RESEARCH ARTICLE

Metabolic rate scaling, ventilation patterns and respiratory water loss in red wood ants: activity drives ventilation changes, metabolic rate drives water loss

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ABSTRACT

Metabolic rate and its relationship with body size is a fundamental determinant of many life history traits and potentially of organismal fitness. Alongside various environmental and physiological factors, the metabolic rate of insects is linked to distinct ventilation patterns. Despite significant attention, however, the precise role of these ventilation patterns remains uncertain. Here, we determined the allometric scaling of metabolic rate and respiratory water loss in the red wood ant, as well as assessing the effect of movement upon metabolic rate and ventilation pattern. Metabolic rate and respiratory water loss are both negatively allometric. We observed both continuous and cyclic ventilation associated with relatively higher and lower metabolic rates, respectively. In wood ants, however, movement not metabolic rate is the primary determinant of which ventilation pattern is performed. Conversely, metabolic rate not ventilation pattern is the primary determinant of respiratory water loss. Our statistical models produced a range of relatively shallow intraspecific scaling exponents between 0.40 and 0.59, emphasising the dependency upon model structure. Previous investigations have revealed substantial variation in morphological allometry among wood ant workers from different nests within a population. Metabolic rate scaling does not exhibit the same variability, suggesting that these two forms of scaling respond to environmental factors in different ways.

KEY WORDS: Intraspecific scaling, Metabolic scaling, Cyclic ventilation, Continuous ventilation, Water loss, Allometry

INTRODUCTION

Metabolism is the sum of anabolic and catabolic processes that enable the formation of biological material and fuel cellular and physiological work (Alexander, 1999; Chown and Nicolson, 2004). As such, the rate at which metabolism occurs is linked intrinsically to how much energy and how many resources are available for movement, growth and reproduction (Brown et al., 2004; Glazier, 2015). These fundamental properties affect other life history traits, such as senescence, and more holistic traits, such as survivability (Burton et al., 2011; Speakman, 2005). Thus, metabolic rate is often thought to be a key determinate of organismal fitness, though direct evidence for this is lacking (Pettersen et al., 2018).

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Movement, growth and reproduction increase metabolic rate in most animals (but see Glazier, 2015), including insects (Basson and Terblanche, 2010; Chown and Nicolson, 2004; Clusella-Trullas et al., 2010; Glazier, 2009; Hammond and Diamond, 1997; Heglund and Taylor, 1988; Lighton, 1990; Niven and Scharlemann, 2005; Weibel and Hoppeler, 2005). Larger body masses also generate higher metabolic rate, although smaller individuals have a higher mass-specific metabolic rate, a pattern reflected in both intraspecific and interspecific relationships (Bartholomew et al., 1988; Brown et al., 2004; Chown et al., 2007; Gillooly et al., 2001; Glazier, 2005). The ultimate cause of this negative allometry is undetermined despite intensive scrutiny of interspecific metabolic rate, which has left intraspecific metabolic rate relatively under-examined (Brown et al., 2004; Burton et al., 2011; Chown et al., 2007; Glazier, 2005; Terblanche et al., 2007; West et al., 1997, 1999). Yet, any population with significant variation in adult body size will experience intraspecific metabolic rate scaling (Glazier, 2005). Therefore, any differences in intraspecific metabolic rate scaling form an important part of explaining behavioural and fitness differences between individuals (Burton et al., 2011).

The metabolic rate of insects is correlated with the pattern of ventilation (Contreras and Bradley, 2009, 2010; Gibbs and Johnson, 2004). There are three principal ventilation patterns observed in insects; continuous, discontinuous and cyclic (Chown and Nicolson, 2004), though the adaptive value of these patterns remains unclear (Chown et al., 2006; Gudowska et al., 2015; Marais et al., 2005; Schimpf et al., 2012; Talal et al., 2016). To date, most studies have focused on discontinuous gas exchange cycles (DGC) (Buck and Kesiter, 1955; Chown et al., 2006; Lighton, 1994; Quinlan and Gibbs, 2006), though the majority of insects do not use DGC (Marais et al., 2005). Therefore, it is important to understand the causes and consequences of the other more prevalent ventilation patterns: cyclic and continuous (Contreras and Bradley, 2009). Increases in metabolic rate induce changes in ventilation pattern, from DGC at the lowest metabolic rates, switching to cyclic ventilation as metabolic rate increases and continuous ventilation at the highest (Basson and Terblanche, 2010; Contreras and Bradley, 2010; Klok and Chown, 2005).

Increases in temperature and activity increase metabolic rate, which has been shown to drive changes in ventilation pattern (Contreras and Bradley, 2010). However, another hypothesis links ventilation type to respiratory water loss (Chown et al., 2006; Lighton, 1994). DGC was originally proposed to be an adaptation to reduce respiratory water loss (Buck and Kesiter, 1955), although contemporary studies have questioned this (Chown and Holter, 2000; Hetz and Bradley, 2005; Lighton and Berrigan, 1995; Matthews and White, 2010). Surprisingly, there is relatively little information addressing the effects of other ventilatory patterns on respiratory water loss (Chown, 2002).

Ants are often ecologically influential (Hölldobler and Wilson, 1990), so their metabolic rate and water loss, along with the factors that affect them, are of broad significance. Metabolic rates in ants scale with negative allometry, both intraspecifically and interspecifically (Bartholomew et al., 1988; Chown et al., 2007; Lighton, 1989; Lighton and Wehner, 1993). Investigations into ant ventilation patterns have shown that they can be DGC, cyclic or continuous (Gibbs and Johnson, 2004; Lighton, 1989; Lighton and Berrigan, 1995; Lighton and Garrigan, 1995; Quinlan and Lighton, 1999). Respiratory water loss in ants has been positively correlated with metabolic rate (Chown, 2002; Schilman et al., 2005) and shown to change with differing ventilation types (Gibbs and Johnson, 2004).

Wood ants are a keystone species that exert effects upon invertebrate and plant populations (Domisch et al., 2009; Hawes et al., 2002; Skinner and Whittaker, 1981), as well as having a role in nutrient cycling (Finér et al., 2013). This impact manifests primarily through workers foraging for aphid honeydew and scavenging other invertebrates (Skinner, 1980). Consequently, the metabolic rate of wood ant workers, among which body size varies considerably, has a direct impact on their environment. Here, we studied the metabolic rate, respiratory water loss and ventilation patterns of wood ant foragers covering a broad range of naturally occurring body sizes. We show that metabolic rate and respiratory water loss both scale with negative allometry. Foragers' respiratory water loss can be explained solely through increases in metabolic rate. The ventilation pattern performed by ants is strongly associated with metabolic rate, independent of any changes induced by increasing mass. However, changes in metabolic rate are not necessary to cause changes in ventilation type; instead, activity is the trigger that induces changes in ventilation pattern in wood ants.

