

RESEARCH ARTICLE

The energetics of running stability: costs of transport in grass-cutting ants depend on fragment shape

Karin Moll^{1,*}, Walter Federle¹ and Flavio Roces²

¹Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK and ²Department of Behavioural Physiology and Sociobiology, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

*Author for correspondence (km498@cam.ac.uk)

Accepted 12 October 2011

SUMMARY

Grass-cutting ants (*Atta vollenweideri*) carry fragments that can be many times heavier and longer than the ants themselves and it is important for them to avoid falling over during load transport. To investigate whether the energetic costs of transport are affected by the need to maintain stability, the rate of CO₂ production was measured in both unladen workers and workers carrying standardized paper fragments of different size and shape. We tested: (1) the effect of mass by comparing workers carrying either light or heavy fragments of the same size, and (2) the effect of shape by comparing short and long fragments of the same mass. Consistent with previous studies, metabolic rate increased but running speed remained constant when ants carried heavier fragments. The net cost of transport (normalized to the total mass of ant and fragment) was the same for heavy and light fragments, and did not differ from the costs of carrying a unit body mass. Ants carrying long fragments showed similar metabolic rates but ran significantly slower than ants carrying short fragments. As a consequence, net cost of transport was significantly higher for long fragments than for short ones, and higher than the costs of carrying a unit body mass. The observed reduction in running speed is likely a result of the ants' need to maintain stability. When the absolute costs of transport were compared, smaller ants required more energy to carry heavier and longer fragments than larger workers, but the opposite was found for lighter and shorter fragments. The absolute costs of transport per unit fragment mass suggest that it is energetically advantageous for a colony to allocate smaller workers for the transport of small fragments and larger workers for large fragments. The present results underline the importance of biomechanical factors for the understanding of leaf-cutting ant foraging strategies.

Key words: energetics, terrestrial locomotion, static stability, load, ant, *Atta*.

INTRODUCTION

Ants are common model organisms for studies of central-place foraging. The foraging success of an ant colony is largely determined by the strategies workers use to exploit the available resources (Traniello, 1989). Foragers are responsible for the energy intake of the entire colony and therefore incur the complete costs of colony food acquisition. The foraging costs include the locomotion costs of foragers from the nest to the food source, the costs of food collection once at the source, the costs of carrying loads back to the nest and, at the colony level, the costs of worker replacement due to increased mortality during foraging (Porter and Jorgensen, 1981; Fewell, 1988; Schilman and Roces, 2006).

Costs of load carriage have been studied in many animals, ranging from vertebrates to insects. Most of these studies have investigated the effects of loads directly attached to the animal's body, or of liquid 'loads' carried internally in the crop (Duncan and Lighton, 1994; Lighton et al., 1993; Taylor et al., 1980). However, animals often carry loads with their limbs or teeth (or mandibles), where the load is located further away from the body center, resulting in a significant displacement of the center of gravity. As static stability during locomotion requires the center of mass to remain within the polygon of the supporting legs (Ting et al., 1994), large off-center loads increase the carrier's risk of falling over. A shift of the center of mass is known to have a strong influence on the running kinematics in ants (Zollikofer, 1994). Although previous studies in

ants have shown that metabolic costs during transport increase with load mass (Duncan and Lighton, 1994; Lighton et al., 1993; Nielsen and Baroni Urbani, 1990), it is still unknown whether stability constraints cause additional costs, and whether these costs influence the ants' foraging decisions. Measurements of energy expenditure during transport of loads that cause a larger or smaller shift of the center of gravity may provide direct evidence for the importance of biomechanical factors in foraging energetics.

Leaf-cutting ant foragers carry leaf fragments that can be larger than their own bodies (Lutz, 1929; Rudolph and Loudon, 1986; Wetterer, 1990). Biomechanical problems associated with the size of the carried fragments are particularly evident in leaf-cutting ants that have specialized in harvesting grass, the so-called grass-cutting ants (Jonkman, 1976; Jonkman, 1979; Robinson and Fowler, 1982). These ants carry fragments of grass blades by holding them with their mandibles in an upright position that is slightly tilted backwards. In our study species, *Atta vollenweideri*, the lengths of grass fragments cut in the field range from approximately 5 to 60 mm (Röschard and Roces, 2003b) and can consequently exceed by many times the ants' body length (e.g. body lengths of foraging ants in our laboratory colony: 5.0–7.6 mm). Ants minimize the backward shift of the center of mass during grass transport by holding long fragments more steeply than shorter ones (Moll et al., 2010). Nevertheless, the transport of long fragments is still associated with a backward shift of the center of mass, which leads to an increased risk of falling over (Moll et al.,

2010). Furthermore, running speed decreases with increasing fragment length (Röschard and Roces, 2002), which is presumably caused by the ants' need to maintain stability.

To investigate whether the energetic costs of load transport are affected by the need to maintain stability, two different experiments were performed. First, we measured metabolic rates in individual *A. vollenweideri* workers that carried standardized paper fragments of similar shape but different mass, for which no marked difference in the position of the fragment's center of mass during transport is expected. Second, metabolic rates were quantified for ants carrying fragments of the same mass but different shape, so as to separate the effects of loading from potential effects arising from stability constraints.

