this range. In Fig. 2, higher tempo ants fell predominately above the mean regression line, indicating a positive effect of tempo on the  $\text{CO}_2$  production. On the other hand, colonies with larger workers fell predominately below the mean regression line, indicating a negative effect of body size on  $\text{CO}_2$  production. This was further tested by removing the effect of colony live weight from the  $\text{CO}_2$  production by using the residuals from this regression.

The log live worker weight (mg) was negatively related to the adjusted  $\text{CO}_2$  production rate (Fig. 3 and Table 2, regression 2), as expected from the commonly reported effect of body size on respiration. A ten-fold increase in mean worker live weight was associated with a 40% decrease in  $\text{CO}_2$  production rate. Considered another way, for the same colony live weight, a colony composed of 10 mg workers respire 40% less than an equal weight colony composed of 1 mg workers. In colonies respiring more than 1 SD above the mean, workers weighed an average of 0.95 mg. Between the mean and +1 SD, workers weighed 2.65 mg; at the mean to  $-1$  SD, they weighed 3.26 mg; and, at less than  $-1$  SD, they weighed 3.9 mg. Nevertheless, within each of these worker sizes, colony  $\text{CO}_2$  production was a linear function of colony weight.

$\text{CO}_2$  production rate was positively related to tempo (Fig. 4 and Table 2, regression 3), such that an increase in tempo of 1 unit was associated with a 1.2-fold increase in adjusted  $\text{CO}_2$  production. For example, for two colonies of the same live weight, the one with a 5 unit greater tempo will respire almost three times as much.

High tempo might increase  $\text{CO}_2$  production because smaller ants have higher respiration rates per gramme of body weight and higher tempos. The effect of tempo on  $\text{CO}_2$  production independent of the effect of worker live weights was tested by using the residuals of regressions of  $\text{CO}_2$  production on worker weight and tempo on worker weight. Size free tempo was positively related to size-free rate of  $\text{CO}_2$  production (Fig. 5 and Table 2, regression 4;  $r^2 = 28\%$ ).

In polymorphic ant species (e.g. *S. invicta* and *Pheidole dentata*), respiration rate of larger workers is lower than that of smaller ones (Calabi & Porter, 1989; Shik, 2010). This negative



**Fig. 2.** The production rate of CO<sub>2</sub> (µL h<sup>-1</sup>) by whole colonies in relation to live colony weight (g). CO<sub>2</sub> production rate is exactly isometric with colony live weight. Each point represents a colony of one of the species, such that species may be represented multiple times. Species with higher tempos tended to fall above the mean regression line and those with higher worker body weights below. (a) symbols coded for tempo, (b) symbols coded for worker size.

effect of worker body size can be seen in Fig. 3 in which a ten-fold increase in individual worker live weight was associated with an approximately 40% decrease in worker respiration rate (µL CO<sub>2</sub> mg<sup>-1</sup> h<sup>-1</sup>) (Table 2, regression 2). The crucial question for the present study is whether tempo contributed to worker respiration rate independent of its association with worker size. Because both tempo and worker respiration are related to worker size, adjusting CO<sub>2</sub> production for both colony and worker size allowed a size-free test of this question (tempo is already size-free). The size-free plot in Fig. 5 shows that higher tempo bears a cost in CO<sub>2</sub> production rate independently of its association with worker size (Table 2, regression 4). A

1-unit increase in tempo was associated with a 1.2-fold increase in size-free worker respiration rate ( $r^2 = 28\%$ ). Thus Fig. 5 illustrates the partial correlation of tempo and respiration (i.e. the unique effect of tempo on respiration). Ants that have evolved a tempo higher or lower than the average expected from their body size also evolved higher or lower than average worker respiration rate.

The accounting of the effects of colony live weight, worker live weight and tempo on CO<sub>2</sub> production can be visualized by tracking the sums of squares associated with each step of the analysis above (Fig. 6). Removal of the effects of colony live weight reduced the sums of squares from 20.5 to 4.69 and the

**Table 2.** Regressions of the predictor variables against the CO<sub>2</sub> production per hour by whole colonies.

| Regression | x-variable(s)<br>(logs)                                   | y-variable<br>(logs)   | Parameter | <i>b</i>      | SE           | <i>t</i> <sub>51</sub> | <i>P</i>        | <i>F</i> <sub>1,54</sub> | Total<br>SS | Regression<br>SS | Error<br>SS | <i>r</i> <sup>2</sup> |
|------------|---|--|-----------|---------------|--------------|------------------------|-----------------|--------------------------|-------------|------------------|-------------|-----------------------|
| <b>1</b>   | Colony live weight (g)                                    | $\mu\text{L CO}_2 \text{ h}^{-1}$  | Intercept | <b>2.694</b>  | <b>0.045</b> | <b>60.41</b>           | <b>0.00000</b>  | 183                      | 20.54       | 15.85            | 4.69        | 77                    |
|            |   |  | Slope     | <b>0.996</b>  | <b>0.074</b> | <b>13.51</b>           | <b>0.00000</b>  |                          |             |                  |             |                       |
| <b>2</b>   | Worker live weight<br>(mg)                                | $\mu\text{L CO}_2 \text{ h}^{-1}$ , adjusted<br>for colony live<br>weight            | Intercept | 0.044         | 0.038        | 1.15                   | 0.254           | 12.0                     | 4.69        | 0.85             | 3.84        | 17                    |
|            |   |  | Slope     | <b>-0.244</b> | <b>0.071</b> | <b>-3.46</b>           | <b>0.001</b>    |                          |             |                  |             |                       |
| <b>3</b>   | Tempo   | $\mu\text{L CO}_2 \text{ h}^{-1}$ adjusted<br>for colony live<br>weight              | Intercept | -0.066        | 0.038        | -1.74                  | 0.0879          | 16.7                     | 4.69        | 1.11             | 3.58        | 22%                   |
|            |   |  | Slope     | <b>0.090</b>  | <b>0.022</b> | <b>4.08</b>            | <b>0.0001</b>   |                          |             |                  |             |                       |
| <b>4</b>   | Tempo   | $\mu\text{L CO}_2 \text{ h}^{-1}$ , adjusted<br>for colony and<br>worker live weight | Intercept | -0.065        | 0.033        | -1.94                  | 0.05718         | 20.9                     | 3.84        | 1.10             | 2.77        | 27%                   |
|            |   |  | Slope     | <b>0.088</b>  | <b>0.019</b> | <b>4.57</b>            | <b>0.00003</b>  |                          |             |                  |             |                       |
| <b>5</b>   | Colony dry weight (g)                                     | $\mu\text{L CO}_2 \text{ h}^{-1}$  | Intercept | <b>3.108</b>  | <b>0.046</b> | <b>67.33</b>           | <b>0.00000</b>  | 130                      | 20.54       | 14.5             | 6.04        | 70%                   |
|            |   |  | Slope     | <b>0.982</b>  | <b>0.086</b> | <b>11.38</b>           | <b>0.00000</b>  |                          |             |                  |             |                       |
| <b>6</b>   | Colony lean weight, g                                     | $\mu\text{L CO}_2 \text{ h}^{-1}$  | Intercept | <b>3.269</b>  | <b>0.053</b> | <b>61.99</b>           | <b>0.00000</b>  | 122                      | 20.54       | 14.2             | 6.30        | 69%                   |
|            |   |  | Slope     | <b>0.967</b>  | <b>0.087</b> | <b>11.05</b>           | <b>0.00000</b>  |                          |             |                  |             |                       |
| <b>7</b>   | Colony metabolic<br>weight (g)                            | $\mu\text{L CO}_2 \text{ h}^{-1}$  | Intercept | <b>3.311</b>  | <b>0.054</b> | <b>61.50</b>           | <b>0.00000</b>  | 128                      | 20.54       | 14.4             | 6.11        | 70%                   |
|            |   |  | Slope     | <b>0.980</b>  | <b>0.087</b> | <b>11.29</b>           | <b>0.00000</b>  |                          |             |                  |             |                       |
| <b>8</b>   | Species means, colony<br>live wt. (g)                     | $\mu\text{L CO}_2 \text{ h}^{-1}$  | Intercept | <b>2.792</b>  | <b>0.075</b> | <b>36.98</b>           | <b>0.00000</b>  | 31.1                     | 2.52        | 1.78             | 0.74        | 71%                   |
|            |   |  | Slope     | <b>0.865</b>  | <b>0.155</b> | <b>5.58</b>            | <b>0.00009</b>  |                          |             |                  |             |                       |
| <b>9</b>   | Change in activity $\times$<br>change in running<br>speed | % change in<br>$\mu\text{L CO}_2 \text{ g}^{-1} \text{ h}^{-1}$                      | Intercept | <b>0.121</b>  | <b>0.034</b> | <b>3.577</b>           | <b>0.001</b>    | 6.31 (1, 26)             | 0.70        | 0.14             | 0.56        | 20%                   |
|            |   |  | Slope     | <b>0.014</b>  | <b>0.006</b> | <b>2.511</b>           | <b>0.019</b>    |                          |             |                  |             |                       |
| <b>10</b>  | Colony live weight (g)                                    | Disturbed $\mu\text{L CO}_2 \text{ h}^{-1}$  | Intercept | <b>5.82</b>   | <b>0.051</b> | <b>113.7</b>           | <b>0.00001</b>  | 145 (1, 40)              | 15.93       | 12.49            | 3.44        | 78%                   |
|            |   |  | Slope     | <b>1.02</b>   | <b>0.084</b> | <b>12.0</b>            | <b>0.000001</b> |                          |             |                  |             |                       |
| <b>11</b>  | Mean worker live<br>weight (mg)                           | Disturbed<br>$\mu\text{L CO}_2 \text{ g}^{-1} \text{ h}^{-1}$                        | Intercept | 0.035         | 0.041        | 0.86                   | 0.3966          | 11.2 (1, 40)             | 3.44        | 0.75             | 2.69        | 20%                   |
|            |   |  | Slope     | <b>-0.261</b> | <b>0.078</b> | <b>-3.34</b>           | <b>0.0018</b>   |                          |             |                  |             |                       |