MATERIALS AND METHODS
Animals
Formica rufa L. (Hymenoptera: Formicidae) foragers were collected from Ashdown Forest, East Sussex, UK (51.0780, 0.0300) on five separate dates from 25 August to 19 September 2016. Four nests were visited and approximately 30 ants were collected from each nest on each collection date. Nest locations were as follows: nest 1: 51.07555, 0.02962; nest 2: 51.07531, 0.03524; nest 3: 51.07552, 0.03475; nest 4: 51.07549, 0.03019. Not all the ants collected were analysed; ants were kept for a maximum of 10 days. Upon collection, workers from each nest were housed separately and provided with a diet of water and sugar water *ad libitum*. Subsequently, the whole of nest 3 was collected and moved to the laboratory on 25 May 2017. The nest was kept under a 12 h:12 h light:dark regime and fed sugar water and frozen crickets *ad libitum*.

Metabolic rate and water loss measurement
Experiment 1
The metabolic rate and water loss of individual ants were measured using an LI-7000 dual-channel CO_2 infra-red gas analyser (LI-COR, Lincoln, NE, USA). Air at room temperature (22–24°C) was pumped using an SS4 Subsampler (Sable Systems International, Las Vegas, NV, USA) through two pairs of scrubbing columns; the first pair contained soda lime to remove CO_2 and the second pair contained Drierite (W.A. Hammond Drierite Co., Xenia, OH, USA) to remove water vapour. Air flow was then split between two identical chambers of 30 ml connected in parallel. Air flow into each chamber was maintained at 100 ml min^{-1}, regulated by two mass flow controllers (GFC17, Aalborg, NY, USA) – one for each

chamber. One chamber served as the reference chamber, the other as the test chamber. The reference chamber remained empty and the test chamber contained a single ant. After passing through the chambers, air flowed into two separate channels of the LI-7000, where output was recorded by LI-COR software. The sample rate was 2 Hz. Individual ants remained in the test chamber for between 30 min and 1 h.

A total of 107 ants were used for the analysis of experiment 1, 29 ants from nest 1, 25 ants from nest 2, 26 ants from nest 3 and 27 ants from nest 4.

Experiment 2
Ants from nest 3 were placed in the test chamber (see above) twice; once restrained with modelling clay (Plasticine®) and once unrestrained. The order of the treatments was randomised. During the restrained treatment only, an equal mass of modelling clay was placed in the reference chamber to ensure that the volume of the two chambers was equal. A total of 24 ants were used for experiment 2.

Ventilation type classification and exclusions
Ventilation type was classified from the metabolic rate traces. Ants showed one of two ventilation types: continuous or cyclic (Fig. 1). Ventilation patterns were classified following Gray and Bradley (2006) and Lighton (1994). Cyclic ventilation was characterised by pronounced, regular peaks and troughs, where CO_2 ventilation dropped to zero but did not remain at zero for an appreciable length of time (Fig. 1). Continuous ventilation had no obvious regularity, CO_2 ventilation never reached zero and carbon dioxide was continuously excreted (Fig. 1). Some ants demonstrated both ventilation types whilst in the chamber. In such cases, the metabolic rate of the ant was calculated from the most prevalent ventilation type.

Four ants had intermediate ventilation types that could not be confidently assigned to one category and, consequently, were excluded from experiments 1 and 2. Four recordings that showed baseline drift were also excluded from experiment 1. In two cases, ants died during or shortly after the end of a recording; these too were excluded from our analysis.

Ant mass
Ants were dried in a Gallenkamp Hotbox Oven Size 1 (Weiss Technik UK, Loughborough, UK) for 7 days at 50°C. Individual ants were then weighed to the nearest 0.1 mg using an AV264C Adventurer Pro Analytical Balance (Ohaus, Greifensee, Switzerland). Subsequently, we removed 22 ants directly from an additional nest, immobilised them on ice and measured their fresh mass. These ants were then dried as above and their dry mass was measured. The ants had a mean fresh water content of 69.7%, similar to values from other studies, which have reported fresh water content to be 66–70% (Lighton and Feener, 1989; Lighton and Wehner, 1993). Consequently, a correction factor of 70% was applied to the dry mass data.

Analysis
The total rate of CO_2 production ($\dot{V}_{CO_2}$) and H_2O loss ($\dot{V}_{H_2O}$) for each ant was calculated using Origin 2016 (https://www.originlab.com/2016) and a mean rate was obtained. All statistical analyses were conducted using R v.3.3.3 (http://www.R-project.org/).

To calculate the rate of CO_2 and H_2O production per ant, the concentration of CO_2 (ppm) was converted to CO_2 fraction and then multiplied by the flow rate (100 ml min^{-1}). The integral of CO_2 min^{-1} versus min was calculated for a stable period of the recording. This value was then divided by the exact measurement
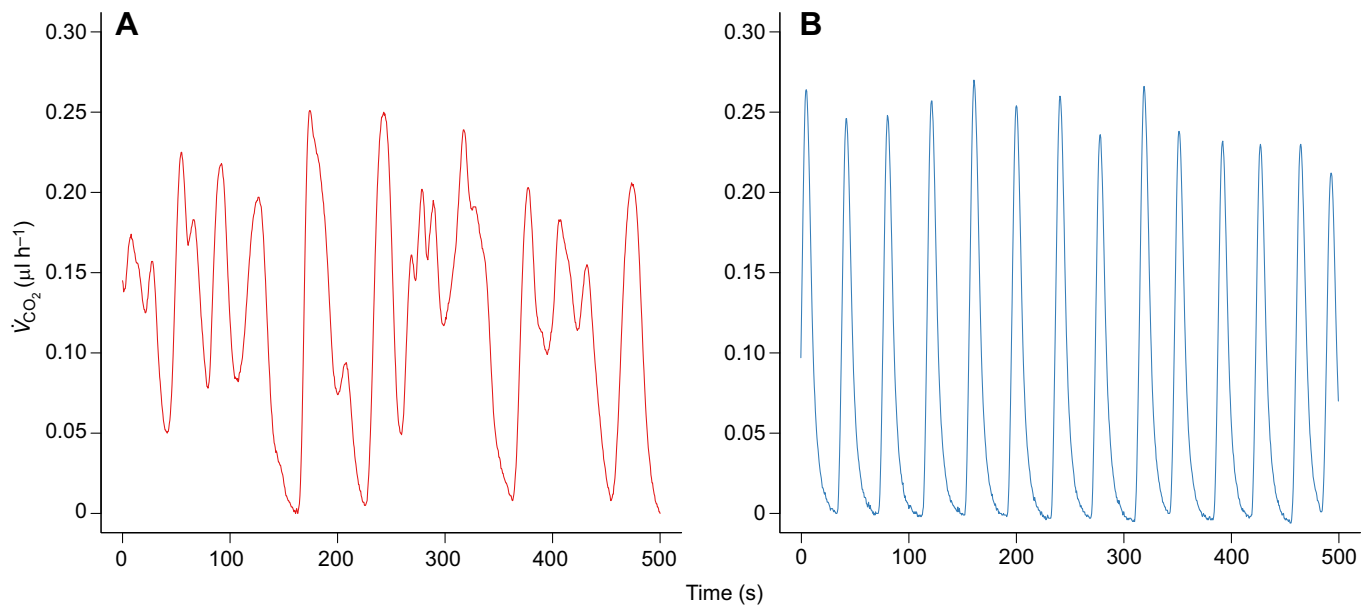
Fig. 1. *Formica rufa* workers use two different ventilation types for gas exchange. Traces of carbon dioxide excretion ($\dot{V}_{CO_2}$) during: (A) continuous gas exchange and (B) cyclic gas exchange.

time for each ant, to give a rate of ml CO_2 h^{-1}. An identical conversion was used for analysing the water loss of each ant, with the additional conversion of volume of H_2O to mass of H_2O following Lighton (2008).