MATERIALS AND METHODS

Study animals

To investigate the influence of fragment mass and length on metabolic costs of load transport, we used indirect calorimetry based on CO₂ production of load-carrying ants. Experiments were carried out with a large laboratory colony of *Atta vollenweideri* (Forel 1893). The colony was kept in a 12 h:12 h light:dark cycle at 24–29°C and 35–55% humidity. Temperature during the experiments ranged from 25.5 to 28°C. Ants were fed with blackberry leaves (*Rubus* spp.) and honey water.

Tested fragments

The influence of fragment mass on metabolic costs of grass transport was investigated by providing standardized paper fragments of different mass (light: 4.80±0.30 mg, *N*=15; heavy: 9.59±0.33 mg, *N*=15; means ± s.d.), but the same length (12.5 mm) and width (4.8 mm). This was achieved using paper of two different thicknesses (80 and 160 g m⁻²). The effect of fragment length on metabolic costs was studied by offering the ants paper fragments of different length (12.5 and 25 mm) but with the same mass (short: 4.89±0.29 mg, *N*=14; long: 4.78±0.23 mg, *N*=15) and width (2.4 mm), with no statistical difference between the achieved fragment masses (*t*-test: *t*₂₇=1.10, *P*=0.28). The light and short fragments also had a similar mass, yet the light fragments were twice as wide as the short fragments. This difference in fragment width had no significant effects on any analyzed parameter and the results of this comparison are therefore not reported here. The fragment lengths were chosen

based on field measurements in *A. vollenweideri* (Röschard and Roces, 2003b); 25 mm is above the mean but within the range of naturally carried grass fragments (approximately 5–35 mm) by workers of the size range of our laboratory colony (body mass range of carriers: 2.9–10.9 mg). Fragments of this length could be carried without constraints along the respirometry running tube as described below. To make the paper fragments sufficiently attractive to the ants, they were soaked in orange juice for at least 1 h and then dried, as has been done previously in field and laboratory experiments (Moll et al., 2010; Röschard and Roces, 2002; Röschard and Roces, 2003a; Röschard and Roces, 2011).

Respirometric measurements

To measure metabolic rates, a high-resolution respirometry system (TR-2, Sable Systems International, Las Vegas, NV, USA; resolution: 0.01 p.p.m. CO₂) was used as described by Lighton (Lighton, 1990). This system allowed CO₂- and H₂O-free air to be drawn through a circular glass tube (25×3 cm, volume: 177 cm³), in which the ants could move freely, at a flow rate of 200 ml min⁻¹ standard temperature and pressure (STP)-controlled by a mass-flow controller (Fig. 1). Advantages and disadvantages of flow-through respirometry systems have been discussed before (Lighton, 2008; Halsey and Lighton, 2011). The CO₂ production rate (\dot{V}_{CO_2}) of a single ant was measured with an IR-CO₂ analyzer (LI-COR 6251, LI-COR, Lincoln, NB, USA) at a sample rate of 1 Hz. The design of the tubular respirometry chamber takes advantage of the stereotyped running behavior many ants exhibit when confined in a closed-end tube (Lighton and Feener, 1989; Schilman and Roces, 2005). They spend long periods of time running or walking from one end to the other. To encourage consistent and voluntary running in load-carrying *A. vollenweideri*, foraging ants were led through the open glass tube prior to the experiments to establish a sufficiently strong pheromone trail within the tube. Slippery Fluon (Whitford, Diez, Germany) on the walls (but not at the bottom) of the tube prevented the ants from climbing up to the ceiling of the tube. After at least 1 h, the tube was disconnected from the colony. Ants that left the tube were individually removed by careful manipulation, so as to avoid the release of alarm pheromone within the tube. This was continued until no ants were left in the tube. The trail-laying procedure was repeated after every second measurement. Because our experiment

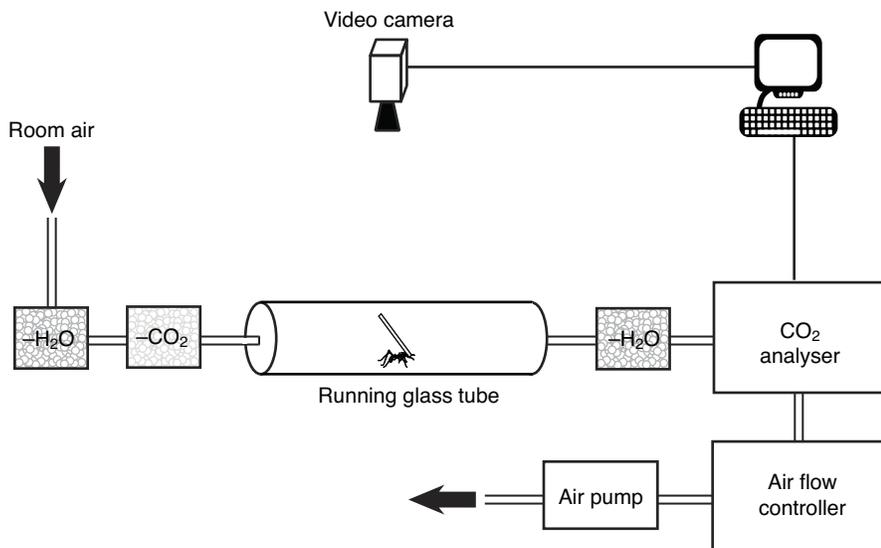


Fig. 1. Experimental setup used to measure the metabolic rate of an individual running laden or unladen ant. Water- and CO₂-free air was drawn through a running tube (volume: 177 cm³) at a flow rate of 200 ml min⁻¹ controlled by a mass-flow controller. The CO₂ produced by the ant was measured by an IR-CO₂ analyzer. During the measurements, ants were filmed from above with a video camera.

involved only voluntary locomotion, we can exclude artefacts potentially arising from repeatedly forcing insects to run while in the tube (Lipp et al., 2005; Nielsen et al., 1982), or forcing them to run at predefined speeds on treadmills (Lighton and Feener, 1989). In *Atta colombica*, \dot{V}_{O_2} and duration of the run were not correlated, suggesting that ants rapidly (within 3 min) reach steady-state \dot{V}_{O_2} during each run (Lighton et al., 1987). A short interval (>3 min) of steady running and CO_2 output was therefore sufficient to quantify metabolic rate at a single speed.

