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## ORIGINAL ARTICLE

# Heterogeneous activity causes a nonlinear increase in the group energy use of ant workers isolated from queen and brood

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**Abstract** Increasing evidence has shown that the energy use of ant colonies increases sublinearly with colony size so that large colonies consume less per capita energy than small colonies. It has been postulated that social environment (e.g., in the presence of queen and brood) is critical for the sublinear group energetics, and a few studies of ant workers isolated from queens and brood observed linear relationships between group energetics and size. In this paper, we hypothesize that the sublinear energetics arise from the heterogeneity of activity in ant groups, that is, large groups have relatively more inactive members than small groups. We further hypothesize that the energy use of ant worker groups that are allowed to move freely increases more slowly than the group size even if they are isolated from queen and brood. Previous studies only provided indirect evidence for these hypotheses due to technical difficulties. In this study, we applied the automated behavioral monitoring and respirometry simultaneously on isolated worker groups for long time periods, and analyzed the image with the state-of-the-art algorithms. Our results show that when activity was not confined, large groups had lower per capita energy use, a lower percentage of active members, and lower average walking speed than small groups; while locomotion was confined, however, the per capita energy use was a constant regardless of the group size. The quantitative analysis shows a direct link between variation in group energy use and the activity level of ant workers when isolated from queen and brood.

**Key words** activity; energetics; image analysis; long term; scaling; simultaneous

## Introduction

Ants cooperate to increase fitness by working in groups to efficiently acquire and use resources (Krause & Ruxton, 2002; Hölldobler & Wilson, 2008). Recent studies on the

metabolic rate of ants from different species potentially provide some new insights into the issue of whole group performance from an energetic viewpoint (Hou *et al.*, 2010; Waters *et al.*, 2010; Shik *et al.*, 2012, 2014; Cao & Dornhaus, 2013; Fewell & Harrison, 2016). These studies found that the colonial metabolic rate is lower than the sum of individual energy usages, and scales sublinearly with colony size, that is, on a mass-specific basis larger colonies consume less energy than smaller ones. The sublinear metabolic scaling indicates that sociality makes a worker more energetically efficient than living on its own, and the larger the colony is, the higher the efficiency is. However, the sublinear scaling was not

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observed in every study on the group energetics of ants. When ant workers were housed in small and/or dark containers where their locomotion was limited, the metabolic scaling became isometric (Lighton, 1989; Waters *et al.*, 2010), that is, the energy consumption of the group was equal to the sum of the individual workers. It was postulated that the isometric scaling was caused by the fact that these workers were grouped with no queen or brood, which “is critical to regulate individual patterns of work output” (Waters *et al.*, 2010). Noticing that in the studies that showed sublinear scaling in whole colonies, ants were allowed to move freely, whereas in the studies that showed isometric scaling, activity was confined, we hypothesize that the key to understanding the scaling powers of group metabolic rate is the activity behavior, and that the isometric scaling was observed, not because ant workers were isolated from queen and brood, but simply because their locomotion was limited.

Ant foraging dynamics are significantly influenced by their colony size (Detrain & Deneubourg, 2006; Detrain & Deneubourg, 2009; Dornhaus *et al.*, 2012). It has been well documented that among all the workers engaged in foraging outside of the nest (ants engaged in other tasks are not included), the activity distribution is highly skewed, as a large fraction of tasks are carried out by these active workers, such as pheromone trail laying, recruiting, and food transport (Traniello, 1977; Beckers *et al.*, 1992; Pinter-Wollman *et al.*, 2011). Interestingly, as the colony size increases, the proportion of active workers decreases (Traniello, 1977; Maillieux *et al.*, 2003; Detrain & Deneubourg, 2006; Naug, 2009; Waters *et al.*, 2010; Dornhaus *et al.*, 2012).

Clearly, activity level among individual ants during foraging is heterogeneous. Our hypothesis suggests that the behavioral heterogeneity gives rise to sublinear energetics. It can be easily understood in the following way. Compared to the ants at rest, the walking ants consume 2–6 times more energy, depending on its speed (Lighton *et al.*, 1987; Fewell, 1988). Thus, a large colony with a disproportionately large fraction of inactive members will have a low average per capita metabolic rate. In their seminal work, Waters *et al.* (2010) found that individuals’ walking speeds exhibit power-law distributions that scale with colony size in *Pogonomyrmex californicus*, that is, in all the investigated colonies with queens and brood, the number of ants with low walking speeds is higher than the number with high speeds, and the fraction of the ants with low speeds increases with colony size. Based on this finding, Waters *et al.* postulated that one of the possible reasons for the sublinear metabolic scaling is that larger colonies exhibit a greater disparity of active and inactive ants than smaller colonies. However, 3 factors limit the explanation

power of Waters *et al.*’s study. First, the recording of the ants’ locomotion trajectories was conducted separately from the respirometry in their study so that the relationship between the speed patterns and the energetic patterns was inferred, instead of being directly linked. Second, Waters *et al.* attributed the sublinear metabolic scaling, and implicitly attributed the disparity of activity, to the social environments, and argued that when workers were isolated from queen and brood (the social environment), which “regulate individual patterns of work output,” the metabolic scaling became linear. However, our hypothesis suggests that being with queen and brood is not the key for the sublinear scaling. If allowed to move freely, instead of being confined in a small container as done in Waters *et al.*’s study, workers without queen or brood may still have a different distribution of speed, and therefore, sublinear metabolic scaling. Third, and finally, for each of the 8 colonies whose locomotion trajectories were recorded, these authors only subsampled a 60-s segment of video with a relatively low sampling resolution and manually digitized each ant’s spatial coordinates for analyzing the speeds. Such a short segment may only reflect a snapshot of the ants’ behaviors. It is nearly impossible to manually monitor hundreds of ants’ locomotion for hours.

In this study, our goal is to overcome these 3 limiting factors to test our hypothesis. Using worker harvester ants (*Pogonomyrmex barbatus*) that are separated from queen and brood, we employ state-of-the-art computer vision techniques and respirometry to study activity and group energetics simultaneously over long periods (hours) to build a direct link between them.