Experiment 1

The allometric scaling of metabolic rate and water loss on nest and ventilation type was assessed with linear mixed effects models using the lme function from the 'nlme' package (https://CRAN.R-project.org/package=nlme). A maximal model (Table 1) incorporating all biologically relevant interactions was fitted initially. Model simplification occurred through stepwise removal of non-significant factors until the minimal adequate model remained (Table 1). Data were log transformed to ensure comparability with previous studies of the allometric scaling of metabolic rate (e.g. Lighton and Bartholomew, 1988; Nicholls et al., 2017; Vogt and Appel, 1999) and to normalise the data. Collection date was included as a random factor to account for any temporal effects on metabolic rate (Table 1).

To determine how scaling exponents might depend upon the structure of the statistical model, we compared the scaling exponent derived from the linear mixed effects model (Table 1) with linear models in which the size of workers was the only independent variable and metabolic rate was the dependent variable. One contained all the ants regardless of ventilation type, another contained ants only using continuous ventilation, and yet another contained ants using only cyclic ventilation (Table 2).

Experiment 2

The likelihood of restraint affecting ventilation type was assessed using a conditional logistic regression, modelled using the 'survival' package (https://CRAN.R-project.org/package=survival). Cyclic and continuous ventilation were scored as 0 and 1, respectively. Changes in metabolic rate were analysed using t-tests and Wilcoxon signed-rank tests from the R base package.

RESULTS
Experiment 1
Metabolic rate scaling

We assessed the metabolic rates of wood ant workers selected from four nests within the same population (see Materials and Methods). The fresh mass of workers ranged from 4.7 to 26.7 mg and, despite being sampled from four distinct nests, the range of worker sizes did not differ significantly (ANOVA, $F_{3,107}=0.69$, $P>0.50$; Fig. S1). To determine their metabolic rates, we placed individual ants in a respirometry chamber and recorded continuously the amount of carbon dioxide that they excreted (see Materials and Methods). Whilst in the respirometry chamber, ants displayed two distinct ventilation patterns: cyclic and continuous (Fig. 1).

The metabolic rate of ants increased with increasing body mass ($t_{100,107}=3.08$, $P<0.002$). Overall, ant metabolic rate was negatively allometric, scaling with an exponent of 0.46±0.15 (mean±s.e.m.), indicating that larger ants have relatively lower metabolic rates than their smaller counterparts. Continuously ventilating ants had a higher metabolic rate than those using cyclic ventilation

Table 1. Structure of linear mixed effects models used for assessing metabolic and water loss rate scaling of *Formica rufa*

Model	Response	Fixed effects	Random effects
Maximal model	log Metabolic rate	(Nest+log dry mass+ventilation type)^2	Collection date
Minimal adequate model	log Metabolic rate	log Dry mass+ventilation type	Collection date
Maximal model	log Water loss	(Nest+log dry mass+ventilation type)^2	Collection date
Minimal adequate model	log Water loss	log Dry mass+ventilation type	Collection date
Maximal model	log Water loss	(Nest+log dry mass+ventilation type+log metabolic rate)^2	Collection date
Minimal adequate model	log Water loss	log Metabolic rate	Collection date

Table 2. Different models used to assess variability of metabolic rate scaling exponents

Model type	Dependent variable(s)	Scaling exponent	Standard error
Linear mixed effects model	Continuous and cyclic ventilation+dry mass	0.48	0.12
Linear model	Continuous and cyclic ventilation+dry mass	0.60	0.17
Linear model	Continuous ventilation	0.52	0.17
Linear model	Cyclic ventilation	0.56	0.23

(Fig. 2A; $t_{100,107}=3.22$, $P=0.001$) but metabolic rate increased with size in a similar way irrespective of the type of ventilation being conducted ($F_{6,111}=0.05$, $P=0.82$). Mean metabolic rate did not differ between nests (Fig. 3; $F_{3,107}=1.61$, $P=0.19$), nor was there any difference in the response of metabolic rate to size

among all four nests ($F_{3,107}=0.30$, $P=0.83$). Mean metabolic rate of the different ventilation types was also similar among nests ($F_{3,107}=0.37$, $P=0.77$).