Prior to and after each recording, the CO_2 signal in the whole system and in the empty glass tube was recorded as baseline. During the analysis, the start and end baselines were subtracted, assuming a linear drift, and the \dot{V}_{CO_2} values were corrected.

Experimental procedure

The four different types of fragments were alternately placed into a foraging arena, which was connected with the nest *via* a 3-m-long flexible tube. Using a small cardboard bridge, ants that returned to the nest carrying a paper fragment were individually led to the test tube, which was disconnected from the respirometry system during this procedure. \dot{V}_{CO_2} recording (as described above) started after at least 4 min after the tube was re-connected to the respirometry system to make sure that any CO_2 not originating from the ant was fully flushed out of the chamber. Each carrier was measured for 3 to 5 min, which represented the duration over which most ants voluntarily carried the fragments. Subsequently, \dot{V}_{CO_2} of the same worker was measured without load. Workers mostly dropped the fragments by themselves. If fragments were not dropped after 10 min, the fragment (not the ant) was held with a pair of tweezers until the ant released it from its mandibles. Any fragment was removed from the tube prior to the recordings of unladen ants. Unladen ants were also allowed to run in the closed tube for at least 4 min before the recording started. Most ants immediately began to run steadily in the tube. Data from ants that were reluctant, i.e. they dropped their fragments too early or ran irregularly, were not used.

To measure running speed during the metabolic measurements, ants were recorded with a video camera (Basler A602f, Basler Vision Technologies, Ahrensburg, Germany) at 10 Hz from above. The ant's two-dimensional position in the tube was digitized in the video recordings every second and mean running speed (total distance/duration) was calculated for each ant in the loaded and the unloaded condition. After the recordings, ants and their loads were collected and weighed to the nearest 0.1 mg.

Body mass of workers in the experimental series ranged from 2.9 to 10.9 mg for the comparison of light and heavy fragments, and 3.7 to 8.1 mg for the comparison of short and long fragments (Table 1). For both comparisons, the ants' body masses did not differ significantly between the two groups (light vs heavy: $t=1.03$, d.f.=28, $P=0.31$; short vs long: $t=-0.56$, d.f.=27, $P=0.58$).

Data analysis

A total of 59 ants were analyzed and mean \dot{V}_{CO_2} was calculated for each ant with and without load over a sequence of 3–4 min each. Unfortunately, minimum cost of transport, i.e. the slope of the relationship between running speed and metabolic rate (Taylor et al., 1970), could not be determined because the limited range of speeds did not allow us to assign a statistically significant slope to this relationship. We therefore compared net cost of transport (C_{net}), which is calculated as the difference in energy uptake during running and resting divided by running velocity and body mass, and describes the net energy required to move a unit body mass over a unit distance (in $Jg^{-1}m^{-1}$). As the y-axis intercept in plots of metabolic rate vs speed is usually higher than the resting metabolic rate (the difference likely representing the cost of maintaining the body posture of locomotion) (see Schmidt-Nielsen, 1972), this estimate of C_{net} may slightly decrease with running velocity. In order to calculate C_{net} , resting or standard \dot{V}_{CO_2} ($\dot{V}_{CO_2,S}$) was estimated from the ant's body mass (M_b), using the allometric equation $\dot{V}_{CO_2,S}=0.0524M_b^{0.618}$ [calculated from $\dot{V}_{O_2,S}=0.074M_b^{0.618}$ and a respiratory quotient (RQ) of 0.71 found in load-carrying *A. colombica* workers] (Lighton et al., 1987). $\dot{V}_{CO_2,S}$ can readily be corrected for temperature assuming a Q_{10} of 2 (Lighton, 1988; Lighton, 1989; Lighton and Bartholomew, 1988) whereas \dot{V}_{CO_2} , which is a function of temperature-sensitive and temperature-insensitive components (Lighton and Feener, 1989), cannot. Thus, net \dot{V}_{CO_2} was calculated by subtracting $\dot{V}_{CO_2,S}$ at the temperature of the running ant from the measured \dot{V}_{CO_2} during running. Net \dot{V}_{CO_2} was converted to energy units (J) based on RQ=0.71 (Lighton et al., 1987). Hence, assuming $6.606 cal ml^{-1} CO_2$ (Brody, 1945) and $4.187 J cal^{-1}$, 1 ml CO_2 corresponds to 27.659 J. C_{net} was obtained by dividing net \dot{V}_{CO_2} by the ant's body mass and running speed. As C_{net} is sensitive to running speed, results need to be considered with respect to this variable.