## Materials and methods

### *Respirometry of ants that were allowed to move freely*

Respirometry was conducted on harvester ant workers (*Pogonomyrmex barbatus*) in 3 different sized groups, namely, small, medium, and large. *P. barbatus* belongs to the same genus as *P. californicus*, used in Waters *et al.*’s study. Also, *P. barbatus* workers are not dimorphic, so the potential effects of members of different size on metabolic scaling is not a concern (Shik, 2010; Waters *et al.*, 2010). For each group size, measurements were taken in the afternoons and repeated 3 times with intervals ranging from 1 to 7 d between each measurement. Each time, ant workers were randomly selected from the same colony of ~900 ants and ants were given approximately 1 h to adjust to the testing environment. The number of workers in each measurement was 30 (for all 3 small groups), 138, 150, and 150 (for medium groups), and 250, 300, and 300

(for large groups), respectively. Body mass of the group was measured after each trial to the nearest 0.1 mg, using a digital microbalance (Perkin-Elmer AD6; Downers Grove, IL, USA).

Ants were housed in an airtight chamber (35 cm × 25 cm × 2 cm), the top of which is made of plexiglass for videotaping. A piece of synthetic diet (Dussutour & Simpson, 2008) with a mass of 3 g was placed at a corner of the chamber. A piece of cotton ball soaked with water was placed at the center of the chamber. The temperature was recorded and controlled at  $26.5 \pm 0.5$  °C. Ants were *ad libitum* fed before the measurements. Lights were kept on during the entire respirometry session. Before all trials, the CA-10 CO<sub>2</sub> analyzer (Sable Systems International, Las Vegas, NV, USA) was calibrated with dry and CO<sub>2</sub>-free air running through a drierite/ascarite II/magnesium perchlorate column, and then was spanned with a gas of known CO<sub>2</sub> concentration (1000 ppm CO<sub>2</sub> in N<sub>2</sub>). At the beginning of the measurements, the dry CO<sub>2</sub>-free air was pumped through the chamber at rate of 2 L/min for 5 min to purge the CO<sub>2</sub> that had accumulated during the preparation. Before the air was sent to the CA-10 CO<sub>2</sub> analyzer, the moisture was removed by a column of magnesium perchlorate (A picture of the setup and explanation are available in Fig. S1). Flow-through respirometry with an incurrent flow measurement was implemented. The flow rate was set at 90 mL/min. For this flow rate and chamber volume, the response time for the washout to reach 95% of the final value is estimated to take about 60 min (Lighton, 2008). A baseline measurement was taken before and after each experimental trial. The respirometry chamber had 3 inlets and 3 outlets to make sure that the air was well mixed (Lighton, 2008). The respirometry was conducted for 3 h for each experimental trial. The behavior monitoring and respirometry data showed that it took about 20–50 min for the activity and the CO<sub>2</sub> production of the ants to reach relatively stable levels. Thus, only data from the last 2 h was analyzed.

#### *Respirometry of ants confined in small syringes*

Four groups of ants with 30, 70, 150, and 300 workers were placed in 60-cc syringe barrels (respirometry chambers). Cotton balls were placed in the syringes with small groups (30 and 70 ants), so that the effect volume was about 40-cc for these groups. Thus, the surface area available for ants to move was about 20 cm<sup>2</sup> for large groups and about 14 cm<sup>2</sup> for small groups. The chambers were placed in a pelt-5 temperature controller (SSI) with the lights on. The methods for calibrating and scrubbing were the same as above (A picture of the setup and ex-

planation are available in Fig. S1). Flow rate was set at 90 mL/min. Ants were provided with synthetic diet and a small tube of water. Respirometry started 1 h after the ants were placed in the temperature controller, and ran for 3 h. A multiplexer (SSI) was used to switch measurements between each chamber.