Effect of different models on metabolic scaling exponent

Several different methods are available for estimating allometric scaling exponents. To determine the extent to which different methods and statistical models influenced scaling exponents, we constructed a number of different biologically relevant models. We found a difference in the allometric scaling exponents estimated by different statistical models (Fig. 2, Table 2). The linear mixed effects model, which included ventilation type as a factor, predicted slopes of 0.46±0.15 (Fig. 2A), whereas a linear model, in which ventilation type was ignored, predicted a slope of 0.59±0.19 (Fig. 2B). Two additional linear models that analysed continuously and cyclically ventilating ants independently also produced
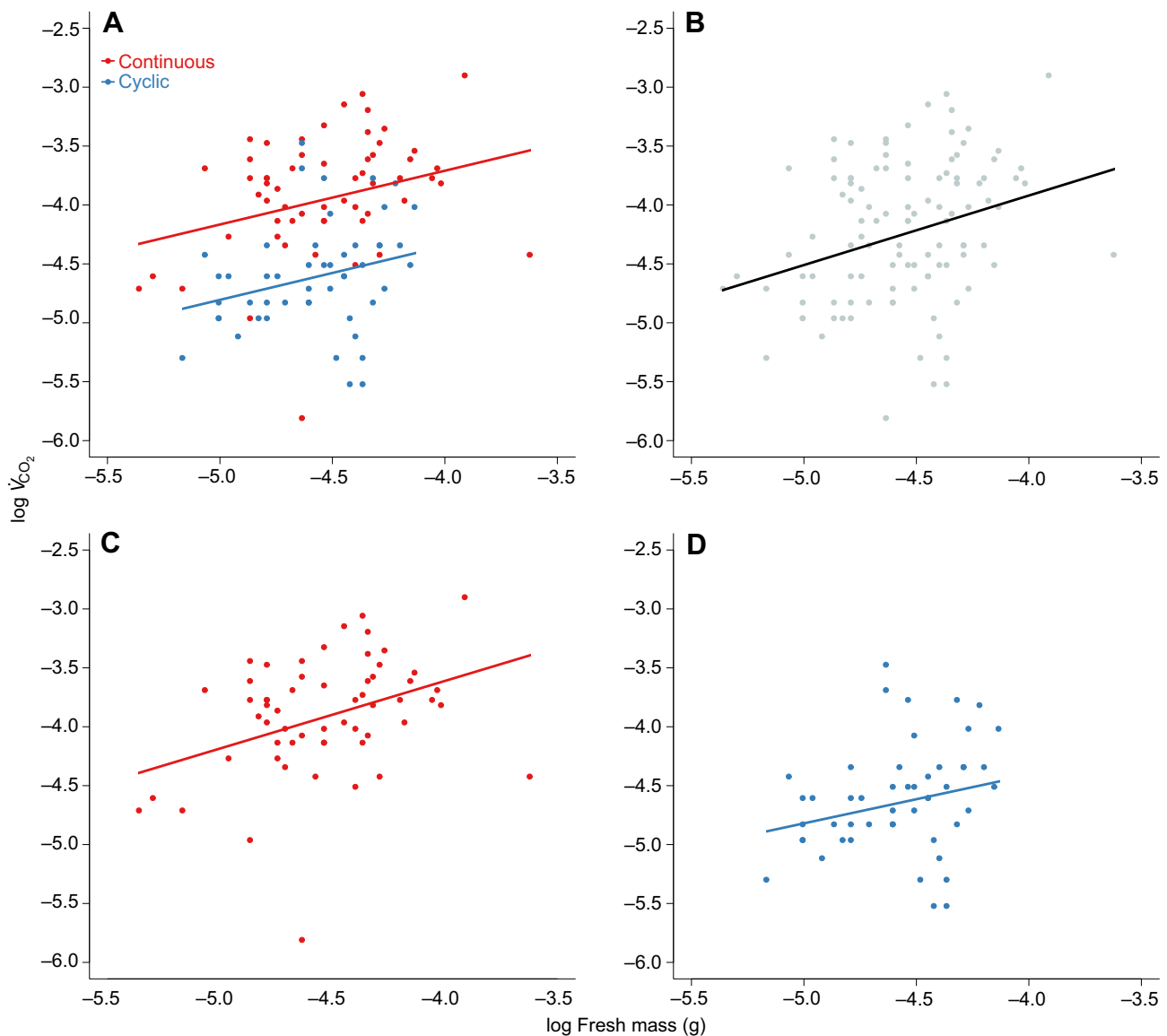

Fig. 2. *Formica rufa* **worker metabolic rate is higher when ventilating continuously versus cyclically and scales with negative allometry.**
Metabolic rate (measured as $\dot{V}_{CO_2}$ in ml h^{-1}) during different ventilation patterns does not differ in response to body size (mass in g) increases.
(A) Parameters estimated from a linear mixed effects model using data from all ants. (B) Parameters estimated from a linear model using data from all ants.
(C) Parameters estimated from a linear model using data from ants using only continuous gas exchange. (D) Parameters estimated from a linear model using data from ants using only cyclic gas exchange.
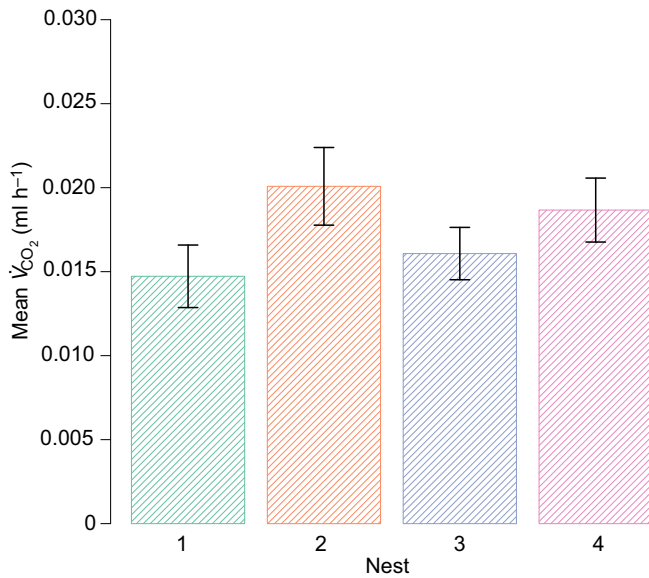
Fig. 3. *Formica rufa* metabolic rate does not differ between nests. Data are mean (±s.e.m.) metabolic rate.

different scaling exponents. The linear model with only continuously ventilating ants predicted a slope of 0.57±0.20 (Fig. 2C) and the linear model with only cyclically ventilating ants predicted a slope of 0.40±0.25 (Fig. 2D). Consequently, the structure of the statistical model could strongly affect the scaling exponent value that is reported.

Water loss scaling

We simultaneously monitored water loss for each ant (Fig. 4) in addition to the carbon dioxide excreted. Water loss increased with increasing body mass (Fig. 5; $t_{3,107}$=2.01, $P<0.05$). Overall, water loss was negatively allometric, scaling with an exponent of 0.53 ±0.26, indicating that larger ants lose relatively less water that their smaller conspecifics. Mean water loss was higher when ants were

ventilating continuously than when they were ventilating cyclically (Fig. 5; $t_{3,107}$=4.77, $P<0.001$), although the scaling of water loss with increasing mass did not differ between the two ventilation types ($F_{1,107}$=0.19, $P=0.66$). Mean water loss ($F_{3,107}$=1.53, $P=0.21$) and the scaling of water loss ($F_{3,107}$=0.34, $P=0.80$) were non-significantly different among all four nests. Mean water loss also did not differ among nests for the two different ventilation types ($F_{3,107}$=1.55, $P=0.21$).