To evaluate the costs of carrying a unit load for the different types of fragments, we calculated $C_{net,tot}$, which describes the net energy required to move a unit mass (body mass + fragment mass)

Table 1. Means \pm s.d. of fragment properties, ant body mass, running speed, metabolic cost for ants that carried one of the four different types of fragments (light, heavy, short and long) as well as unladen ants (pooled)

	Fragments of different mass			Fragments of different length		
	Unladen	Light	Heavy	Unladen	Short	Long
Fragment mass (mg)	–	4.80 \pm 0.30	9.59 \pm 0.33	–	4.98 \pm 0.29	4.78 \pm 0.23
Fragment length (mm)	–	12.5	12.5	–	12.5	25
Fragment width (mm)	–	4.8	4.8	–	2.4	2.4
Body mass (mg)	6.97 \pm 2.02	7.51 \pm 2.31	6.73 \pm 1.75	5.48 \pm 1.15	5.36 \pm 1.29	5.60 \pm 1.05
Running speed (mm s ⁻¹)	10.70 \pm 2.75	11.11 \pm 1.93	9.52 \pm 3.09	10.84 \pm 2.97	10.23 \pm 1.67	7.89 \pm 2.27
$\dot{V}_{CO_2,S}$ ($\mu l CO_2 h^{-1}$)	2.22 \pm 0.40	2.33 \pm 0.49	2.18 \pm 0.32	1.91 \pm 0.24	1.89 \pm 0.26	1.93 \pm 0.23
\dot{V}_{CO_2} ($\mu l CO_2 h^{-1}$)	4.71 \pm 1.15	6.23 \pm 1.10	8.25 \pm 1.79	3.85 \pm 0.77	5.63 \pm 1.47	6.25 \pm 1.13
C_{net} ($J g^{-1} m^{-1}$)	0.29 \pm 0.14	0.42 \pm 0.240	0.89 \pm 0.67	0.28 \pm 0.14	0.55 \pm 0.22	0.88 \pm 0.45
$C_{net,tot}$ ($J g^{-1} m^{-1}$)	0.29 \pm 0.14	0.23 \pm 0.07	0.34 \pm 0.19	0.28 \pm 0.14	0.28 \pm 0.10	0.45 \pm 0.19
N	30	15	15	29	14	15

C_{net} , net cost of transport (i.e. energy required to move a unit body mass over a unit distance); $C_{net,tot}$, net cost of transport with respect to the total mass (ant + fragment) moved; N, sample size; \dot{V}_{CO_2} , CO_2 production rate; $\dot{V}_{CO_2,S}$, standard (resting) rate of CO_2 production estimated from the allometric equation $\dot{V}_{CO_2,S}=0.0524M_b^{0.618}$ (adapted from Lighton et al., 1987), where M_b is mass in g.

Table 2. Comparison of running speed, \dot{V}_{CO_2} , C_{net} and $C_{net,tot}$ between ants carrying light or heavy fragments and unladen ants, and between ants carrying short or long fragments and unladen ants

	Fragments of different mass			Fragments of different length			
	d.f.	<i>t</i>	<i>P</i>	d.f.	<i>t</i>	<i>P</i>	
Running speed				Running speed			
Unladen–light*	14	–1.17	0.52	Unladen–short*	13	–0.25	>0.9
Unladen–heavy*	14	2.46	0.06	Unladen–long*	14	3.11	<0.05
Light–heavy	28	1.69	0.20	Short–long	27	3.13	<0.01
\dot{V}_{CO_2}				\dot{V}_{CO_2}			
Unladen–light*	14	–4.25	<0.01	Unladen–short*	13	–4.50	<0.01
Unladen–heavy*	14	–8.10	<0.001	Unladen–long*	14	–10.76	<0.001
Light–heavy	28	–4.41	<0.001	Short–long	27	–1.19	0.49
C_{net}				C_{net}			
Unladen–light*	14	–3.17	<0.01	Unladen–short*	13	–3.88	<0.01
Unladen–heavy*	14	–4.96	<0.001	Unladen–long*	14	–6.39	<0.001
Light–heavy	28	–3.69	<0.01	Short–long	27	–3.13	<0.01
$C_{net,tot}$				$C_{net,tot}$			
Unladen–light*	14	1.42	0.35	Unladen–short*	13	2.06	0.12
Unladen–heavy*	14	–0.95	0.71	Unladen–long*	14	–4.03	<0.01
Light–heavy	28	–2.27	0.06	Short–long	27	–3.38	<0.01

Asterisks indicate paired *t*-tests.

over a unit distance (in $Jg^{-1}m^{-1}$). Thus, $C_{net,tot}$ was obtained by dividing net \dot{V}_{CO_2} by the sum of body and fragment masses and running speed. $C_{net,tot}$ was converted to energy units as described above. Similar values of $C_{net,tot}$ in laden and unladen workers indicate that transport costs per unit load are equal to the transport costs of a unit body mass, whereas smaller or higher values in laden workers indicate lower or higher mass-specific costs of load carriage, respectively.

All data are reported as means \pm s.d. Regression analysis was by least squares and slopes are reported with standard errors (s.e.m.). Regression slopes were compared by analysis of covariance (ANCOVA, test for homogeneity of slopes) and, if not significantly different, were used to correct for differences in body masses. Workers with and without loads were compared using paired *t*-tests, and ants with short vs long fragments and light vs heavy fragments were compared using independent *t*-tests. To avoid α inflation, *P*-values were corrected with the Bonferroni method. The significance level for all tests was $P < 0.05$.