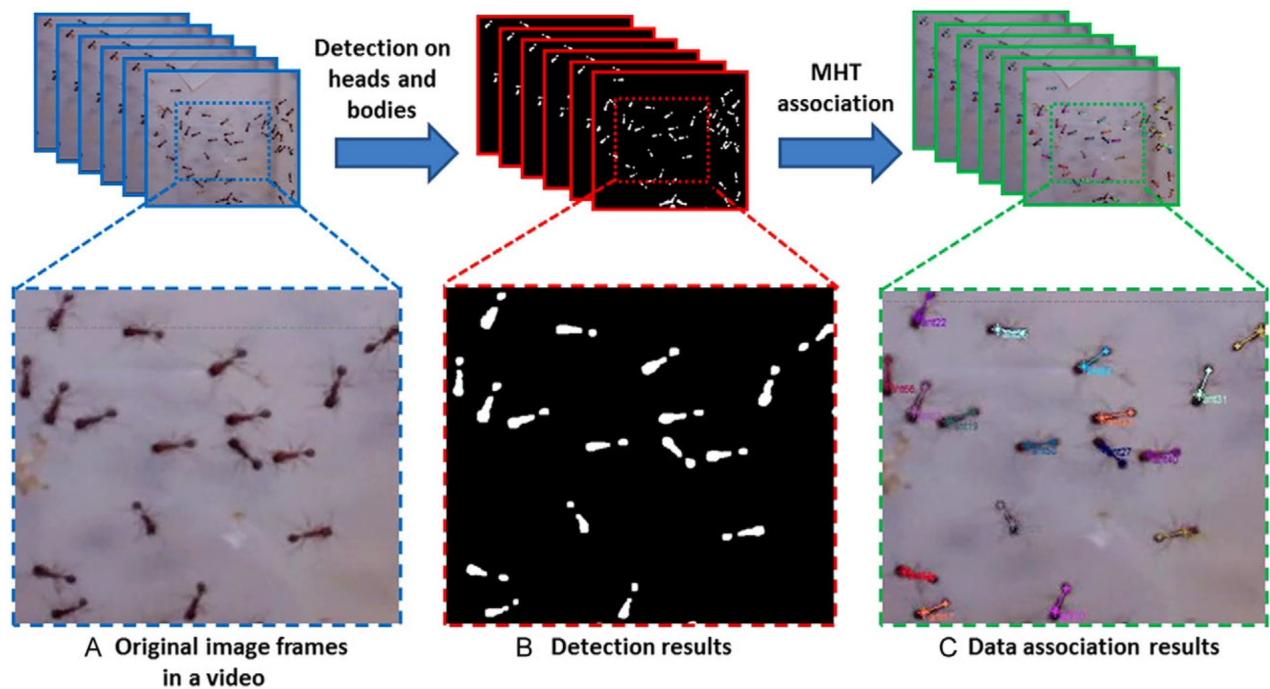
#### *Behavior filming*

The activity of ants in the large chamber (35 cm × 25 cm × 2 cm) was monitored. A camera connected to a computer was set 35 cm above the respirometry chamber. Filming was conducted simultaneously with the respirometry. The behavioral data from the last 2 h were analyzed. Due to the body overlapping problem in the syringes, the ants whose locomotion was confined were not monitored.

#### *Image analysis*

To automatically track multiple ants with computer vision methods, generally 2 types of challenges need to be overcome. The first challenge is to detect each individual ant accurately, despite the issues including different background–foreground contrast, overlapping of ant bodies, and shape inconsistency of the ants. The second challenge is to associate ants in different frames to tackle the “who is who” problem, given ant detection results in each frame. Regarding these challenges, several methods have been proposed for ant tracking, such as gap filling (Fasciano *et al.*, 2013), occlusion tunnels methods (Fasciano *et al.*, 2014), video playback and fragment correction methods (Poff *et al.*, 2012), but these methods could not solve issues such as ant body overlapping and low contrast problems.

In this study, a novel multiple ant tracking method was implemented, which solves the above issues by implementing a part-based detection and a multihypothesis testing (MHT) scheme for data association. The sampling rate was 15 frames/s. The overview of the workflow is shown in Figure 1. The detection stage focused on separating the ants' bodies and heads. While fitting to eclipses, the head and body have different shapes, as shown in Figs. 1(A) and (B), which allows the separating. By using our method, individual ants can be tracked by analyzing their exposed body parts even when overlapping occurs. In the data association stage with MHT, all the detected heads and bodies of ants across frames were linked by first formulating all possible hypotheses, and then finding the optimal hypotheses combination, as shown in Figs. 1(B) and (C). Two types of constraints on spatial and



**Fig. 1** Overview of workflow. (A) Original frames in video; (B) part-based detection results obtained from (A); and (C) ant trackers generated from (B).

temporal domains are leveraged to ensure consistency. First, 1 body (or head) can be linked to at most 1 body (or head) in the previous frame, and at most 1 body (or head) in the next frame. Second, 1 head can be linked to at most 1 body in the same frame with their spatial distance less than  $D$  (the longest possible length of an ant). After the detection and data association stages, accurate trackers are generated to represent all the ants and their locations across all frames. These trackers make it possible to analyze the recorded trajectories, and give quantitative data on the following behavioral matrices. More details of image analysis method is available in the online Supporting Information.

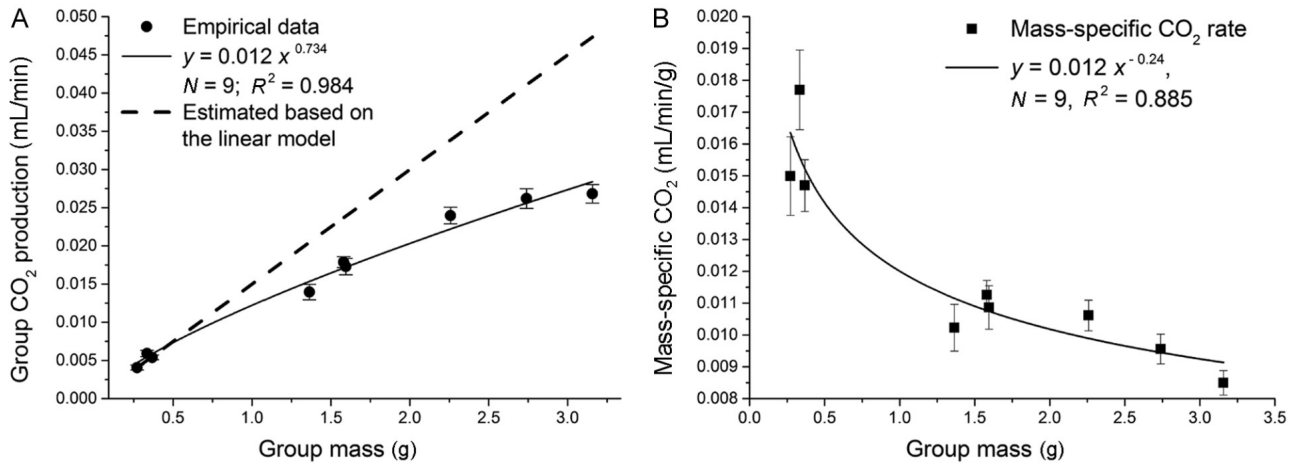
Two kinds of quantitative behavioral data were analyzed.

- (1) Percentage of active ants. The temporal samplings were taken as 0.1 min (6 s) segments. Within each temporal segment, an ant that made a move longer than 5 pixels (0.313 cm) was counted as an active ant. Since each ant was individually tracked, the algorithm was able to avoid double counting the moving ants, that is, an ant that made 1 move, paused, and then made another move within the same temporal segment was only counted once in this segment. The percentage of active ants in a group for each

segment was estimated as the ratio of the number of the active ants and the total number of ants. For each measurement, a time series of the percentage of active ants was recorded, which had 1200 data points (2 h/6 s). To compare the overall behaviors between the small, medium, and large groups, the data points from 3 independent measurements of the similar sized groups were combined, and treated as a 6-h long time series. The values of the average and standard deviation of the 6-h long series were compared. ANOVA was performed to compare the difference between the 6-h series from the groups with different series.