Response of water loss to metabolic rate

Having determined that water loss and metabolic rate responded similarly to body size, nest affiliation and ventilation type, we assessed the response of water loss to metabolic rate. Given that ventilation type and size proved important in the previous models, we fitted a maximal model that included dry mass, nest affiliation and ventilation type to ascertain the response of water loss in the context of all potentially relevant variables (Table 1). Metabolic rate was, however, the only significant predictor of water loss (Fig. 6; $F_{1,107}$=60.41, $P<0.001$). Water loss increased with increasing metabolic rate (Fig. 6; $t_{101,107}$=7.77, $P<0.001$) with an isometric relationship (slope=0.95±0.12), showing that respiratory water loss is driven only by metabolic rate; there is no difference in water loss among ventilation types when metabolic rate is accounted for. Mean water loss did not differ significantly between nests ($F_{3,107}$=1.04, $P=0.38$) or ventilation types ($F_{3,107}$=1.53, $P=0.22$). The scaling of water loss with metabolic rate ($F_{1,107}$=0.40, $P=0.75$) and mass ($F_{3,107}$=0.19, $P=0.90$) also did not differ among nests. Mean water loss did not differ significantly among ants from different nests using different ventilation patterns ($F_{1,107}$=1.49, $P=0.22$). Mean water loss was also not significantly different between ants using continuous and cyclic ventilation independent of nest affiliation ($F_{1,107}$=1.53, $P=0.22$). Water loss scaling with both metabolic rate ($F_{1,107}$=1.17, $P=0.28$) and mass ($F_{1,107}$=1.68, $P=0.20$) was not significantly different among ventilation types. Increases in mass did not cause an increase in water loss ($F_{1,107}$=0.39, $P=0.53$). Furthermore, increases in water loss in response to mass were unaffected by simultaneous increases in metabolic rate ($F_{1,107}$=0.26, $P=0.61$).
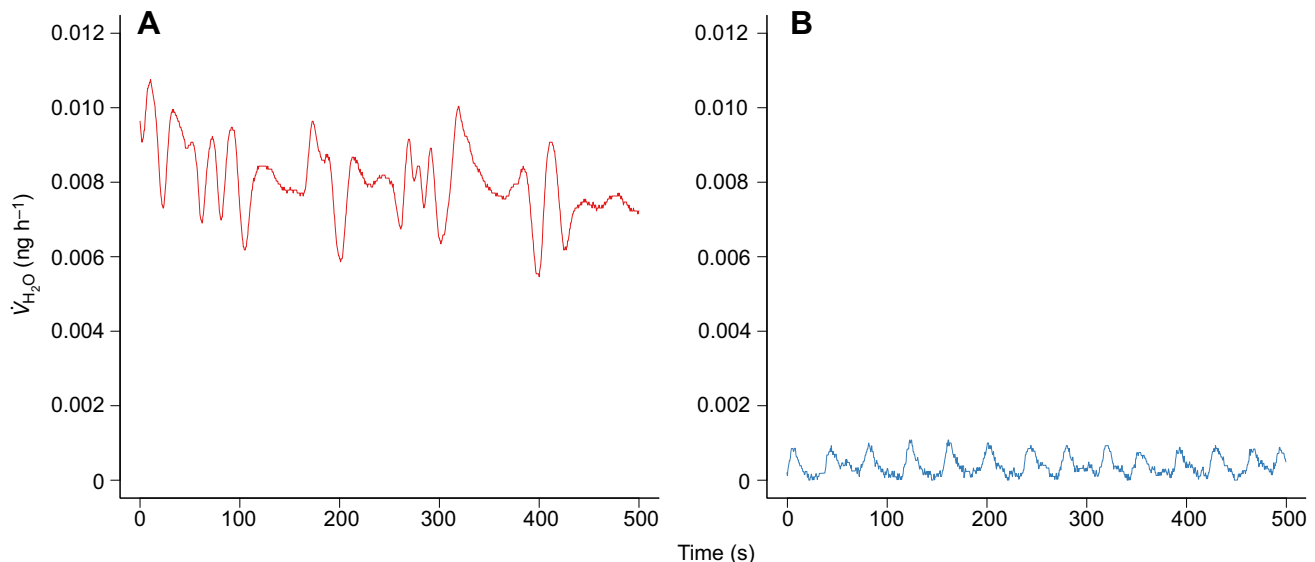

Fig. 4. *Formica rufa* workers use two different ventilation types for gas exchange, which is reflected in the patterns of respiratory water loss. Respiratory water loss ($\dot{V}_{H_2O}$) during: (A) continuous gas exchange and (B) cyclic gas exchange.
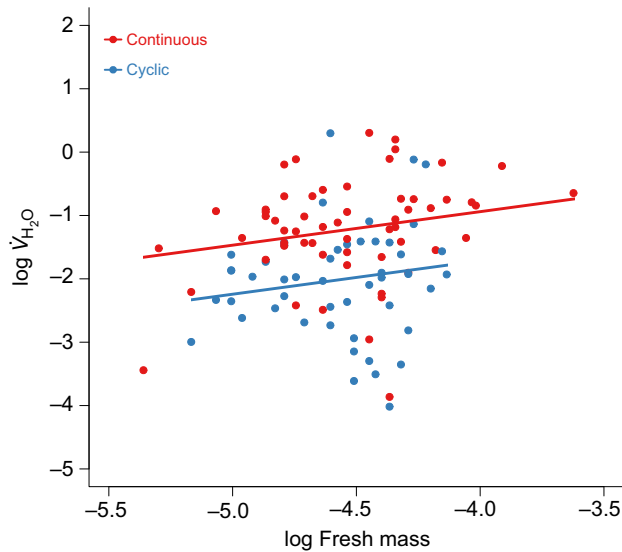
Fig. 5. Respiratory water loss of *F. rufa* workers scales with negative allometry and is higher when ventilating continuously versus cyclically. Water loss rates ($\dot{V}_{H_2O}$ in ng h^{-1}) during different ventilation patterns do not differ in response to body size (mass in g) increases.

Fig. 7. *Formica rufa* workers do not change their metabolic rate when restrained or unrestrained. Black circles and bar show mean (±s.e.m.) metabolic rate and paired green circles show individual ant metabolic rate.