RESULTS

Running speed

When carrying fragments of different mass, the mean running speeds (Table 1) in laden and unladen workers did not differ significantly (Table 2). Ants that carried light and heavy fragments ran at similar speeds (Table 2). By contrast, ants with long fragments were significantly slower than unladen ants and ants that carried short fragments of the same mass (Table 2). Running speeds of ants with short fragments did not differ from those of unladen ants (Table 2).

Running speed increased significantly with body mass for ants that carried the heavy and long fragments (heavy: $R^2=0.39$, $t_{13}=2.88$, $P < 0.05$; long: $R^2=0.50$, $t_{13}=3.63$, $P < 0.01$; Fig. 2), but not for ants with light or short fragments and unladen ants [light: $R^2=0.04$, $t_{13}=0.77$, $P=0.46$; short: $R^2=0.24$, $t_{12}=1.93$, $P=0.08$; unladen (pooled): $R^2=0.002$, $t_{57}=-0.38$, $P=0.71$]. Although the absence of a dependence on body size for light and short fragments as well as for unladen ants may be based on the limited range of body sizes investigated, its presence for heavy and long fragments is likely explained by a reduction in running speed for small ants with relatively high loading ratios (body mass lower than 5–6 mg).

CO₂ production

In both comparisons, \dot{V}_{CO_2} (Table 1) was significantly correlated with body mass ($F_{1,109}=55.20$, $P < 0.001$; Fig. 3), with smaller ants producing less CO₂ per unit time than larger ants. The scaling of \dot{V}_{CO_2} with body mass did not differ significantly between experimental conditions ($F_{7,102}=0.48$, $P=0.85$), with an overall relationship of $\dot{V}_{CO_2} \sim M_b^{0.47 \pm 0.06}$. To correct our data for different ant body sizes, we used $\dot{V}_{CO_2}/M_b^{0.47}$ for further analysis.

For all experimental conditions, laden workers produced significantly more CO₂ per unit time than unladen workers (Table 2, Fig. 3). Ants carrying heavy fragments produced significantly more CO₂ per unit time than ants carrying the light fragments (Table 2, Fig. 3A), whereas \dot{V}_{CO_2} did not differ significantly between ants carrying short and long fragments (Table 2, Fig. 3B).

Cost of transport

The costs of transport for fragments of different mass and length were analyzed by comparing C_{net} . The resting metabolic rates predicted from body mass and $\dot{V}_{CO_2,S}$ are shown in Table 1. For fragments of both different mass and different length, C_{net} decreased with body mass ($F_{1,109}=30.98$, $P < 0.001$; Fig. 4A,B) and the scaling coefficients did not differ significantly between experimental conditions ($F_{7,102}=1.69$, $P=0.12$), with an overall relationship of $C_{net} \sim M_b^{-0.82 \pm 0.15}$. This scaling coefficient is not significantly different from the value of -0.602 found in *A. colombica* (Lighton et al., 1987) ($F_{1,109}=2.21$, $P=0.14$). As described above, the scaling coefficient was used to correct for differences in body size.

C_{net} was significantly increased for any laden workers compared with unladen ones (Table 2, Fig. 4A,B). C_{net} increased significantly with both load mass and load length (Table 2). In case of the heavier fragments, C_{net} primarily increased because of the higher metabolic rate (Fig. 3A), with no significant changes in running speed (Fig. 2A). Conversely, in case of the longer fragments, C_{net} was higher mainly because of the decreased running speed (Fig. 2B), and not because of an increase in metabolic rate (Fig. 3B).

As C_{net} does not take into account the fragment mass carried, the costs of transport for the different fragment types relative to the transport costs of a unit mass (ant + fragment) were evaluated by comparing $C_{net,tot}$ (Table 1). $C_{net,tot}$ also decreased with body mass ($F_{1,109}=16.19$, $P < 0.001$; Fig. 4C,D) with a common scaling coefficient

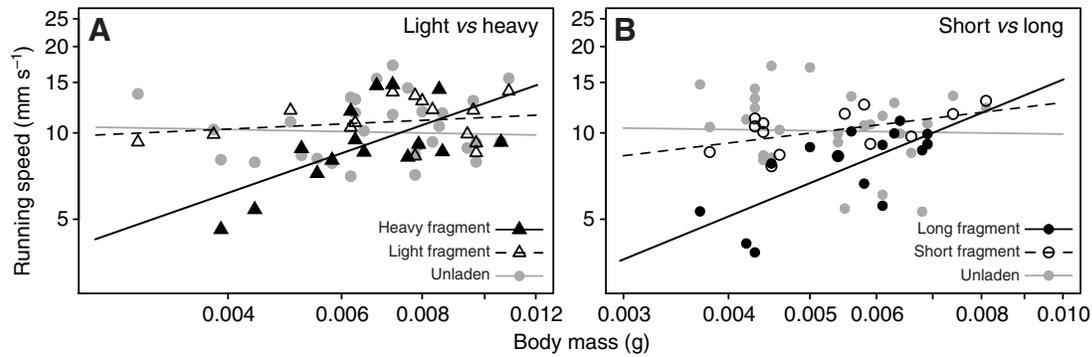


Fig. 2. Log-linear relationship between running speed and body mass (M_b) of ants that carried (A) light and heavy fragments and (B) short and long fragments and the corresponding unladen ones (pooled). Regression lines: (A) light: $17.79M_b^{0.10\pm0.13}$, heavy: $478.19M_b^{0.79\pm0.27}$; (B) short: $64.82M_b^{0.35\pm0.18}$, long: $3741.9M_b^{1.19\pm0.33}$; (A,B) unladen: $8.24M_b^{-0.04\pm0.12}$.