- (2) Walking speed. Within each temporal segment of 6 s, the distance of each active ant was recorded, and the walking speed of each ant was estimated as the distance divided by 6 s. Two kinds of walking speeds were recorded. The first one is the average speed within a segment over all the active ants in a group, and the second one is the average speed within a segment over all the ants in a group, including the ones at rest. The speeds were recorded as time series of 2 h for each measurement.

To compare the overall behaviors between the small, medium, and large groups, the data points from 3



**Fig. 2** CO<sub>2</sub> production rate of free moving ants in groups with different sizes. For each size, 3 groups of ants were randomly chosen and measured (see “Methods” section). (A) Total CO<sub>2</sub> rate of each group. The solid line shows the best fitting of a power law function, which gives  $y = 0.012x^{0.734}$  ( $R = 0.984$ ). The dashed line represents the hypothetical estimate based on the assumption that group CO<sub>2</sub> rate increases linearly with group size (linear model), as (group mass/mass of the smallest group) × measured CO<sub>2</sub> of the smallest group. (B) Mass-specific CO<sub>2</sub> rate of 9 groups of ants. The black dots are the average values over the 2-h measurements, and the error bars are the standard deviations. The solid line shows the best fitting of a power law function, which gives  $y = 0.012x^{-0.24}$  ( $R = 0.885$ ).

independent measurements of the similar sized groups were combined, and treated as a 6-h long time series (2 h × 3). The values of the average and standard deviation of the 6-h long series were reported. ANOVA was performed to compare the difference between the 6-h series from the groups with different series.

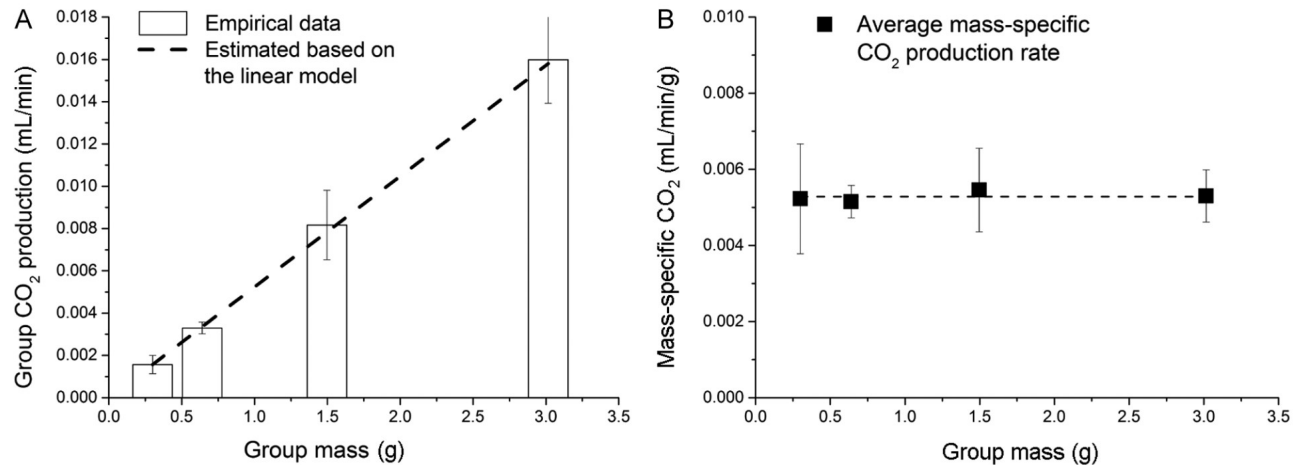
Two tracking methods with different temporal resolutions and filming periods were compared. The first method sampled 15 frames of video per second, which was used in this study. The second method sampled 1 frame/s, which was taken in a previous study (Waters *et al.*, 2010). A 2-h long filming of a small group of ants was analyzed by these 2 methods with different sampling rates. The results of the percentage of active members and the average walking speeds of all ants were compared.

The behavioral matrices of ants may vary greatly during a long period. Thus, it is possible that a short observation interval is only a snapshot, and the values for activity level given by the snapshot may not represent the values over a long period. Previously, Waters *et al.* (2010) subsampled 1-min long videos of 8 colonies. In this study, each ant group was filmed over a 2-h long period. To compare the results from the short and long observations, the average speeds of a small ant group during 1-min segments were estimated over a 2-h period. The distribution of the speeds during 1-min segments was analyzed. A narrow variation of the distribution would indicate relatively constant behaviors, in which case short observations would be sufficient.

## Results

### CO<sub>2</sub> production rate of free moving ants

When ants were allowed to move freely, the CO<sub>2</sub> production rate (metabolic rate) did not increase proportionally with the group size. The total masses of the large groups (250–300 workers) were 8–10 times larger than those of the small groups (30 ants), but the CO<sub>2</sub> production rates of the larger groups were only 5–6 times higher (Fig. 2A). Using the CO<sub>2</sub> value of the group with the smallest mass as the reference, we estimated the CO<sub>2</sub> values of other groups assuming a linear increase in CO<sub>2</sub> with mass (the dashed line in Fig. 2A), as (group mass/mass of the smallest group) × measured CO<sub>2</sub> of the smallest group. Fig. 2(A) shows that the hypothetical values based on the linear model are higher than the empirical values (empty bars), and the difference between the hypothetical and empirical values increases with group mass. The nonlinear energetics of ant groups can also be seen in the mass-specific CO<sub>2</sub> production rate. For each group size, we calculated the average values of body masses and mass-specific CO<sub>2</sub> production rates over 3 measurements. Fig. 2(B) shows that, mass specifically, the CO<sub>2</sub> rate ranged between 0.0147–0.0177, 0.0102–0.0113, and 0.0085–0.0106 mL/min/g in the small, medium and large groups, respectively, and it scales with group mass negatively as  $y = 0.012x^{-0.24}$  ( $n = 9$ ,  $R^2 = 0.885$ ).



**Fig. 3** CO<sub>2</sub> production rate of ants confined in syringes in groups of different sizes. (A) Total CO<sub>2</sub> rate of each group. The dashed line represents the hypothetical estimate based on the assumption that group CO<sub>2</sub> rate increases linearly with group size (linear model), as (group mass/mass of the smallest group) × measured CO<sub>2</sub> of the smallest group. (B) Mass-specific CO<sub>2</sub> rate. The black dots represent the average values over the 2-h respirometry session of each group. The error bars represent the standard deviations.