Experiment 2

The differences in metabolic rate and water loss between ants performing continuous and cyclic ventilation may have been due to activity within the respiratory chamber. To test whether movement was indeed linked to changes in ventilation type, we examined the effects of restraining ants within the chamber. Unrestrained ants were 11±1.04 times more likely to engage in continuous gas exchange than in cyclic gas exchange (conditional logistic regression; $z_{1,48}=2.30$, $P<0.02$), suggesting that continuous gas exchange is linked to activity whereas cyclic gas exchange occurs when ants are stationary. Surprisingly, there was no difference in the mean metabolic rate of restrained and unrestrained ants (Fig. 7;
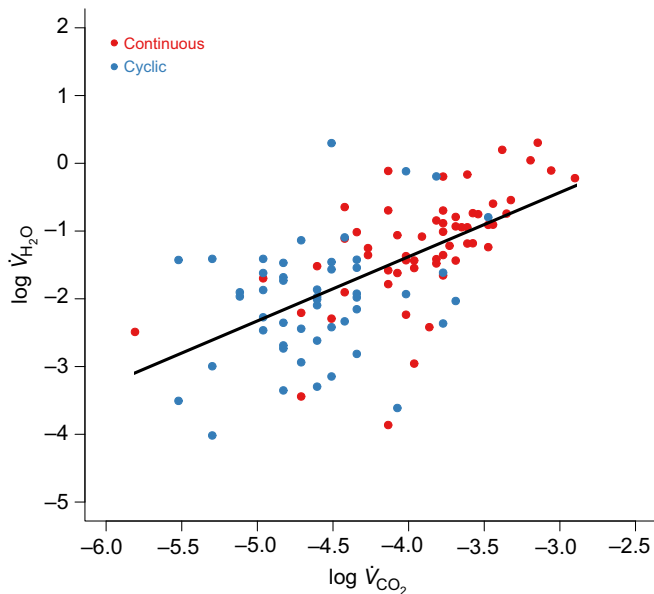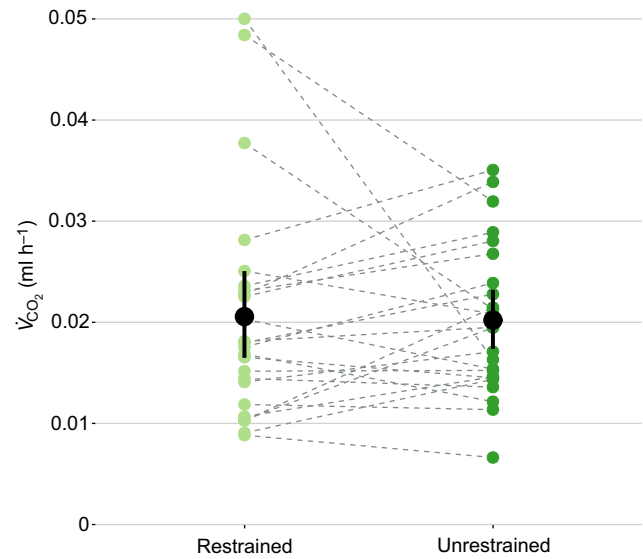
paired *t*-test, $t_{23,48}=0.17$, $P=0.87$) and individual ants did not increase their metabolic rate when restrained compared with when they were unrestrained (Fig. 7; Wilcoxon signed-rank test, $V=181$, $P=0.39$). Moreover, water loss was also not significantly different when ants were restrained or unrestrained (paired *t*-test, $t_{23,48}=0.14$, $P=0.89$). Consequently, changes in ventilation type are driven by activity but are independent of increases in metabolic rate.

To explain the absence of a significant difference in metabolic rate in the restrained versus the unrestrained ants, we compared the mean metabolic rate of the restrained ants performing cyclic ventilation ($N=16$) from experiment 2 with an equal number of size-matched unrestrained cyclically ventilating ants from experiment 1. There was a significant difference in the mean metabolic rate between the two groups of ants (Fig. 8; *t*-test, $t_{29,32}=3.51$, $P<0.002$); restrained ants from experiment 2 had a higher mean metabolic rate than unrestrained ants from experiment 1 (Fig. 8). This suggests that restraining ants elevated their metabolic rate compared with that of
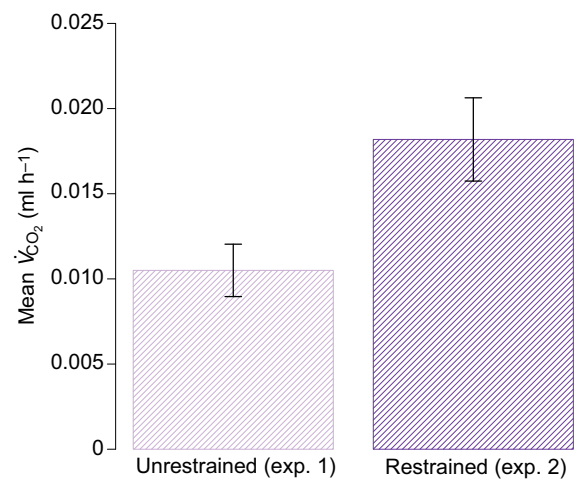

Fig. 6. Respiratory water loss is driven entirely by increases in metabolic rate. Different ventilation patterns do not differentially contribute to respiratory water loss; water loss ($\dot{V}_{H_2O}$ in ng h^{-1}) and changes in ventilation pattern coincide with changes in metabolic rate ($\dot{V}_{CO_2}$ in ml h^{-1}).

Fig. 8. *Formica rufa* metabolic rate differs between restrained and unrestrained cyclically ventilating ants. Mean (±s.e.m.) metabolic rate of unrestrained *F. rufa* workers from experiment 1 compared with that of restrained *F. rufa* workers from experiment 2.

stationary but unrestrained ants, emphasizing that activity, not elevated metabolic rate, causes the change in ventilation type.

DISCUSSION

The relationships between metabolic rate, water loss, ventilation pattern, activity and size in insects are uncertain with numerous, sometimes conflicting, interpretations (Chown, 2002; Chown et al., 2006; Gibbs and Johnson, 2004). We explored the interactions between these factors in foragers of the red wood ant, *Formica rufa*. Although large foragers had higher metabolic rates than their smaller counterparts, metabolic rate was negatively allometric so that larger ants had relatively lower metabolic rates than small ants. Foragers performed either cyclic or continuous ventilation. Ants performing cyclic ventilation had lower metabolic rates than equivalently sized ants performing continuous ventilation but an increase in metabolic rate did not drive the switch between these two ventilation patterns. Rather, the switch from cyclic to continuous ventilation is driven by activity; foragers perform cyclic ventilation whilst stationary at the same mean metabolic rate as they perform continuous ventilation whilst active.

Respiratory water loss, like metabolic rate, was negatively allometric so that larger ants lost absolutely more, but relatively less, water than their smaller counterparts. Ants performing cyclic ventilation lost less water than equivalently sized ants performing continuous ventilation. However, metabolic rate was the primary driver of respiratory water loss, with higher metabolic rates causing greater water loss. The independence of water loss from the ventilation pattern was confirmed by comparing restrained and unrestrained ants; similar rates of water loss occurred at similar metabolic rates despite differences in ventilation pattern. This emphasises that within wood ant foragers, both metabolic rate and respiratory water loss are independent of ventilation pattern, which is itself driven by activity.

Ventilation pattern is independent of metabolic rate

Higher metabolic rates in insects have been associated with distinct ventilation patterns (Contreras and Bradley, 2010; Gibbs and Johnson, 2004), with continuous ventilation being used during periods of high metabolic demand to ensure adequate gas exchange for cellular respiration (Chown, 2002; Gibbs and Johnson, 2004). In wood ant foragers too, continuous ventilation is associated with higher metabolic rates than is cyclic ventilation. However, increasing metabolic demand in wood ants occurs simultaneously with switching from cyclic to continuous ventilation because both are induced by the onset of movement, an interpretation supported by comparisons of restrained and unrestrained ventilation patterns. Unrestrained workers are far more likely to perform continuous gas exchange than restrained workers despite having similar metabolic rates, implying that ventilation pattern is independent of metabolic rate and is driven by activity. During experiment 2, activity was inferred rather than directly recorded and this presents a limitation on the conclusions drawn. However, our observations indicate that ants were immobile during the restrained treatment and that unrestrained ants moved freely around the chamber. Thus, stationary workers perform cyclic ventilation, with movement triggering continuous ventilation rather than an increase in metabolic rate.