Numbers in bold italics are statistically significant.

Predictors included the logs of colony live, dry, lean and metabolic weight; individual worker mean live, dry, lean and metabolic weights; tempo and mean activity. Activity was used only in regression 1.

further removal of the effects of worker live weight reduced it to 3.84. Finally, removal of the effects of tempo reduced the CO<sub>2</sub> production sums of squares to 2.77. Together, these three variables thus accounted for 87% of the total variance in CO<sub>2</sub> production.

Because the literature reports often use dry weight and/or lean weight, production rate of CO<sub>2</sub> was tested against these measures of colony weight. The relationship of CO<sub>2</sub> production rate ( $\mu\text{L h}^{-1}$ ) to colony dry, lean and metabolic weight was almost identical to that using colony live weight (Table 2, regressions 5–7). Species means for these measures can be found in the Supporting information (Appendix S2).

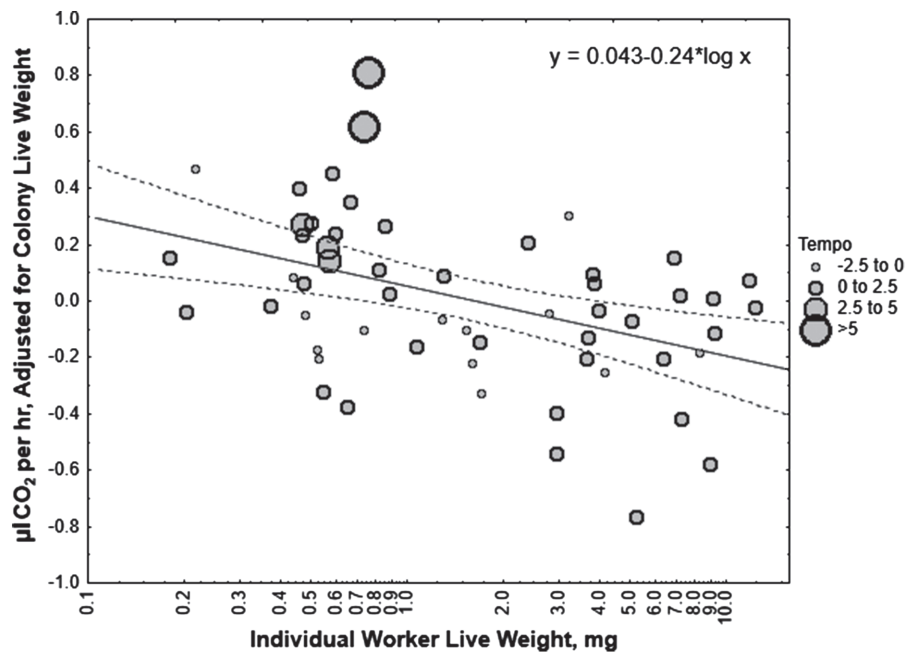
Activity was low in all colonies, 80% (45 of 56) showing less than 10% of workers active, and 59% (33 of 56) with 5% or less. The mean percentage of active workers ranged from 1% for *P. longicornis* to 15% for *P. gracilis*. When activity was regressed against the final residuals noted above, it explained no additional variance, and this was also the case when it was entered into a multiple regression against CO<sub>2</sub> production, either alone or with other variables. Activity was deleted from further analysis.

When live brood weight (or percentage brood) was regressed against the final residuals noted above, it explained no additional variance. Similarly, inclusion of log brood live weight in

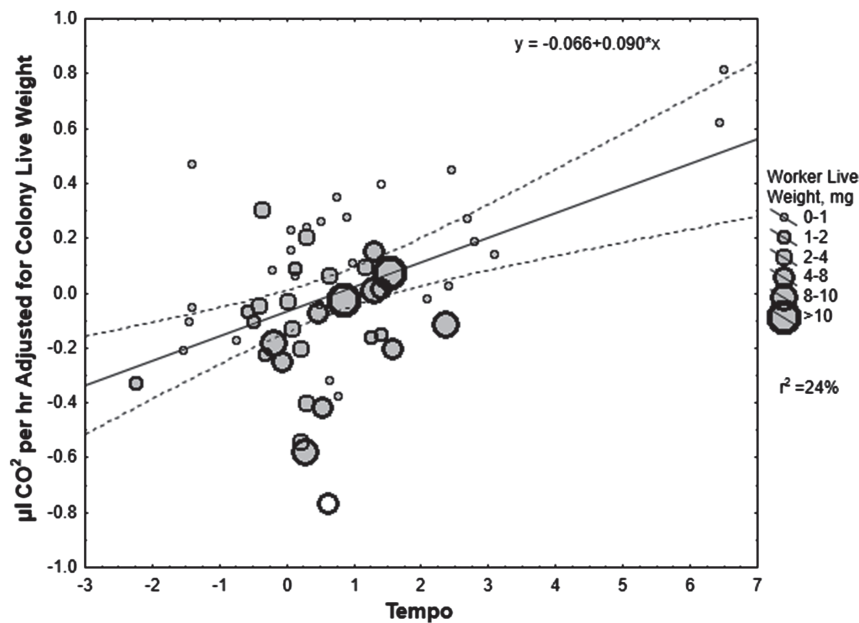
the regression of log colony CO<sub>2</sub> production versus log whole colony live weight did not explain additional variation (partial correlations,  $P > 0.1$ ). This was probably because, despite our attempts to standardize brood, there was considerable variation, and the live weight of brood was isometric with the total live weight of workers (log–log slope = 0.91; 95% confidence interval = 0.17). Brood was therefore deleted from further analysis.