#### CO<sub>2</sub> production rate of ants confined in small syringes

When the activity of the ants was confined in small syringes, however, the group CO<sub>2</sub> production rate increased linearly with group size (Fig. 3A). The estimated CO<sub>2</sub> values based on the linear model agree remarkably with the empirical data. Accordingly, the mass-specific CO<sub>2</sub> rates of all 4 groups were at a similar level, 0.00528 mL/min/g (Fig. 3B).

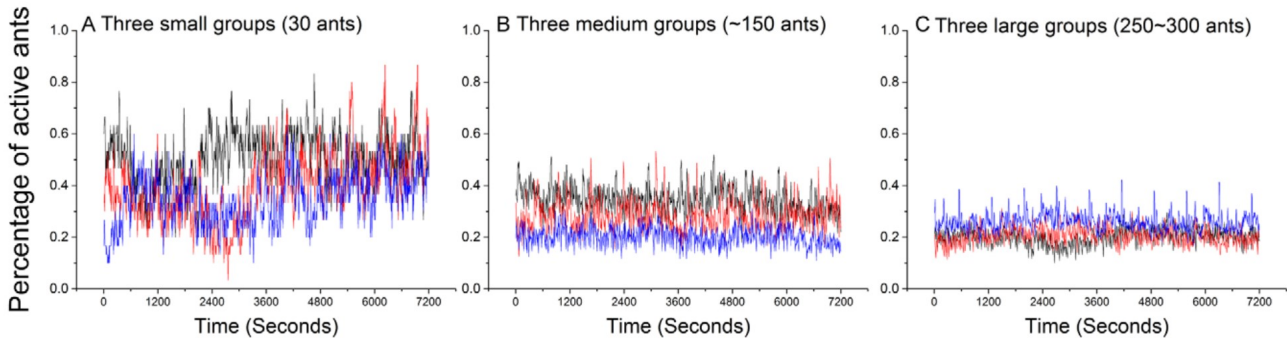
#### Percentage of active workers

Through the 2-h measurements, 3 small groups had 50.8% ± 10%, 35.1% ± 10%, and 40.0% ± 13% active members in every 6-sec segment, respectively (Fig. 4). Combining the data from 3 small groups as a 6-h long time series, the overall average percentage of active members was 42.0% ± 13%. The percentage of the active workers in 3 medium groups were 34.8% ± 5%, 20.6% ± 3%, and 28.8% ± 5% with an average over the combined 6-h long time series of 28.1% ± 8% (Fig. 4). The values of 3 large groups were 20.2% ± 3%, 26.1% ± 4%, and 20.7% ± 3% with an overall average of 22.3% ± 4% (Fig. 4). Comparing the 6-h long time series, the percentage of active members in the small group was significantly higher than that in the medium groups (ANOVA  $F_{(1,7198)} = 3069.2$ ,  $P < 0.001$ ) and the large groups (ANOVA  $F_{(1,7198)} = 7477.9$ ,  $P < 0.001$ ).

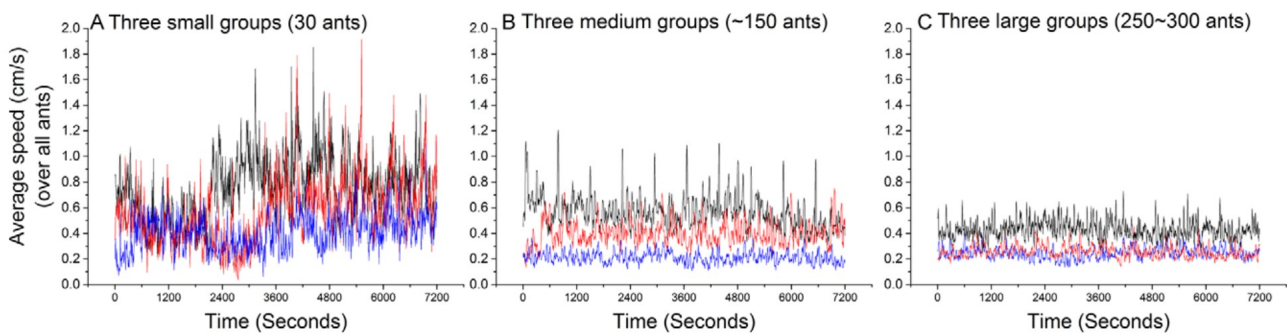
#### Walking speed

Figure 5 shows the walking speed averaged over all workers (including both active and inactive) of each group size. Through the 2-h measurements, the average speeds of 3 small groups were 0.760 ± 0.244, 0.405 ± 0.14, and 0.572 ± 0.25 cm/s, respectively. The average value over the combined 6-h time series was 0.579 ± 0.26 cm/s. Three medium groups had average speeds 0.571 ± 0.13, 0.213 ± 0.05, and 0.405 ± 0.10 cm/s with an overall average of 0.397 cm/s. Those of the large groups were 0.247 ± 0.05, 0.432 ± 0.07, and 0.260 ± 0.05 cm/s with an overall average 0.313 ± 0.10 cm/s. The average speed of the small groups is significantly higher than that of the medium groups (ANOVA,  $F_{(1,7198)} = 1193.0$ ,  $P < 0.001$ ), and the large groups (ANOVA,  $F_{(1,7198)} = 3221.8$ ,  $P < 0.001$ ).