Previous investigations that explicitly examined the switch from cyclic to continuous ventilation concluded that it is driven primarily by increasing metabolic rate (Basson and Terblanche, 2010; Gibbs and Johnson, 2004; Käfer et al., 2015; Moerbitz and Hetz, 2010; Nicholls et al., 2017). The activity-induced switch that we observed in wood ant foragers cannot explain all these observations. For

example, experimenters studying tsetse flies removed periods of activity from their analysis but still observed switching from cyclic to continuous ventilation (Basson and Terblanche, 2010). Consequently, metabolic rate in these flies appears to be the primary cause of switching ventilation patterns (Basson and Terblanche, 2010). However, Matthews and White (2013) also demonstrated changes in ventilation pattern without changes in metabolic rate in cockroaches. Studies of other taxa are more equivocal. Harvester ant queens have higher metabolic rates when ventilating continuously versus cyclically (Gibbs and Johnson, 2004). However, their activity was not recorded, raising the possibility that differences in activity drive changes in ventilation pattern.

Although we show a discrete switch between cyclic and continuous ventilation, the role of activity in directly driving ventilatory patterns could be subtler. There is evidence to suggest that ventilation patterns vary along a continuum rather than being discrete entities (Contreras and Bradley, 2010), and therefore differential activity could drive changes in cycle frequency, which would also account for our observations of discrete ventilation pattern switching. European paper wasps also change their ventilation pattern with increasing metabolic rate (Käfer et al., 2015). The increase in the frequency of ventilatory cycles with increasing metabolic rate coincided with an increase in activity (Käfer et al., 2015). This raises the possibility that movement could be responsible for the changes in ventilation pattern of paper wasps, just as in wood ants. Similar observations were made of weevils (Klok and Chown, 2005), where changes in cycle frequency, caused by increasing temperature, were accompanied by concurrent changes in activity (Klok and Chown, 2005). Activity drives metabolic rate, rather than the reverse (Glazier, 2015). Consequently, increased activity should trigger changes in ventilation pattern simultaneously, to ensure that adequate gas exchange can occur.

The absence of discontinuous gas exchange

Formica rufa workers did not perform DGC despite it being found in the workers (Lighton and Wehner, 1993; Quinlan and Lighton, 1999) and queens (Gibbs and Johnson, 2004; Lighton and Berrigan, 1995) of other ant species. This may not be surprising given that *F. rufa* workers' mean mass-specific metabolic rate during cyclic gas exchange is 1.15 ml CO_2 g^{-1} h^{-1} (±0.66), an order of magnitude higher than that of workers of other ant species that do perform DGC (Lighton and Berrigan, 1995; Lighton and Garrigan, 1995; Quinlan and Lighton, 1999). The large difference in mass-specific metabolic rate is probably due to the difference in size between these ant workers, *F. rufa* workers being substantially smaller. *Formica rufa* mass-specific metabolic rate may be too high to permit DGC.

Several aspects of wood ant ecology suggest DGC may be unnecessary. For example, wood ant workers are not at risk from dehydration because their mesic habitats have relatively high rainfall and their diet consists of honeydew (Collingwood, 1979), allowing lost water to be swiftly replaced. *Formica rufa* nests are formed of large mounds of twigs, leaves and needles; very little of the nest is subterranean, unlike nests of many other ants (Hölldobler and Wilson, 1990). Workers spend time on the surface of the nest or foraging; thus, workers are unlikely to suffer from poor respiratory gas diffusion due to claustral living (Chown et al., 2006; Lighton and Berrigan, 1995; White et al., 2007).

It is feasible that there are no adaptive functions of DGC (or cyclic gas exchange) (Contreras and Bradley, 2010) and that all ventilation patterns are a continuum, responding to fixed gaseous set-points

(Chown and Holter, 2000). The set-point hypothesis posits that the opening and closing of spiracles respond to local concentrations of carbon dioxide and oxygen independently, producing the observed ventilation patterns. However, this hypothesis cannot explain changes in ventilation pattern without an increase in metabolic rate. If spiracles were only responding to set-points, increased metabolism would be required to change the partial pressures of CO_2 and O_2 within the tracheae and induce changes in ventilation type. Instead, we observed changes in ventilation type independent of metabolic rate.

Metabolic rate drives respiratory water loss independent of ventilation pattern

There has been considerable debate about the cause of respiratory water loss in insects, although there is a consensus that larger insects with higher metabolic rates incur greater losses than smaller insects, and that open spiracles are associated with higher amounts of water loss (Addo-Bediako et al., 2001; Chown, 2002). Respiratory water loss in wood ant foragers increased with body mass, scaling isometrically. Continuous ventilation in these ants incurred greater water loss than cyclic ventilation but the primary driver was metabolic rate. The lower rates of water loss during cyclic ventilation are due to lower metabolic rates, rather than to any reduction caused by the pattern itself (Gibbs and Johnson, 2004). This is consistent with the strong link between water loss and metabolic rate reported across insects (Chown, 2002).

Homogeneous physiological scaling among colonies

The homogeneity of scaling in metabolic rate and respiratory water loss among workers from *F. rufa* nests contrasts with the heterogeneity in morphological scaling demonstrated by the implementation of different growth rules for compound eye growth (facet size versus facet number) in different nests (Perl and Niven, 2016). An explanation for this difference is that environmental effects causing variation in morphological scaling do not affect metabolic scaling. Morphological variability of ant workers is likely to be a consequence of exposure to different environments (e.g. nutrition) during growth and development. However, in honey bees, larval starvation has no effect on imago metabolic rates under normal feeding conditions (Wang et al., 2016), raising the possibility that metabolic scaling is resilient to environmental factors that generate variability in morphological scaling. Furthermore, the homogeneity of metabolic rate scaling among colonies indicates that any differences in fitness between nests are unlikely to be mediated by forager metabolic rate.

Some studies have suggested that metabolic rate scaling is caused by differential increases in body size through cell number or cell size (Davison, 1955; Kozłowski et al., 2003). Growth mediated by increased cell number produces isometric scaling whereas growth through increased cell size produces an exponent of 0.67 (Chown et al., 2007; Davison, 1955; Kozłowski et al., 2003). This idea has been tested within ants, predicated on the idea that relative cell number (facet number) and cell size (facet size) in compound eyes are an accurate proxy for the entire body (Chown et al., 2007). Were this true in *F. rufa*, heterogeneity in the scaling of worker eyes among nests (Perl and Niven, 2016) would reflect scaling of their entire bodies, producing different metabolic scaling exponents among nests. The absence of differences in metabolic scaling among nests despite differences in the contributions of facet number and size to compound eye scaling (Perl and Niven, 2016) suggests that this does not apply to wood ants. However, direct measurements of both metabolic and morphological scaling from the same nests are necessary to confirm this fully.