of -0.58 ± 0.14 for all conditions (no significant difference between conditions, $F_{7,102}=0.98$, $P=0.45$). Again, this scaling coefficient was used to correct for differences in body size. $C_{\text{net,tot}}$ was similar for ants that carried light and heavy fragments and did not increase compared with unladen ants (Table 2, Fig. 4C). Consequently, the costs of carrying a unit fragment mass did not differ from the costs of carrying a unit body mass, neither for the light nor for the heavy fragments. By contrast, $C_{\text{net,tot}}$ depended significantly on the length of the fragment. As for light and heavy fragments, $C_{\text{net,tot}}$ did not change significantly for short fragments compared with unladen ants (Table 2). However, $C_{\text{net,tot}}$ was significantly higher for ants carrying long fragments than for unladen ants and ants with short fragments (Table 2, Fig. 4D). This shows that the costs of carrying a unit mass were particularly high for long fragments.

When the absolute costs per unit distance for ants of different sizes are compared (i.e. the energy output of an individual expressed in Joules per unit distance; Fig. 5A,B), ants carrying heavier and longer fragments incurred higher costs than workers carrying light and shorter fragments, a trend similar to that seen for C_{net} (Fig. 4A,B). In contrast to the result for C_{net} , however, a difference between the slopes is now apparent ($F_{7,102}=3.88$, $P<0.001$). The absolute costs per unit distance of unladen ants increased significantly with body mass ($t_{57}=4.30$, $P<0.001$, scaling coefficient: 0.64 ± 0.15 ; Fig. 5A,B). For ants with light and short fragments, scaling coefficients were also positive, but the relationship was not significant (light: $t_{13}=1.40$, $P=0.19$, scaling coefficient: 0.21 ± 0.15 ; short: $t_{12}=0.83$, $P=0.42$, scaling coefficient:

0.26 ± 0.32 ; Fig. 5A,B). By contrast, the absolute costs per unit distance decreased significantly with body mass for ants carrying long fragments, and a similar (but not statistically significant) trend was present for ants with heavy fragments (long: $t_{13}=-2.28$, $P<0.05$, scaling coefficient: -0.86 ± 0.38 ; heavy: $t_{13}=-0.99$, $P=0.34$, scaling coefficient: -0.35 ± 0.35 ; Fig. 5A,B).

If the absolute costs per unit distance are normalized for fragment mass, they are similar for light and heavy fragments, and higher for long than for short fragments (Fig. 5C,D), consistent with the results for $C_{\text{net,tot}}$ (Fig. 4C,D). However, the scaling with body mass varied again between the four types of fragments ($F_{3,51}=3.17$, $P<0.05$). Scaling coefficients were positive for ants with light and short fragments, but negative for long and heavy ones, the trend being significant only for the long fragments (light: $t_{13}=1.69$, $P=0.17$, scaling coefficient: 0.22 ± 0.13 ; short: $t_{12}=0.70$, $P=0.49$, scaling coefficient: 0.22 ± 0.30 ; long: $t_{13}=-2.57$, $P<0.05$, scaling coefficient: -0.97 ± 0.36 ; heavy: $t_{13}=-1.03$, $P=0.32$, scaling coefficient: -0.38 ± 0.37 ; Fig. 5C,D). The negative scaling coefficient for the long fragments is mainly based on the high costs for workers smaller than 5–6 mg, whereas for larger workers the costs of carrying short and long fragments were similar (Fig. 5D).

DISCUSSION

In this study, fragment mass directly affected CO_2 production of the carrying ant, whereas fragment length showed no direct effect. Because of similar mean running speeds, values of $C_{\text{net,tot}}$ were the

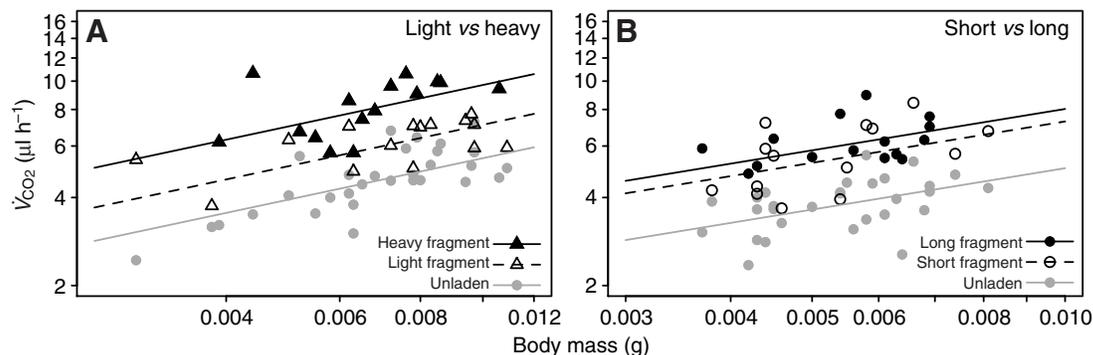


Fig. 3. Log-linear relationships between CO_2 production rate (\dot{V}_{CO_2}) and body mass (common scaling coefficient: 0.47 ± 0.06). (A) Workers with light and heavy fragments and the corresponding unladen ones (pooled). (B) Workers with short and long fragments of equal mass and the corresponding unladen ones (pooled).