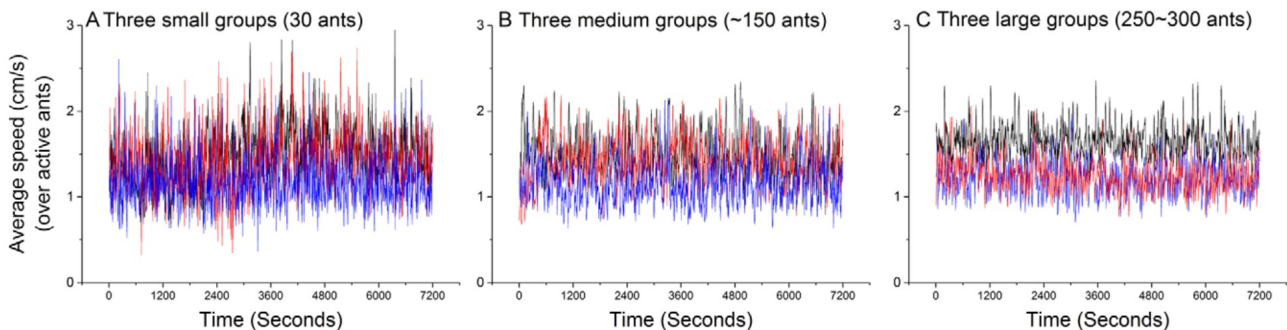
Interestingly, the average speeds of all walking ants (excluding the inactive ones) were at a similar level, regardless of the group size (Fig. 6). Three small groups had speeds 1.481 ± 0.36, 1.164 ± 0.30, and 1.410 ± 0.35 cm/s with an overall average of 1.351 ± 0.35 cm/s. The speeds of the medium groups were 1.512 ± 0.26, 1.146 ± 0.25, and 1.418 ± 0.26 cm/s with an overall average of 1.359 ± 0.30 cm/s. Finally, the large groups had speeds of 1.233 ± 0.21, 1.267 ± 0.20, and 1.662 ± 0.18 cm/s with an overall average of 1.389 ± 0.28 cm/s. The overall average speed of the small group was significantly larger than that of the large group (ANOVA,  $F_{(1,7198)} = 23.1$ ,  $P < 0.001$ ), but the difference



**Fig. 4** The percentage of active ants in small (A), medium (B), and large groups (C). In each panel, the black, red, and blue curves show independent measurements of 3 similar sized groups over 2-h period.



**Fig. 5** Walking speed averaged over all ants in small (A), medium (B), and large groups (C). In each panel, the curves (black, red, and blue) show the speeds averaged over all the ants, active and inactive, in a group every 6 sec.



**Fig. 6** Walking speed averaged over active ants in small (A), medium (B), and large groups (C). In each panel, the curves (black, red, and blue) show the speeds estimated from active ants only, and the resting ones were excluded.

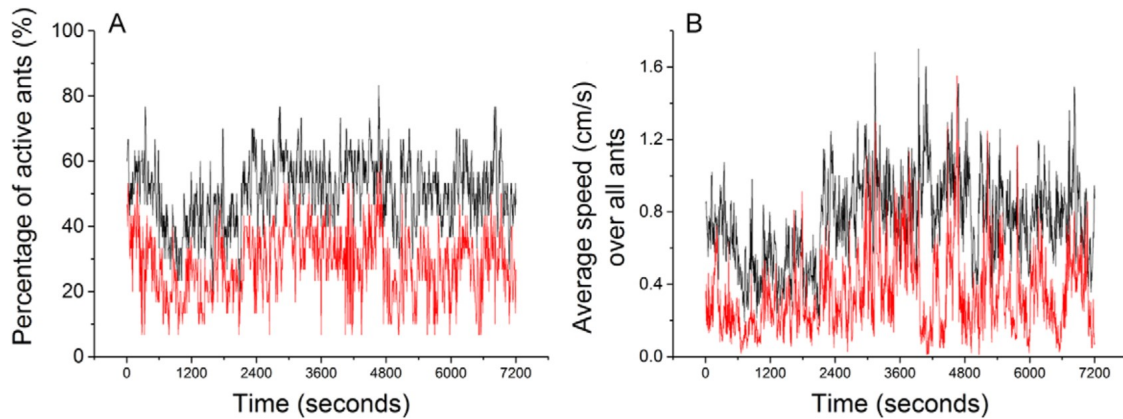
between the small and medium groups was insignificant (ANOVA,  $F_{(1,7198)} = 0.924$ ,  $P < 0.337$ ).

#### Comparison between 2 tracking methods with different temporal resolutions

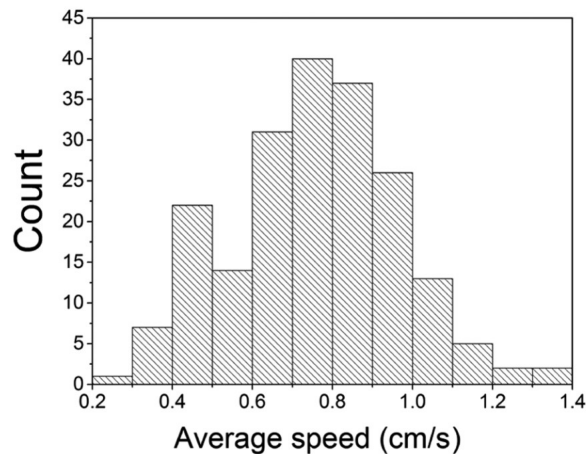
Two tracking methods with sampling rates of 15 frames/s and 1 frame/s, respectively, gave different

results from the same period of video. Figure 7 shows an example from a 2-h long video of a small group (30 ants). The higher temporal resolution estimated the percentage of active ants over these 2 h to be  $50.9\% \pm 10\%$ , which is 2 times higher than the value given by the low temporal resolution ( $28.7\% \pm 10\%$ ). There was a 2 fold difference in the average speed over all ants (active and inactive) given by the high and low resolutions:  $0.76 \pm 0.24$  cm/s (high resolution) and  $0.349 \pm 0.22$  cm/s (low resolution).





**Fig. 7** Comparison between tracking methods with different temporal resolution. (A) Percentage of active ants and (B) walking speed averaged over all ants. In both panels, the black curves show the results from the high temporal resolution of 15 frames/s, and the red curves show the results from the low resolution of 1 frame/s.



**Fig. 8** Distribution of average speed over all ants in a small group (30 ants) during 1-min segments over a 2-h period. The speed ranges from 0.2 to 1.4 cm/s with an average  $0.76 \pm 0.24$  cm/s.