There was considerable variation of the dependent and independent measures within species (see Supporting information, Appendix S2), suggesting the use of species means (logged), instead of colony means, to weight all species equally and remove sample-size bias. The use of species means reduced the sample size from 54 colonies to 15 species and decreased the predictive power compared with the colony means analysis. However, this did not change the relationships: log CO<sub>2</sub> output per hour was linearly related to log colony live weight (i.e. slope not significantly different from 1.0) (Fig. 7) and mean tempo and mean worker live weight had similarly significant effects on CO<sub>2</sub> production. The positive effect of tempo on CO<sub>2</sub> production shown in Fig. 4 can be seen in Fig. 7 as the tendency of species with higher tempos to lie above the regression line (and those with lower tempo below). Using species or colony means in these analyses yielded similar results and so colony means were used to increase statistical power in our analyses.





**Fig. 3.** The effect of worker body size (live weight) on worker respiration rate ( $\mu\text{L g}^{-1} \text{h}^{-1}$ ) was significantly negative: a ten-fold increase in live weight reduced respiration rate by approximately 40%. High tempo ants were predominately smaller than low tempo ones.



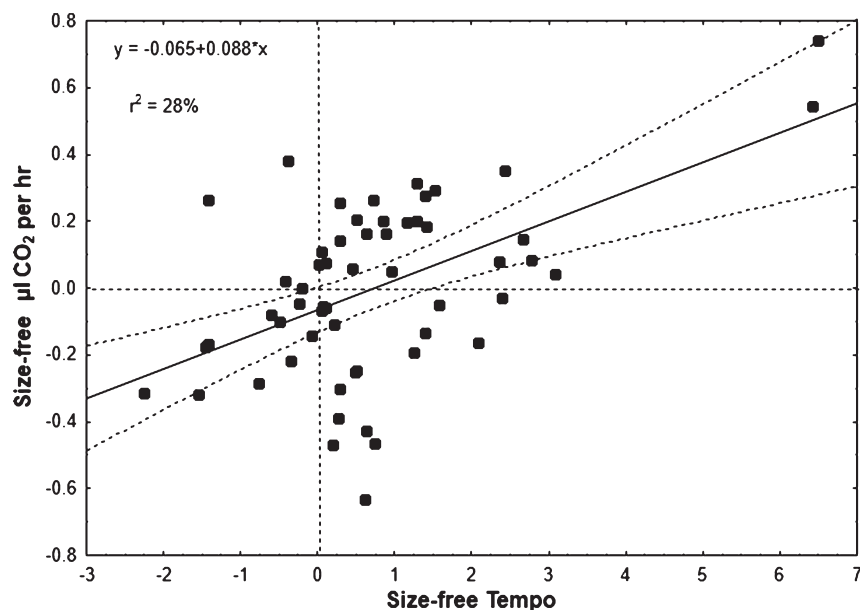
**Fig. 4.** As worker tempo increases, respiration of whole colonies increases beyond the increase associated with colony weight. This increased  $\text{CO}_2$  production is greater for colonies with smaller workers than larger ones, and their values fall (respectively) above and below the average regression.

#### Weight-specific respiration

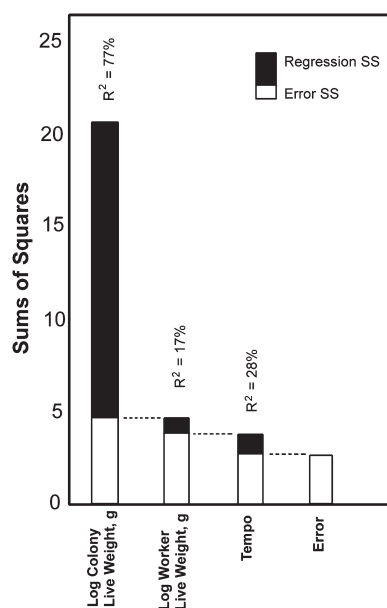
The weight-specific respiration rates for whole colonies ranged from 85 to 3244  $\mu\text{L CO}_2 \text{ g}^{-1} \text{h}^{-1}$  (based on live weight). Table 3 shows the means and range for specific respiration rates based on whole colony live, dry, lean and metabolic weights. These rates compare reasonably with the weight-specific

respiration rates reported in the literature (Shik, 2010; Waters *et al.*, 2010; Shik *et al.*, 2014).

Partial correlations were used to describe the effect of an independent variable (tempo for example) on respiration with the effects of body size, worker specific respiration rate, colony live weight and percentage brood removed. Partial correlation coefficients showed that whole colony live weight explained 78%



**Fig. 5.** Size-free worker respiration rate (derived by adjusting for the effect of colony live weight and worker body size) plotted against tempo (which is also size-free) yields the size-free relationship shown here. Tempo has a positive effect on worker respiration rate, independent of the effect of body size on both.



**Fig. 6.** Variance of  $\mu\text{L CO}_2 \text{ h}^{-1}$  associated with whole colony live weight, worker live weight and tempo. Of the unexplained variance left over after adjusting for whole colony weight, 17% is associated with worker weight and, of that left-over variance, 28% is associated with tempo, leaving only 13% of the variance in  $\text{CO}_2$  production unexplained. No other tested factors explained additional variance.

of the variance in whole colony  $\text{CO}_2$  production that remained after removing the effects of all the other variables (partial correlation coefficient  $-0.48$ ). Similarly, tempo (coefficient  $0.34$ ) explained 33% of the remaining variance, and worker specific

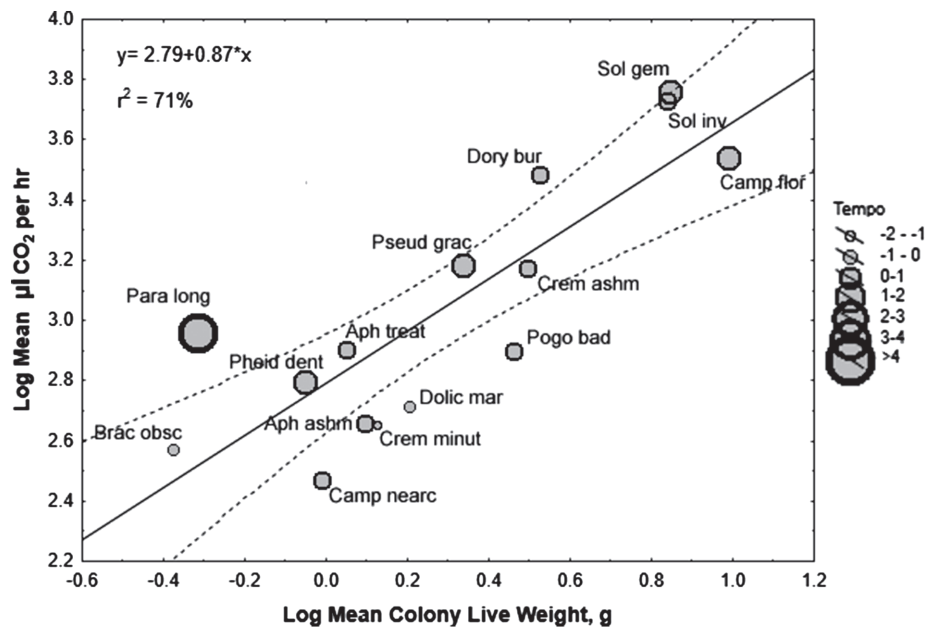
**Table 3.** Comparison of mean and ranges of weight-specific whole-colony respiration rates based on live, dry, lean and metabolic weights.

| Whole colony specific respiration, $\mu\text{L CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ | Mean | Minimum | Maximum |
|---|------|---------|---------|
| Based on live weight  | 621  | 85.1    | 3244    |
| Based on dry weight   | 1653 | 302     | 8831    |
| Based on lean weight  | 2482 | 360     | 14 997  |
| Based on metabolic weight   | 2765 | 397     | 16 789  |

respiration (coefficient  $0.82$ ) explained 81% of the remaining variance (Table 4). Thus, each of these variables uniquely explained part of the variance in whole colony  $\text{CO}_2$  production rate. Other factors entered into the regression included percentage fat, percentage brood and activity (all log or square root transformed), although none had a significant effect on colony respiration in any combination. Individual worker live weight had an effect only when tempo was removed from the regression. The overall outcome of these additive and opposing relationships is that whole live colony  $\text{CO}_2$  production increased in exact proportion to whole colony live weight (slope  $1.00$ ). The most basic biological characteristic underlying these relationships is worker size (weight) because both tempo and metabolic rate decrease with increasing worker size.