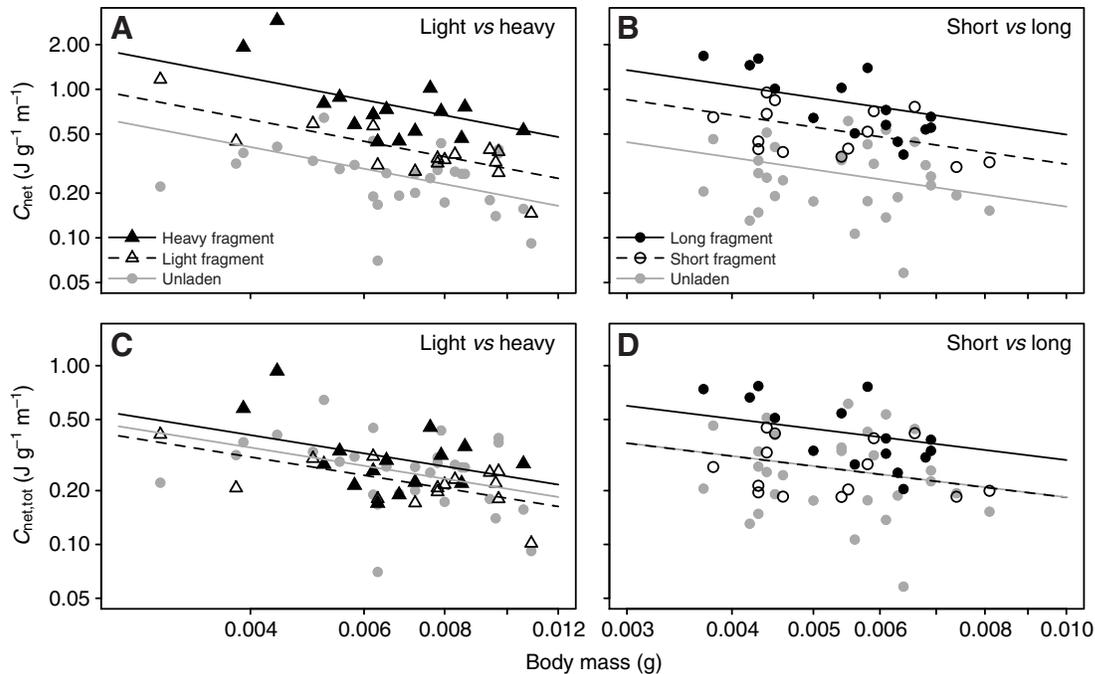


Fig. 4. Log-linear relationships between net cost of transport (C_{net} , i.e. the net energy required to move a unit body mass over a unit distance) and body mass (A,B) and between the net costs with respect to the total mass ($C_{net,tot}$, ant + fragment) and body mass (C,D). (A,C) Workers with light and heavy fragments and the corresponding unladen ones. (B,D) Workers with short and long fragments and the corresponding unladen ones (common scaling coefficients: C_{net} : -0.82 ± 0.15 , $C_{net,tot}$: -0.58 ± 0.1).

same for heavy and light fragments, and did not differ from the costs of carrying a unit body mass. By contrast, ants carrying long fragments showed similar metabolic rates but ran significantly slower than ants carrying short fragments. As a consequence, the net costs of carrying a unit load over a unit distance were significantly higher for long fragments than for short fragments, and higher than the costs of carrying a unit body mass. To maximize the fragment transport rate, foragers are expected to run as fast as possible. In a previous study, we found that *A. vollenweideri* ants fall over more often when carrying long fragments (Moll et al., 2010). The reduced velocity observed in the present study therefore suggests that workers carrying long fragments are running close to their limit of stability, and that this condition increases $C_{net,tot}$. Therefore, the shape of a fragment, independent of its mass, has important effects on foraging energetics.

An increase of metabolic rate with the mass of the carried load has been observed for mammals and other arthropods (Duncan and Lighton, 1994; Keren et al., 1981; Kram, 1996; Kram and Taylor, 1990; Lighton et al., 1987; Lighton et al., 1993; Marsh et al., 2006; Nielsen and Baroni Urbani, 1990; Schilman and Roces, 2005; Soule et al., 1978; Taylor et al., 1980). We found no effect of load mass on running speed. Previous studies have shown a reduction in running speed for laden ants (Bartholomew, 1988; Lighton et al., 1993; Rissing, 1982), but the reported changes were small for low loading ratios (Lighton et al., 1987). In some species, running speed was not affected or even increased with loading (Duncan and Lighton, 1994; Schilman and Roces, 2005). Running speed is not only a function of load but may also depend on other factors, such as the nutritional state of the colony, food quality and the phase of the daily foraging cycle (Bollazzi and Roces, 2011; Roces, 1993; Roces and Núñez, 1993).