#### Comparison between short and long observations

Average walking speed over all ants in a small group (30 ants) was taken as an example to compare the short and long observation. To compare with the previous study (Waters *et al.*, 2010), which took 1-min segments for analysis, the speed in this study was estimated during 1-min long temporal segments over a 2-h long period. The distribution of speed ( $n = 2 - \text{h}/1 \text{ min} = 120$ ) is shown in Figure 8. It ranged from 0.2 cm/s to 1.4 cm/s with an average of 0.76 cm/s and standard deviation of 0.24 cm/s.

#### Discussion

As shown in Figure 7, high and low temporal resolutions gave different results for the ants' behaviors. The estimated percentage of active members and walking speed were 2 fold higher when the sampling rate was improved from 1 frame/s to 15 frames/s. One of the possible reasons for this is that the ants' trajectories are highly convoluted, and ants often make sudden turns (Adler & Gordon, 1992; Pinter-Wollman *et al.*, 2011). Thus, sampling with low temporal resolution has high chance of missing considerable amount of the distance travelled by ants between 2 successive time points, and therefore underestimate the motion and speed. Over the 2-h period, we observed a large variation in the average walking speed in 1-min segments, from 0.2 cm/s to 1.4 cm/s (Fig. 8). This indicates it is likely that the analyses of 1-min observations give only momentary snapshots, and may not well represent the ants' behaviors over long periods. These comparisons highlight the advantage of our automatic computerized tracking technique, because it is difficult, if possible, to manually digitize coordinates of individual ants with high temporal resolution over a long period.

In their pioneering work, Waters *et al.* (2010) postulated "isolated worker groups (isolated from queen and brood) exhibited isometric metabolic rate scaling, suggesting that the social environment of the colony is critical to regulating individual patterns of work output." However, our results suggest that the reason for the linear energy–size relationship observed in the worker groups by Waters *et al.* (2010) is perhaps not because the workers were isolated from queen and brood, but simply because their activity

was confined (Fig. 3). Although the purpose of this study was not to estimate the scaling power of the energy–size relationship, and we only conducted respirometry on a limited number of groups with different sizes, we can still fit the 9 data points of metabolic rate in Figures 2(A) and (B) with power laws. The results of the fitting give scaling powers of 0.734 ( $n = 9$ ,  $R^2 = 0.984$ ) and  $-0.24$  ( $n = 9$ ,  $R^2 = 0.885$ ) for whole group and mass-specific metabolic rate respectively, which are remarkably close to the value estimated by (Waters *et al.*, 2010).

The decreased percentage of active ants with increasing group size has been seen in other ant species, for example, *Lasius niger* (Mailleux *et al.*, 2003), but these studies did not measure the energy costs of the ants. More recently, Waters *et al.* (2010) showed that large *Pogonomyrmex californicus* colonies had relatively more fast walking ants than small colonies, and suggested a link between colonial energy use and disparity between active and inactive members. However, the link in their study can only be considered indirect, because the walking speed data came from 60-s snapshots of the ants' locomotion in 8 colonies, which were not under respirometry.

Our study, for the first time, analyzed the ants' locomotion and CO<sub>2</sub> production rate simultaneously, for long time periods (2 h  $\times$  9 groups), and with much higher temporal resolution. Thus, we are able to pair the behavioral and respirometry data from the same groups for the following quantitative analysis. The mass-specific CO<sub>2</sub> production rate of the resting ants in each group, regardless of the group size, was a constant, 0.00528 mL/min/g (Fig. 3). Noticing that the walking speed of the active ants in each group was also roughly a constant (Fig. 6), we assume that the mass-specific CO<sub>2</sub> rate induced by walking is a constant,  $X$ , for each active ant regardless of the group size. Thus, the mass-specific CO<sub>2</sub> rate for an active group, MS-CO<sub>2</sub>, can be estimated as  $MS - CO_2 = 0.00528 + X \cdot F$ , where  $F$  (= mass of active ants/mass of total ants) is the percentage of active ants. We now take the data from the large groups to estimate the value of  $X$ . In the large groups, MS-CO<sub>2</sub> was 0.00956 mL/min/g (Fig. 2), and the percentage of active ants  $F$  was 22.3% (Fig. 4). Thus,  $X$  is estimated to be 0.0192 mL/min/g. Using the estimated value of  $X$  and the measured values of  $F$  in the small and medium groups, 42% and 28.5%, respectively, we estimate the mass-specific CO<sub>2</sub> values to be 0.0133 and 0.0107 mL/min/g for the small and medium groups respectively, which are very close to the measured values, 0.0158 and 0.0108 mL/min/g (Fig. 3). Note that, the estimated value of the mass-specific CO<sub>2</sub> rate induced by walking (in addition to the resting cost),  $X = 0.0192$  mL/min/g, is about 4 times larger than the resting value

(0.00528 mL/min/g), in agreement with the results from previous studies that walking ants consume 2–6 times more energy than the resting ants (Lighton *et al.*, 1987; Fewell, 1988). This means that energywise, 1 walking ant is equivalent to 5 resting ants. Thus, if a group has 20% active members, this group would consume 180% more energy than a similar sized group with all inactive members (80% resting + 5  $\times$  20% active), as we see in Figures 2 and 3.

It is possible that the allometry of group energetics is caused by some unknown mechanisms other than the heterogeneity in activities, and the linear increase in group CO<sub>2</sub> in small syringes (Fig. 3) is due to elevated per worker CO<sub>2</sub> production under unnaturally stressed crowding in large groups, and not due to allometries of worker activity, per se. However, there is no evidence suggesting that crowdedness would cause elevated metabolic rate in our case. It is natural for ants to crowd in nests. In fact, the mass-specific metabolic rate of the ants in the syringes,  $\sim 0.00528$  mL/min/g, is almost 2 fold lower than the lowest value observed in the active ants in the large chamber ( $\sim 0.0090$  mL/min/g in the large groups), which indicates that the ants in the syringes may not be under stress. Moreover, the variation in the mass-specific metabolic rate of all 4 groups in the syringes is very small (Fig. 3). If the ants in the large groups were under stress that elevated metabolic rate, it is unlikely that the metabolic rates were elevated to the exactly same level in all groups with different sizes. Thus, since there is no other known mechanism that would cause the sublinear energetics in ants groups, we suggest that the heterogeneity in activity level is a parsimonious explanation.