#### Disturbance

Disturbance caused a large increase in running speed in most species (Fig. 8a). Increases ranged from approximately  $10 \text{ mm s}^{-1}$  in *C. minutissima* to between  $80$  and  $90 \text{ mm s}^{-1}$  for *C. floridanus* and *P. longicornis*. Thus, for all species,



**Fig. 7.** The relationship of the species means of  $\text{CO}_2$  production rates to live colony weight. The log–log slope of 0.87 is not significantly different from 1.0, and the relationship is similar to that using colony means in Fig. 2. High tempo species tended to fall above the mean regression line and low tempo below, as in Fig. 2.

undisturbed running speed was well below the maximum. Mean percentage increase in activity (Fig. 8a) was the highest for *P. longicornis* (553%). The lowest mean increase was observed in *P. dentata* (24%). Three species, *C. ashmeadi*, *C. minutissima* and *C. nearcticus* showed mean decreased activity in response to disturbance. This may be the effect of the behavioural response of these species to remain motionless when their nests are disturbed as can be seen in the field (K. S. Mason, unpublished observations).

Disturbance did not change the relationship of  $\mu\text{L CO}_2 \text{ h}^{-1}$  to colony live weight (Table 2, regression 10). In both disturbed and undisturbed colonies,  $\mu\text{L CO}_2 \text{ h}^{-1}$  was isometric with colony weight (i.e. log–log slope = 1.0). Moreover, after adjusting for colony weight by taking the residuals from this regression, the effect of worker body size (logged) on respiration was similar for disturbed (Table 2, regression 11) and undisturbed colonies (Table 2, regression 2) (slopes =  $-0.26$  versus  $-0.24$ , respectively). These relationships held despite the significant increase in running speed and activity that resulted from disturbance.

By themselves, neither the change in activity upon disturbance, nor the change in running speed were significantly related to the percentage change in  $\mu\text{L CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ . However, the product of these two changes combined the effects of both and was positively related to the change in respiration (Fig. 8b; Table 2, regression 9;  $r^2 = 20\%$ ). The mean percentage increase in live worker specific respiration rate ( $\mu\text{L mg}^{-1} \text{ h}^{-1}$ ) was largest and most variable for *C. floridanus* ( $63 \pm 52.6\%$ ). The smallest increase was recorded for *C. ashmeadi* ( $0.18 \pm 17.3\%$ ) and was not significantly different from 0. Figure 8(b) suggests that the increase in worker respiration rate during disturbance greatly outweighed any effect of brood respiration. Moreover, it is likely that the observed relationship among tempo, activity

and respiration is consistent throughout all aspects of ant life such as foraging, defending territories or nests, etc.

The role of activity in these analyses was difficult to decipher, perhaps because activity was usually low: the significance of activity's effect came and went depending on other predictors in the regression. This inconsistency contrasted with the mostly consistent effects of tempo, colony weight and worker body weight.

## Discussion

By contrast to many reports on individual ant respiration and related variables, the present study is based on collection and analysis of whole colonies (or large fragments) in which all demographic groups and stages are represented for 15 species of ants. Live weights are determined from the actual specimens used in each experiment, not from literature reports, museum specimens or small worker groups (Gallé, 1978; Bartholomew *et al.*, 1988; Lighton & Bartholomew, 1988; Shik, 2008; Hou *et al.*, 2010). All colonies are collected in the field and produce brood in the laboratory, indicating they are healthy and have an ovipositing queen and therefore approximate colonies in nature. Collecting physiological and behavioural data in the laboratory from a range of species under controlled conditions allows poignant questions to be asked and answered about superorganismal function and evolution.

### Interspecific patterns at the colony-level

At the whole colony level and across species, size-dependent worker attributes combine in such a way that whole colony

**Table 4.** Partial correlation parameters for multiple regressions of log  $\mu\text{L CO}_2 \text{ h}^{-1}$  against log colony live weight, tempo, activity, log worker live weight and log brood live weight.

| Regression summary: log corrected col $\mu\text{L CO}_2$ production $\text{h}^{-1}$ . Adjusted $r^2 = 0.84$ ; $F_{5,49} = 59.01$ , $n = 55$ . |                 |                     |                      |           |            |          |          |
|---|-----------------|---------------------|----------------------|-----------|------------|----------|----------|
|   | $b$             | SE of $b$           | $t_{49}$             | $P$       |            |          |          |
| Intercept   | 2.77            | 0.140               | 19.8                 | 0.000001  |            |          |          |
| Log live colony weight (g)  | 0.95            | 0.153               | 6.19                 | 0.000001  |            |          |          |
| Tempo   | 0.087           | 0.022               | 3.96                 | 0.0003    |            |          |          |
| Log worker live weight (mg)   | −0.215          | 0.066               | −3.25                | 0.002     |            |          |          |
| Log brood live weight (g)   | 0.045           | 0.1356              | 0.33                 | 0.745     |            |          |          |
| Square root of activity   | −0.293          | 0.359               | −0.815               | 0.419     |            |          |          |
| Partial correlations: log corrected col $\mu\text{L CO}_2$ production $\text{h}^{-1}$ .   |                 |                     |                      |           |            |          |          |
|   | $b^*$ in        | Partial correlation | Semipart correlation | Tolerance | $r^2$      | $t_{49}$ | $P$      |
| Log live colony weight (g)  | 0.852           | 0.662               | 0.334                | 0.153     | 0.847      | 6.19     | 0.000001 |
| Tempo   | 0.236           | 0.493               | 0.214                | 0.817     | 0.183      | 3.96     | 0.0003   |
| Log worker live weight (mg)   | −0.187          | −0.421              | −0.175               | 0.877     | 0.124      | −3.25    | 0.003    |
| Log brood live weight (g)   | 0.045           | 0.047               | 0.018                | 0.154     | 0.846      | 0.328    | 0.745    |
| Square root of activity   | −0.047          | −0.116              | −0.044               | 0.865     | 0.135      | −0.815   | 0.41     |
| Analysis of variance: log corrected col $\mu\text{L CO}_2$ production $\text{h}^{-1}$ .   |                 |                     |                      |           |            |          |          |
|   | Sums of squares | d.f.                | Mean squares         | $F$       | $P$ -value |          |          |
| Regression  | 16.1            | 5                   | 3.20                 | 59.01     | 0.000001   |          |          |
| Residual  | 2.66            | 49                  | 0.054                |           |            |          |          |
| Total   | 18.7            |                     |                      |           |            |          |          |

Significant effects are shown in bold.

respiration increases in exact proportion to colony weight (live, dry, lean or metabolic). The species sampled are samples of convenience, although they represent a broad range of habits, subfamilies, colony sizes and lifestyles, making it likely that increasing the species sample would not substantially change the outcome.

Metabolic rates of unitary species scale to weight with an exponent of 0.75 and, as is reported by Lighton (1989), so do the rates of individual worker ants in the present study. However, in contrast to the findings of Hou *et al.* (2010) who claim that whole colony metabolism scales to weight with an exponent of 0.75, it is shown in the present study that the respiration of whole colonies of ants scales to their live weight with a consistent exponent of 1.0, irrespective of the size of the individual ants making up those colonies. Again, in contrast to Hou *et al.* (2010), the present findings suggest that the per-gramme operating cost of a whole colony does not strictly decrease as colony weight increases but decreases when that weight is composed of larger workers. Similarly, per-gramme operating costs of large colonies increase when that weight is composed of smaller workers. There is thus an energetic advantage to evolving larger worker size, as proposed by King (2010), who shows that ant colonies composed of many large workers dominate the ecosystems investigated. This has implications for energy flow at the ecological scale, and possibly for the factors favouring the evolution of high tempo in social organisms.