Intraspecific metabolic scaling in wood ant foragers

The metabolic rate of wood ant foragers is negatively allometric. However, depending on the statistical model fitted, the scaling exponent differed numerically. Fitting a linear mixed effects model incorporating ventilation pattern estimated a scaling exponent of 0.47, lower than intraspecific metabolic rate scaling exponents of ant workers (Table 3) and other invertebrate taxa, aside from ribbon worms (0.48) (Glazier, 2005). The exponents for individual linear models for each ventilation type (0.52 and 0.56 for continuously and cyclically ventilating ants, respectively; Table 2) were also lower than estimates from other invertebrates (Glazier, 2005) but were similar to estimates from *Camponotus fulvopilotus* (Table 3; Chown et al., 2007; Lighton, 1989). However, a linear model estimated a scaling exponent of 0.60 (Table 2), which is consistent with metabolic rates previously reported for workers of *F. rufa* and other species (Table 3; Chown et al., 2007). All the scaling exponents are also lower than interspecific scaling relationships among ants (Table 3; Chown et al., 2007; Lighton and Wehner, 1993) and other insects (Addo-Bediako et al., 2002; Chown et al., 2007; Lighton et al., 2001; Niven and Scharlemann, 2005), a common pattern when comparing interspecific and intraspecific metabolic rate scaling (Glazier, 2005; Harrison, 2017). The probable cause of the relatively lower intraspecific metabolic rate scaling exponents is the smaller range of body sizes available within a species, compared with the ranges available interspecifically. The numerical differences between exponent estimates are important if scaling

Table 3. Summary of scaling exponents derived from different ant workers

Species	Scaling exponent	Ventilation pattern	Ant activity	Reference
Camponotus fulvopilosus	0.55	Undefined	Uncontrolled	Lighton, 1989
Eciton hamatum	0.84	Undefined	Uncontrolled	Bartholomew et al., 1988
Pogonomyrmex rugosus	0.70	Undefined	Controlled	Lighton and Bartholomew, 1988
Anoplolepis steinergroeveri	0.61	Undefined	Uncontrolled	Chown et al., 2007
Atta columbica	0.64			
Camponotus fulvopilosus	0.56			
Camponotus maculatus	0.60			
Eciton hamatum	0.84			
Formica rufa	0.69			
Messor capensis	1.28			
Messor pergandei	0.61			
Interspecific scaling	0.76	–	–	Mason et al., 2015
Interspecific scaling	0.93	–	–	Lighton and Wehner, 1993
Interspecific scaling	0.80	–	–	Vogt and Appel, 1999

relationships are to be used as a predictive tool (Glazier, 2005). One explanation for the differences in scaling exponent is whether the statistical model incorporated the ventilation pattern. This demonstrates that estimates of scaling exponents that ignore ventilation pattern (Vogt and Appel, 1999) and do not control for activity (Lighton and Bartholomew, 1988) should be treated with caution.

Data availability
Data are available from the figshare digital repository: https://figshare.com/s/cb6954372acd7730d749

Supplementary information
Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.182501.supplemental

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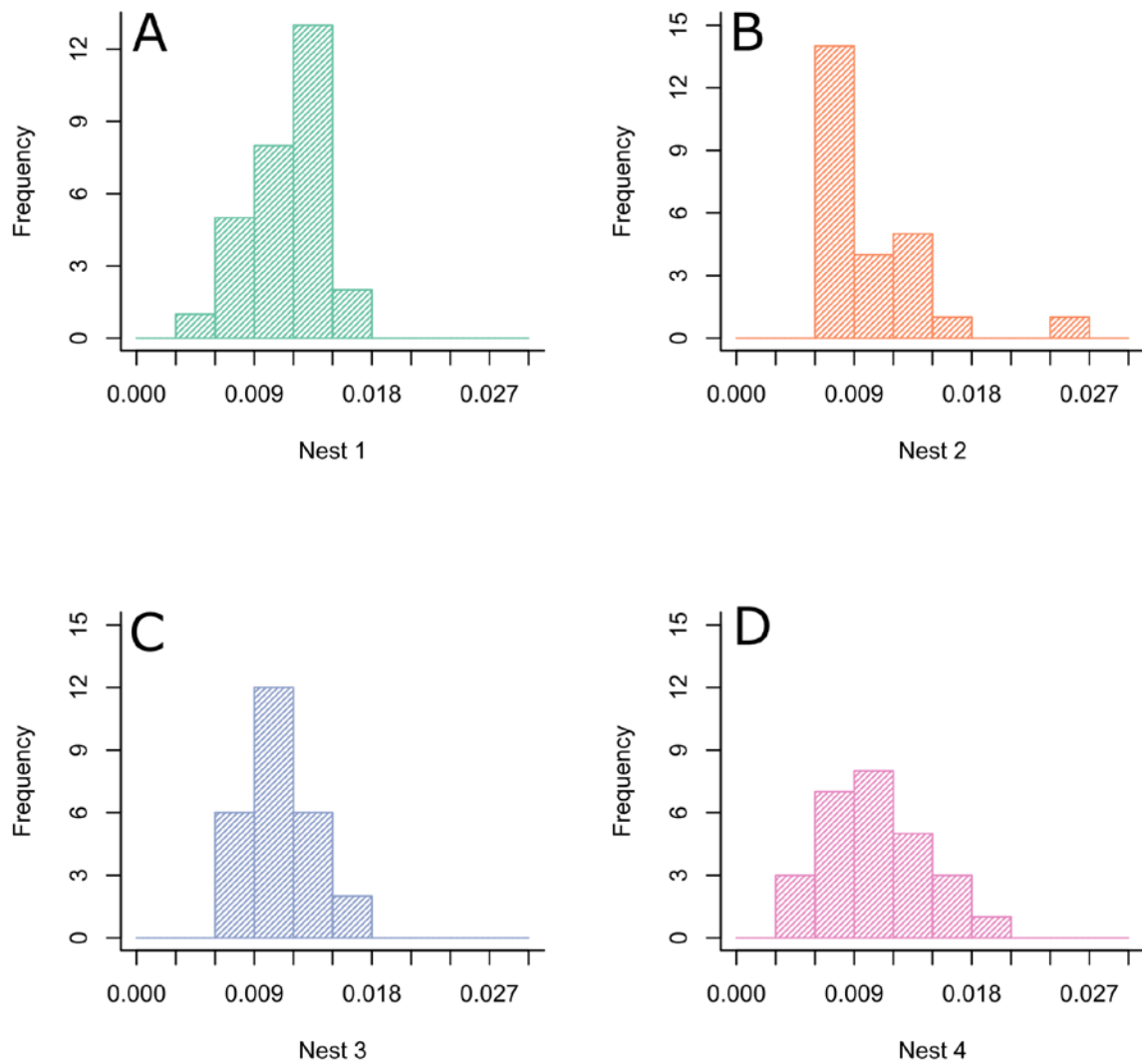
Figure S1. *Formica rufa* worker size did not differ among nests. Size distributions of workers from all four nests: A) Nest 1; B) Nest 2; C) Nest 3, D) Nest 4.