In a recent insightful review on the energetic scaling of ant colonies, Fewell and Harrison (2016) associated the nonlinear energetics with organizational changes in task performance. Since the energy costs of conducting different tasks are different, the sublinear scaling power rises from the facts that colony size affects the number of tasks, the number of workers specialized in a certain task, and the proportions of workers allocated to each task. Fewell and Harrison (2016) also discussed the link between energy efficiency and colony productivity. In our study, the workers were randomly chosen from a large brood-less colony that was housed in a simple artificial nest. Moreover, the measurements were conducted when workers moved in a relatively small arena (surface area = 875 cm<sup>2</sup>). Without the complex task division (e.g., nest maintenance, brood care, and defence), the worker groups still manifested the nonlinear energy use. Here, the task allocation was reduced to be simply bimodal: ants were either at rest or walking at roughly the same speed. Thus, as a simplest extreme and a solid example of what Fewell and Harrison discussed, our

results suggest that the nonlinear energetics stem from the variation in activity levels in groups with different sizes.

It is intuitive that colonies have inactive members, because these members may serve a backup role (buffer), which would be activated when colonies are under stress, such as urgent need of nest maintenance or defence (Charbonneau & Dornhaus, 2015). But the question is why do large groups have disproportionately low percentages of active members. Moreover, organisms' production rate is associated with metabolic rate, and larger colonies often have lower per capita productivities, which has been considered a paradox in the evolution of colony size (Hou *et al.*, 2010; Kramer *et al.*, 2014). Since the sublinear scaling powers of metabolic and production rate have also long been seen and intensively studied in solitary organisms (e.g., Savage *et al.*, 2004), the models developed for explaining the sublinearity in solitary organisms may shed light on the issue in social insect colonies. One of such models (West *et al.*, 1997) assumed that the structure and dynamics of the metabolite transport network (such as cardiovascular and pulmonary systems) in solitary organisms have evolved in such a way that the energy cost of transporting metabolites is minimized. As a result of this minimization, when animals are at rest, only a portion of the transport network is active, and the fraction of active elements, such as opened blood capillaries, increases more slowly than the increase of body mass, which leads to the sublinear metabolic scaling power. When animals are under stress, especially while doing heavy exercises, the inactive elements are activated (e.g., the closed capillaries are recruited) (Weibel & Hoppeler, 2005; Hou & Mayo, 2011), so that the metabolic scaling becomes close to linear. Here, we postulate that similar energy optimization rules may also apply to ant colonies, although the details are far from clear, and what might be under optimization is not the physiological network in ant's body, but the interaction network between ants. Hou (2016) postulated that, while colonies try to maximize their resource acquisition, the time and energy of foraging is minimized to reduce both the risk of predation (Mailleux *et al.*, 2003; Dornhaus *et al.*, 2012) and energy expenditure (Jun *et al.*, 2003; Gillooly *et al.*, 2010; Dornhaus *et al.*, 2012). Maximizing resource acquisition would require most individuals to be highly active, but would also result in high energy expenditure and long average foraging time. In contrast, minimizing time and energy expenditure would require most individuals to be inactive, but would also result in low resource acquisition. Either of these optimization rules would give rise to minimal heterogeneity in activity levels, because foragers will be either all inactive or all active, and therefore, the

group energetics would be linear with group size. Thus, we postulate that the nonlinear energetics emerges from the compromise between these 2 optimization rules which are balanced by the coordination of the forager's interactions. However, what role the interactive network among ants plays in this optimization is unclear. We call for future theoretical modeling on ant communication to test this hypothesis.

A few lines of empirical research with dynamic image analyses will be helpful to develop the theoretical models. First, in this study, food was placed at a fixed location in a relatively small foraging arena. Variables such as long distance of foraging and variation of food location need to be studied in the future, because the size of the foraging territory and the way that food is distributed, that is, clumped versus scattered, affect the foraging rate in harvester ants (Flanagan *et al.*, 2012). Second, the ants in this study were fed *ad libitum* before each measurement. The foraging behaviors may change if the ants were under food restriction (Traniello, 1977). As postulated above, under such a stress, the inactive members may be activated, and therefore the metabolic scaling may become closer to linear. And third, ambient temperature greatly affects ant metabolic rate (Lighton, 1989), and presumably, the walking speed. It is unknown how changes in temperature affect the activity levels in groups with different sizes. These experiments will greatly advance our understanding of the interplay between the behaviors of individual ants, their interactive network, and group energetics.

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## Disclosure

The authors have no conflict of interest to declare.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** Overview of ant detection method.

**Fig. S2.** Multihypothesis testing sample.

**Fig. S3.** (A) Respirometry of ants confined in syringes. Compressed air went through (1) a mass flow controller (Aalborg Instruments and Controls, Inc., NY, USA), (2) a water–CO<sub>2</sub>–water scrubber column, (3) an 8-channel multiplexer (SSI, Las Vegas, USA), (4) syringes hosted in (5) a temperature control chamber (SSI), (6) a small column of water scrubber after the animal chambers, and (7) the CA-10 CO<sub>2</sub> analyzer (SSI). The back pump (8) sent CO<sub>2</sub>-free air through multiplexer to the animals in the channels that were not open to the gas analyzer. The whole system was connected to a computer through a UI-2 unit (9, SSI). (B) Respirometry of ants freely moving in a large chamber. Compressed air went through the mass flow controller and a water–CO<sub>2</sub>–water scrubber column, and then was sent through the animal chamber (10) (35 cm × 25 cm × 2 cm). After the animals, the air was sent through a small water scrubber column and then the gas analyzer. A temperature pad (black) was placed under the chamber, which kept the temperature in the chamber at 26–27 °C. Temperature was recorded by a thermometer (11, TC-1000, SSI) with a probe inside the chamber. A video camera (12) was placed 35-cm above the chamber. The whole system was controlled by a computer via a UI-2 unit.