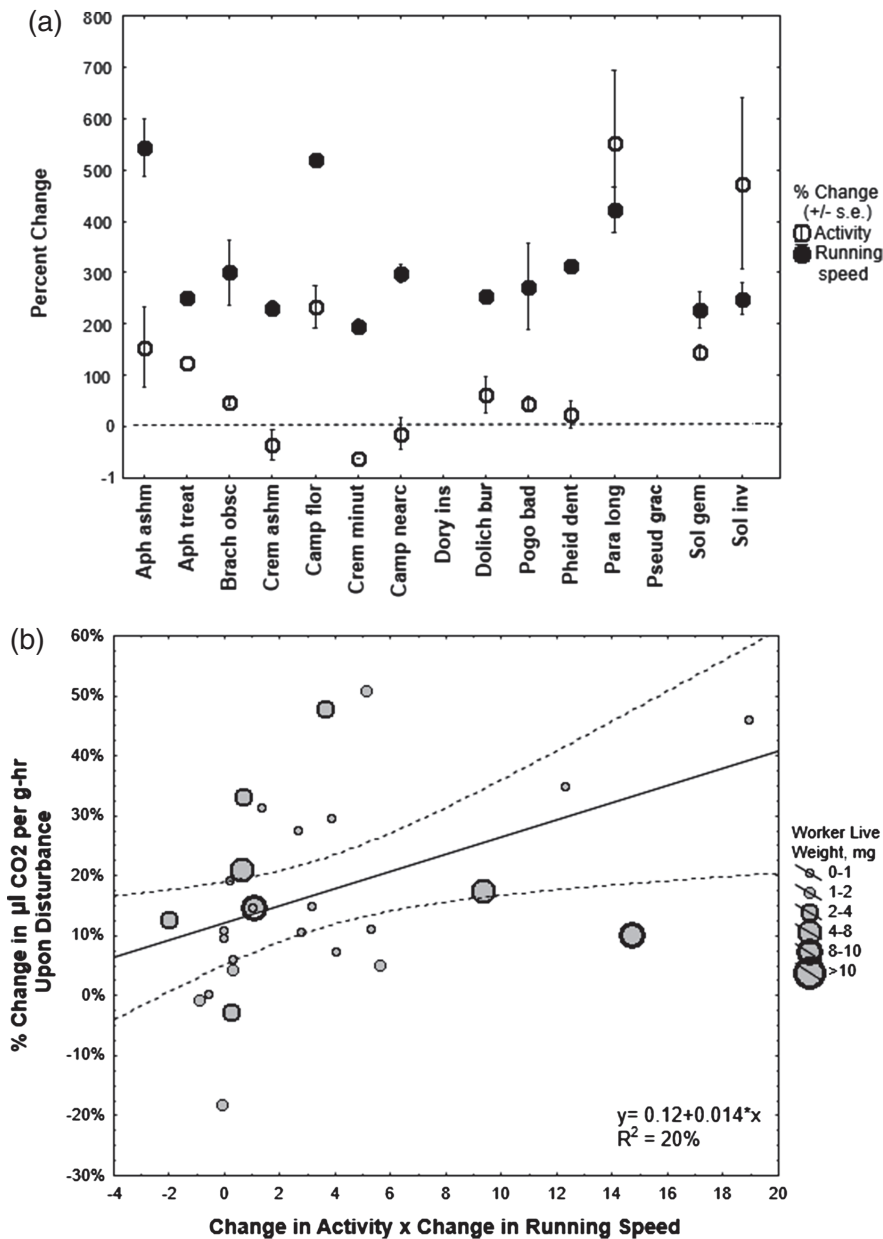
In addition to the obvious and dominant effect of colony weight, tempo is a significant, consistent and positive predictor

of respiration rate, regardless of whether the data are analyzed by colony or by species, or whether colonies are disturbed or undisturbed. Tempo decreases with increasing worker size but, for similar body size, high tempo ants have higher respiration rates than low tempo ants. Worker per-milligramme respiration rate increases more slowly than tempo (log–log slope 0.77), suggesting that the respiratory costs of an increase in tempo are lower for high tempo ants than those with low tempo. This means that high tempo species may be able to evolve still higher tempos because of this disproportionately lower respiratory cost than for low tempo species.

The present study also indicates that there is a metabolic cost for operating at high tempo, independent of the cost of being small, even if this cost decreases relatively as tempo increases. It follows that the decline of specific respiration rate with body size may in part be the result of the associated decreasing tempo and not solely a result of the limitations between surface area and volume as proposed by Kleiber (1961).

#### Intraspecific variation

Strong patterns are observed in how different factors affect respiration across species, despite considerable within-species variation in all measures (respiration, tempo, activity, percentage brood, and colony and worker weight). This may reflect colony-level idiosyncracies similar to that for other important colony-level traits (Tschinkel, 2006; Waters *et al.*, 2010). In *P. dentata* and *Myrmica punctiventris*, foraging tempo increases



**Fig. 8.** The effect of disturbance. (a) Percentage increase in activity and running speed by species. (b) Percentage increase in respiration in relation to the product of change in activity times running speed. Key to species: *Aphaenogaster ashmeadi*; *Aphaenogaster treatae*; *Brachymyrmex obscurior*; *Camponotus floridanus*; *Camponotus nearcticus*; *Crematogaster ashmeadi*; *Crematogaster minutissima*; *Dolichoderus mariae*; *Paratrechina longicornis*; *Pheidole dentata*; *Pogonomyrmex badius*; *Solenopsis geminata*; *Solenopsis invicta*. *Pseudomyrmex gracilis* and *Dorymyrmex bureni* are not shown because they were not disturbed during experimentation.

with increasing group size (Burkhardt, 1998) and running speed ( $\text{mm s}^{-1}$ ) of searching ants is faster in large colonies than small colonies (Herbers & Choiniere, 1996). For polymorphic species such as *S. invicta*, *S. geminata* and *C. floridanus*, mean worker size increases greatly with (natural) colony size (Tschinkel, 1988) and the changing distribution of worker size during colony growth and the seasonal cycle is likely to affect colony energetics. The present study is unable to address this question because the worker size distribution in the experimental nests

simply reflects the worker size distribution of the source colonies (of unknown age).

For species with the most replication, there is no detectable seasonal effect, although such effects cannot be tested in the full data set. Overall, there appears to be considerable colony-level flexibility in tempo, perhaps because of changing labour needs of a colony over the annual brood-rearing cycle and during colony growth (Tschinkel, 2010; Holbrook *et al.*, 2011; Kwapich & Tschinkel, 2013).



If brood is separated from tending workers, the respiration metabolism of brood might be reduced (Martin, 1991), although it is also suggested that isolated brood show increased respiration (Takahashi-Del-Bianco *et al.*, 1998). However, the results of the present study show colony composition (percentage or live weight brood) has no effect on whole colony respiration or on whole colony per-gramme respiration. Given the lack of sensitivity of whole-colony respiration to percentage brood, it is likely that brood respire much less than do workers. Larvae, however, may contribute to colony respiration indirectly because workers increase foraging or tending activity when brood are present (Kwapich & Tschinkel, 2013).

A direct comparison of the respiration rates of the present study with previously published rates is difficult because only two of the species in the present study have been measured previously, and these studies report  $O_2$  consumption. However, the respiratory quotient (RQ) (i.e. the ratio of  $CO_2$  produced to  $O_2$  consumed) ranges from 1.0 for a carbohydrate diet to 0.7 for a diet composed of fats (Knoebel, 1971). Although the present study measures only  $CO_2$  production, colonies are fed a mixed diet and so RQ is likely very close to 1.0. This allows a comparison with studies that measure  $O_2$  consumption. Published measurements of respiration in the fire ant *S. invicta* generally range from 1000 to 2000  $\mu L O_2 g^{-1}$  (dry weight)  $h^{-1}$  at 30 °C (Porter & Tschinkel, 1985; Elzen, 1986; Calabi & Porter, 1989). A comparable mean specific respiration rate (using dry weight) is found in the present study for *S. invicta* ( $1980 \pm 98 \mu L CO_2 g^{-1} h^{-1}$ ). Porter (1986) reports respiration in *P. badius* to be approximately 800–1250  $\mu L O_2 g^{-1}$  (dry weight)  $h^{-1}$  at 30 °C. Again, a comparable rate is found in the present study for *P. badius*,  $821 \pm 508 \mu L CO_2 g^{-1}$  (dry weight)  $h^{-1}$ . Such agreement suggests that the methods used in the present study are comparable with those used in other investigations.

The relationship between phylogeny and tempo is not specifically tested, although inspection of Fig. 1 suggests that, if present, the effects are weak, and that body size is a more important predictor. Of the four subfamilies sampled, both Myrmicinae and Formicinae include species that range in size from the smallest to the largest in the study, and are grouped with small and large species from other subfamilies, respectively. Within each of the four genera represented by two or more species, genera cluster together on the same graph, although this similarity is also correlated with body size (Fig. 1), with no genus falling outside of the normal range for its cross-subfamily group. Therefore, it is unlikely that more power would be gained from adding phylogeny to our analysis.

### The ecology of tempo

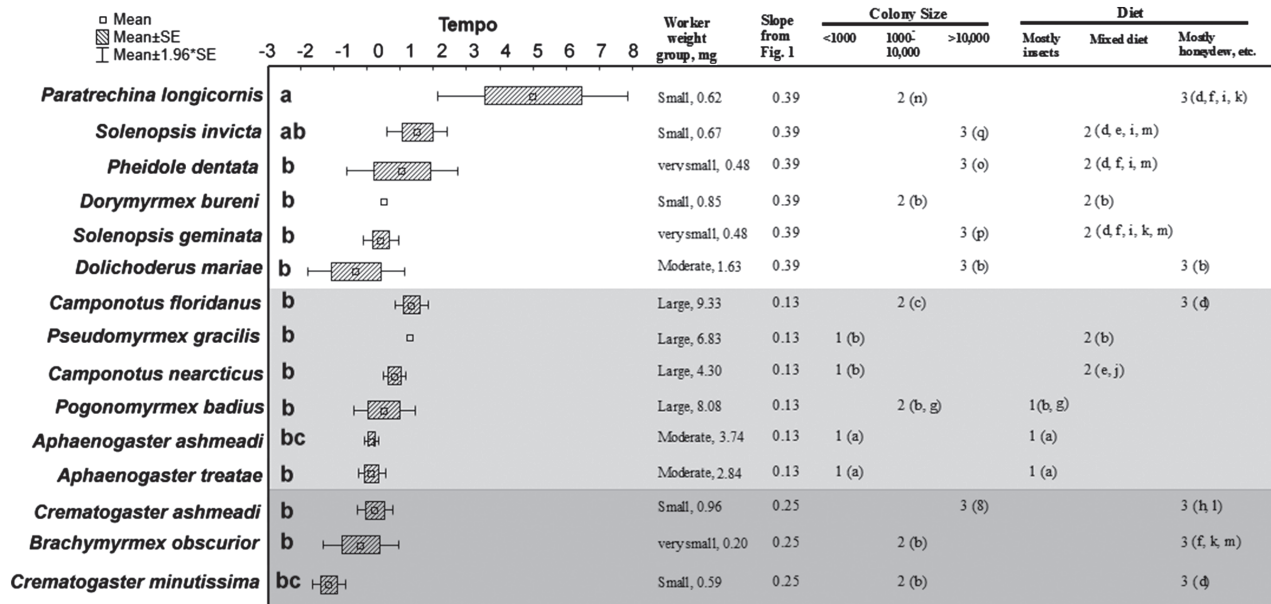
Tempo is a measure of relative running speed, although absolute speed may be more critical because larger ants running at a higher absolute speed may find resources faster than smaller ants. However, in ant communities, body size and foraged items are positively related and this leads to direct competition primarily between species of similar body size (Bernstein & Bernstein, 1969; Hansen, 1978; Bernstein, 1979; Fellers, 1987). Tempo may be important in structuring ant

communities, especially in species rich, highly competitive ant communities.

Oster & Wilson (1978) expect a suite of characters to be associated with high tempo: large colony size, well-defined castes (including polymorphic workers) and dependence on unpredictable or varied resources. By contrast, they expect low tempo ants to have small colonies, little caste differentiation and to use predictable or specialized resources. In Fig. 9, Oster & Wilson's (1978) predictions are compared with the tempo, ecological characters and colony attributes of the species from the present study as described in the literature. A basic problem in making these comparisons is that Oster & Wilson (1978) provide only a qualitative, unit-less definition of tempo, and the current size-free measure used in the present study may differ from their concept. In Fig. 9, the species are arranged primarily by the three different slopes in Fig. 1 and secondarily by mean tempo rank. The group whose tempo increases most rapidly with running speed (group 1) are predominately very small to small ants (one mid-sized), whereas the group with the lower slope (group 2) consists of mid-sized to large ants. The slow-running group (group 3) has an intermediate slope and contains very small to small ants. The rankings by mean tempo alone are not highly consistent. In modest agreement with the predictions of Oster & Wilson (1978), for the relationships between tempo and colony and worker sizes, four of the six species in group 1 (high tempo) have large colonies and two have mid-sized colonies. *Paratrechina longicornis*, the highest tempo species, has mid-sized colonies, as does the slower *D. bureni*. In the low tempo group 2, two of the six species have small to mid-sized colonies, and mid-sized to large workers. The three low tempo group 3 colonies have medium to large colonies, and two of the three have low mean tempo, and all have small workers.

Oster & Wilson (1978) contend that high tempo species often show physical castes (worker polymorphism), although the present study finds no association of polymorphism with high tempo. Polymorphism occurs in *S. geminata*, *S. invicta*, *P. dentata*, *C. floridanus* and *P. badius*, although the first three are in the higher tempo group 1, whereas the last two are in the lower tempo group 2 (Wheeler, 1910; Van Pelt, 1947). Moreover, the workers of the highest tempo species are not polymorphic (*P. longicornis*).

Oster & Wilson (1978) also propose that high tempo should be associated with feeding generalists, and low tempo with specialized feeders on predictable sources such as honeydew. By contrast, Davidson & Patrell-Kim (1996) note an association of 'homopteran tending' with high tempo, and suggest a nutrient balance hypothesis in which species that use predictable carbohydrate sources (sugars) obtain excess energy with which they fuel high tempo movement, high activity levels, alertness and aggressiveness. Tests of these predictions are obscured by the tendency of most ants to feed opportunistically as generalists (Fig. 9) (Van Pelt, 1950; Sudd, 1982). Species in the present study that tend homopterans range from high to low in tempo (e.g. *P. longicornis* and *C. floridanus*, respectively). Homopteran tending is common in many ants, although it does not appear to be associated with particular tempos in the present study.



**Fig. 9.** Tempo and ecological characteristics of species in the present study. Species are grouped according to their slopes in Fig. 1, and ranked by tempo within each slope group. Worker size is for individual workers, with mean worker weight in milligram. Colony size is the estimated mature colony size in number of workers. Letters indicate statistically different mean tempos, although this varied somewhat with different post-hoc tests. Diet reflects the proportion of homopteran honeydew and/or nectar in the diet: 1 = diet is mostly insect carrion and/or prey; 2 = mixed diet of insects and nectar/honeydew; 3 = diet consists largely of honeydew and/or nectar. Sources are given in parentheses: (a) Carroll (1975); (b) KSM, personal observation; (c) Schneirla (1944); (d) Nielsson *et al.* (1971); (e) McLain (1983); (f) Larsen *et al.* (1991); (g) W. R. Tschinkel (personal observation); (h) Leuthold (1968); (i) Whitcomb *et al.* (1972); (j) Beckmann, and Stucky (1981); (k) Sirjusingh *et al.* (1992); (l) Koptur (1992); (m) Richman *et al.* (1983); (n) Passera (1994); (o) Wilson (1976); (p) McInnis (1994); (q) Tschinkel (1993).

#### The ecology of worker body size

By contrast to unitary organisms, social insects possess two interdependent measures of body size: the workers and the colony. Therefore, at the ecosystem level, with respect to ants, the pattern of biomass and energy-use among the ecosystem's components must consider both worker and colony size. Damuth (1981) proposes that, because of the opposite effects of body size on metabolic rate and population density, population energy use should be independent of body size (the 'energetic equivalence rule'), whereas King (2010) shows that, in five Florida ecosystems, and in contrast to the expectation of Damuth (1981), the relative population energy use increases with the size of the workers, as does the population biomass.

The findings of the present study suggest that, for ants, population energetic use should increase somewhat more slowly than biomass because the per-gramme energy use of larger workers is lower. Therefore, the present study provides compelling evidence that population-level studies of ants must take into account not only colony size and density, but also worker size as well.

#### Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/phen.12099

**Appendix S1.** If the relationship between running speed ( $y$ ) and body length ( $x$ ) does not pass through the origin (i.e.  $y=0$  when  $x=0$ ), the tempo computed by simply dividing running speed by body length is increasingly biased as body length decreases. The example shows tempo computed for three different running speeds and a range of body size. In this example, the intercept of running speed versus body length was  $13 \text{ mm s}^{-1}$  and, in the unbiased example, it is zero. The ordinate shows the difference from the unbiased tempo.

**Appendix S2.** The basic data, shown as species means with the coefficient of variation (COV).

**Appendix S3.** Correlations among major variables.

#### References

- Bartholomew, G.A., Vleck, D. & Vleck, C.M. (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.
- Bartholomew, G.A., Lighton, J.R.B. & Feener, D.H. Jr. (1988) Energetics of trail-running, load carriage, and emigration in the column-raiding army ant *Eciton hamatum*. *Physiological Zoology*, **61**, 57–68.
- Beckmann, R.L. Jr. & Stucky, J.M. (1981) Extrafloral nectaries and plant guarding in *Ipomea pandurata* (L.) G. F. W. Mey. (Convolvulaceae). *American Journal of Botany*, **68**, 72–79.
- Bernstein, R.A. (1979) Evolution of niche breadth in populations of ants. *American Naturalist*, **114**, 533–544.

- Bernstein, S. & Bernstein, R.A. (1969) Relationships between foraging efficiency and the size of the head and component brain and sensory structures in the red wood ant. *Brain Research*, **16**, 85–104.
- Boomsma, J.J. & Isaacs, J.A. (1985) Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, **18**, 19–27.
- Burkhardt, J.F. (1998) Individual flexibility and tempo in the ant *Pheidole dentata*. *Journal of Insect Behavior*, **11**, 493–505.
- Calabi, P. & Porter, S.D. (1989) Worker longevity in the fire ant *Solenopsis invicta*: ergonomic considerations of correlations between temperature, size and metabolic rates. *Journal of Insect Physiology*, **35**, 643–649.
- Cao, T. & Dornhaus, A. (2008) Ants under crowded conditions consume more energy. *Biology Letters*, **4**, 613.
- Carroll, J.F. (1975) *Biology and ecology of ants of the genus Aphaenogaster in Florida*. PhD Dissertation, University of Florida.
- Chown, S.L., Marais, E., Terblanche, J.S. et al. (2007) Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Functional Ecology*, **21**, 282–290.
- Cole, B.J. (1992) Short-term activity cycles in ants: age-related changes in tempo and colony synchrony. *Behavioral Ecology and Sociobiology*, **31**, 181–187.
- Damuth, J. (1981) Population density and body size in mammals. *Nature*, **290**, 699–700.
- Davidson, D.W. & Patrell-Kim L. (1996). Tropical ants: why so abundant? *Neotropical Diversity and Conservation*, UCLA Botanical Garden Publication No. 1 (ed. by A. C. Gibson), pp. 127–140. UCLA Botanical Garden, Los Angeles, California.
- Elzen, G.W. (1986) Oxygen consumption and water loss in the imported fire ant *Solenopsis invicta* Buren. *Comparative Biochemistry and Physiology*, **84A**, 13–17.
- Fellers, J.H. (1987) Interference and exploitation in a guild of woodland ants. *Ecology*, **68**, 1466–1478.
- Fewell, J.H., Harrison, J.F., Lighton, J.R.B. & Breed, M.D. (1996) Foraging energetics of the ant, *Paraponera clavata*. *Oecologia*, **105**, 419–427.
- Fiedler, K. & Maschwitz, U. (1988) Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae) II. Lycaenid larvae as trophobiotic partners of ants – a quantitative approach. *Oecologia*, **75**, 204–206.
- Fonck, C. & Jaffe, K. (1996) On the energetic cost of sociality. *Physiology and Behavior*, **59**, 713–719.
- Gallé, L. Jr. (1978) Respiration as one of the manifestations of the group effect in ants. *Acta Biologica*, **24**, 111–114.
- Gillooly, J.F., Hou, C. & Kaspari, M. (2010) Eusocial insects as superorganisms: insights from metabolic theory. *Communicative and Integrative Biology*, **3**, 360–362.
- Hansen, S.R. (1978) Resource utilization and coexistence of three species of *Pogonomyrmex* ants in an Upper Sonoran grassland community. *Oecologia*, **39**, 109–117.
- Herbers, J.M. (1981) Reliability theory and foraging by ants. *Journal of Theoretical Biology*, **89**, 175–189.
- Herbers, J.M. & Choiniere, E. (1996) Foraging behavior and colony structure in ants. *Animal Behavior*, **51**, 141–153.
- Heusner, A.A. (1985) Body size and energy metabolism. *Annual Review of Nutrition*, **5**, 267–293.
- Holbrook, C.T., Barden, P.M. & Fewell, J.H. (2011) Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology*, **22**, 960–966.
- Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Belknap Press, Harvard University, Cambridge, Massachusetts.
- Holm-Jensen, I., Jensen, T.F. & Nielsen, M.G. (1980) The influence of temperature upon the rate of CO<sub>2</sub> carbon dioxide production in enflurane anaesthetized worker ants of *Formica rufa* L. *Insectes Sociaux*, **27**, 180–185.
- Hou, C., Kaspari, M., Vander Zanden, H. & Gillooly, J. (2010) Energetic basis of colonial living in social insects. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 3634–3638.
- Hoy, J.B., Porter, S.D. & Koehler, P.G. (1997) A microcomputer-based activity meter for multiple animals. *Journal of Neuroscience Methods*, **72**, 183–188.
- Hurlbert, S.H. (1984) Pseudoreplication and the design of field experiments. *Ecological Monographs*, **54**, 187–211.
- Jensen, T.F. (1978) Annual production and respiration in ant Formicidae populations. *Oikos*, **31**, 207–213.
- King, J.R. (2010) Size–abundance relationships in Florida ant communities reveal how ants break the energetic equivalence rule. *Ecological Entomology*, **35**, 287–298.
- Kleiber, M. (1961) *The Fire of Life*. John Wiley, New York, New York.
- Kneitz, G. (1967) Stechproben zur jahreszeitlichen dynamik des wald-ameisenstaates (*Formica polycetena* Foerst). *Progress in Soil Biology: Proceedings of the Colloquium on Dynamics of Soil Communities*, pp. 241–248. North Holland Publishing, The Netherlands.
- Knoebel, L.K. (1971) Energy metabolism. *Physiology* (ed. by E. E. Selkurt), pp. 635–650. Little, Brown and Company, Boston, Massachusetts.
- Koptur, S. (1992) Plants with extrafloral nectaries and ants in the everglades habitats. *Florida Entomologist*, **75**, 38–50.
- Kwapich, C.L. & Tschinkel, W.R. (2013) Demography, demand, death and the seasonal allocation of labor in the Florida harvester ant (*Pogonomyrmex badius*). *Behavioral Ecology and Sociobiology*, **67**, 2011–2027.
- Larsen, K.J., Vega, F.E., Moya-Raygoza, G. & Nault, L.R. (1991) Ants (Hymenoptera: Formicidae) associated with the leafhopper *Dalbulus quinquevittatus* (Homoptera: Cicadellidae) on gamagrasses in Mexico. *Annals of the Entomological Society of America*, **84**, 498–501.
- Leonard, J.G. & Herbers, J.M. (1986) Foraging tempo in two woodland ant species. *Animal Behavior*, **34**, 1172–1181.
- Leuthold, R.H. (1968) A tibial gland scent trail and trail laying behavior in the ant *Crematogaster ashmeadi* Mayr. *Psyche*, **75**, 233–248.
- Lighton, J.R.B. (1989) Individual and whole-colony respiration in an African formicine ant. *Functional Ecology*, **3**, 523–530.
- Lighton, J. R. B. (1991) Insects: measurements. *Concise Encyclopedia on Biological and Biomedical Measurement Systems* (ed. by P. A. Payne), pp. 201–208. Pergamon Press, U.K.
- Lighton, J.R.B. & Bartholomew, G.A. (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 United States of America*, **85**, 4765–4769.
- Lighton, J.R.B. & Feener, D.H. Jr. (1989) A comparison of energetics and ventilation of desert ants during voluntary and forced locomotion. *Nature*, **342**, 174–175.
- Lighton, J.R.B. & Fielden, L.J. (1995) Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. *Physiological Zoology*, **68**, 43–62.
- Lighton, J.R.B., Bartholomew, G.A. & Feener, D.H. (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.
- MacKay, W.P. & Sassaman, C.A. (1984) Paradoxical acclimation and seasonal comparisons of oxygen consumption rates in three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Journal of Comparative Physiology B*, **154**, 399–407.

- Martin, P.J. (1991) Respiration of the ant *Leptothorax unifasciatus* (Hymenoptera, Formicidae) at individual and society levels. *Journal of Insect Physiology*, **37**, 311–318.
- McInnis, D.A. (1994) *Comparative ecology and factors affecting the distribution of north Florida fire ants*. PhD Dissertation, Florida State University, Tallahassee, Florida.
- McLain, K.L. (1983) Ants, extrafloral nectaries and herbivory on the Passion vine, *Passiflora incarnata*. *American Midland Naturalist*, **110**, 433–439.
- Myers, R.H. (1990) *Classical and Modern Regression with Applications*. PWS-Kent, Boston, Massachusetts.
- Nielsen, M.G. (1986) Respiratory rates of ants from different climatic areas. *Journal of Insect Physiology*, **32**, 125–131.
- Nielsson, R.J., Bhaktar, A.P. & Denmark, H.A. (1971) A preliminary list of ants associated with aphids in Florida. *Florida Entomologist*, **54**, 245–248.
- Oster, G.F. & Wilson, E.O. (1978) *Caste and Ecology in the Social Insects. Monographs in Population Biology, No. 12*. Princeton University Press, Princeton, New Jersey.
- Packard, G.C. & Boardman, T.J. (1999) The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comparative Biochemistry and Physiology Part A*, **122**, 37–44.
- Passera, L. (1994) Characteristics of tramp species. *Exotic Ants: Biology, Impact, and Control of Introduced Species* (ed. by D. F. Williams), pp. 24–43. Westview Press, Boulder, Colorado.
- Passera, L., Keller, L., Grimal, A. *et al.* (1990) Carbohydrates as energy source during the flight of sexuals of the ant *Formica lugubris* (Hymenoptera: Formicidae). *Entomologica Generalis*, **15**, 25–32.
- Peakin, G.J. & Josens G. (1978) Respiration and energy flow. *Production Ecology of Ants and Termites* (ed. by M. V. Brian), pp. 111–163. Cambridge University Press, U.K.
- Porter, S.D. (1986) Revised respiration rates for the southern harvester ant, *Pogonomyrmex badius*. *Comparative Biochemistry and Physiology*, **83A**, 197–198.
- Porter, S.D. & Tschinkel, W.R. (1985) Fire ant polymorphism: the ergonomics of brood production. *Behavioral Ecology and Sociobiology*, **16**, 323–336.
- Richman, D.B., Whitcomb, W.H. & Buren, W.F. (1983) Predation on neonate larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Florida and Puerto Rico Citrus Groves. *Florida Entomologist*, **66**, 215–222.
- Rogers, L., Lavigne, R. & Miller, J.L. (1972) Bioenergetics of the western harvester ant in the shortgrass plains ecosystem. *Environmental Entomology*, **1**, 763–768.
- Schmid-Hempel, P. (1990) Reproductive competition and the evolution of work load in social insects. *American Naturalist*, **135**, 501–526.
- Schneirla, T.C. (1944) Results of the Archbold expeditions. No. 51. Behavior and ecological notes on some ants from south-central Florida. *American Museum Novitates*, **1261**, 1–5.
- Shik, J.Z. (2008) Ant colony size and the scaling of reproductive effort. *Functional Ecology*, **22**, 674–681.
- Shik, J.Z. (2010) The metabolic costs of building ant colonies from variably sized subunits. *Behavioral Ecology and Sociobiology*, **64**, 1981–1990.
- Shik, J.Z., Santos, J.C., Seal, J.N. *et al.* (2014) Metabolism and the rise of fungus cultivation by ants. *American Naturalist*, **184**, 364–373.
- Sirjusingh, C., Kermarrec, A., Mauleon, H. *et al.* (1992) Biological control of weevils and whitegrubs on bananas and sugarcane in the caribbean. *Florida Entomologist*, **75**, 548–562.
- Sudd, J.H. (1982) Ants: foraging, nesting, brood behavior, and polyethism. *Social Insects* (ed. by H. R. Hermann), pp. 107–155. Academic Press, New York, New York.
- Takahashi-Del-Bianco, M.J., Hebling, A. & Bueno, O.C. (1998) Respiratory metabolism of *Camponotus rufipes* ants: brood and adults. *Comparative Biochemistry and Physiology*, **119A**, 529–532.
- Tschinkel, W.R. (1988) Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta* Buren. *Behavioral Ecology and Sociobiology*, **22**, 103–115.
- Tschinkel, W.R. (1991) Insect sociometry, a field in search of data. *Insectes Sociaux*, **38**, 77–82.
- Tschinkel, W.R. (1993) Sociometry and sociogenesis of the fire ant *Solenopsis invicta* through one annual cycle. *Ecological Monographs*, **64**, 425–457.
- Tschinkel, W.R. (2006) *The Fire Ants*. Belknap/Harvard University Press, Cambridge, Massachusetts.
- Tschinkel, W.R. (2010) The organization of foraging in the fire ant, *Solenopsis invicta*. *Journal of Insect Science*, **10**, 26.
- Van Pelt, A.F. Jr (1947) *Ants of the Gainesville region with special reference to ecology and taxonomy (Hymenoptera: Formicidae)*. MS Thesis, University of Florida.
- Van Pelt, A.F. Jr (1950) *The ecology of the ants of the Welaka Reserve, Florida (Hymenoptera: Formicidae)*. PhD Dissertation, University of Florida.
- Waters, J.S., Holbrook, C.T., Fewell, J.H. & Harrison, J.F. (2010) Allometric scaling of metabolism, growth, and activity in whole colonies of the seed harvester ant, *Pogonomyrmex californicus*. *The American Naturalist*, **176**, 501–510.
- Wheeler, W.M. (1910) *Ants: Their Structure, Development and Behavior*. Columbia University Press, New York, New York.
- Whitcomb, W.H., Denmark, H.A., Bhaktar, A.P. & Greene, G.L. (1972) Preliminary studies of the ants of Florida soybean fields. *Florida Entomologist*, **55**, 129–142.
- Wilson, E.O. (1976) Enemy specification in the alarm-recruitment system of an ant. *Science*, **190**, 798–800.

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