Transporting a unit load was found to be cheaper than transporting a unit body mass in many mammals (including humans) and arthropods (Goldman and Iampietro, 1962; Herreid and Full, 1986;

Lighton et al., 1993; Maloiy et al., 1986; Nielsen and Baroni Urbani, 1990; Taylor et al., 1980), but no difference was found in several ant species (Bartholomew, 1988; Duncan and Lighton, 1994; Lighton et al., 1987; Nielsen et al., 1982). According to our result for $C_{net,tot}$, i.e. the net cost of transport per unit body + fragment mass, carrying a heavier fragment costs approximately the same amount of energy per unit load as carrying a light one or a unit body mass. However, when the absolute costs of transport are considered, smaller ants required more energy per unit distance than larger ants to carry heavy fragments, and less energy to carry light ones (Fig. 5A). This relationship at the individual level is also relevant at the colony level, when cutting behavior and colony-wide intake of the harvested plants are considered. A given mass of plant material can hypothetically be carried back to the nest either by smaller ants as a large number of small pieces, or by larger ants as a small number of large pieces. Fig. 5C shows that both ways would result in similar costs for the colony. Hence, it may be energetically advantageous for polymorphic species such as leaf-cutting ants to allocate small workers for the carriage of lighter fragments, and larger workers for the carriage of heavier fragments. Such a matching between ant body size and load size has been found repeatedly, not only in those leaf-cutting ants that cut semicircular fragments from dicotyledonous leaves (Cherrett, 1972; Lutz, 1929), where the matching may directly result from their geometric mode of cutting, but also in the grass-cutting ant species of the present study (Röschard and Roces, 2003b).

In contrast to heavier fragments, long fragments caused a reduction in the mean running speed, confirming previous results in grass-cutting ants (Röschard and Roces, 2002). This reduced speed led to an increase in the $C_{net,tot}$ to a level significantly higher than the cost of carrying a unit body mass. The cost of carrying a unit load over a unit distance has been shown to increase with decreasing speed in vertebrates (Taylor et al., 1982; Taylor et al., 1970) and cockroaches (Herreid and Full, 1984), suggesting that low running speeds are energetically

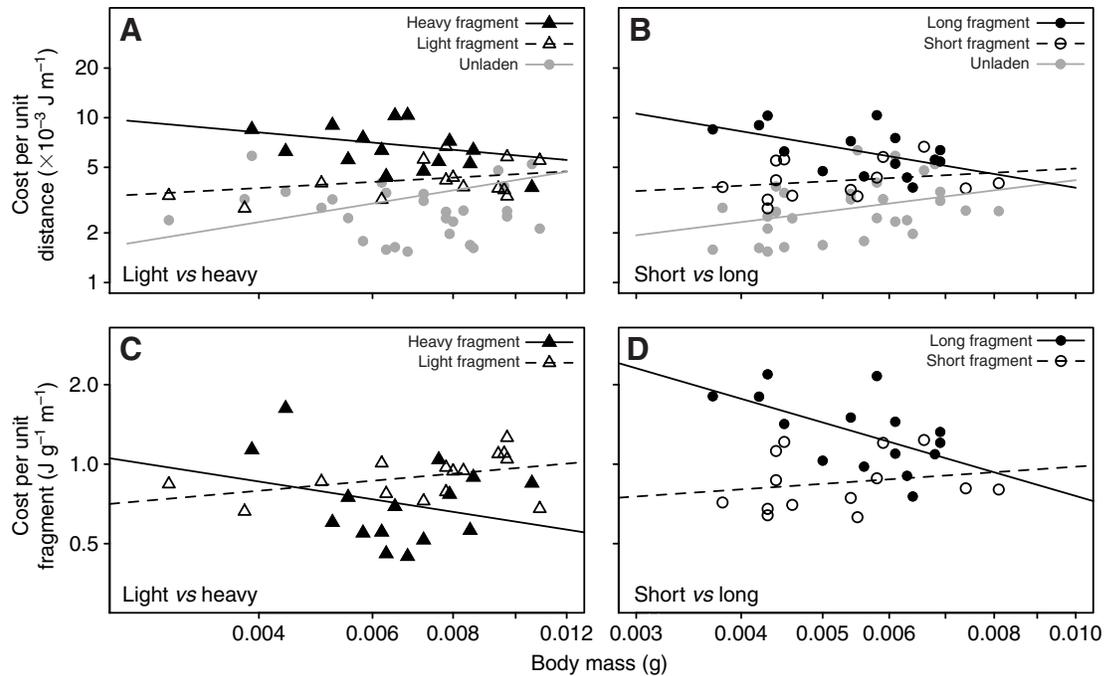


Fig. 5. Log-linear relationships between body mass and (A,B) the absolute cost per distance and (C,D) the absolute cost per unit fragment (and per unit distance) for workers with light and heavy fragments (A,C) and the corresponding unladen ones (A) and workers with short and long fragments (B,D) and the corresponding unladen ones (B) [scaling coefficients: (A) light: 0.21 ± 0.15 , heavy: -0.35 ± 0.35 ; (B) short: 0.26 ± 0.32 , long: -0.86 ± 0.38 ; (A,B) unladen: 0.64 ± 0.15 ; (C) light: 0.22 ± 0.13 , heavy: -0.38 ± 0.37 ; (D) short: 0.22 ± 0.30 , long: -0.92 ± 0.36].

disadvantageous. In grass-cutting ants, the need to maintain stability when carrying long fragments likely limits their maximum running speed. Even though workers reduce the backward shift of the center of mass when carrying long fragments by holding them more steeply than short ones, they do not fully compensate for this shift (Moll et al., 2010). Workers carrying long fragments further increase stability by reducing their stride length and increasing the number of legs simultaneously in ground contact, both of which limits their maximum running speed; these kinematic adjustments will be described in a separate study.

Apart from carrying loads, leaf-cutting ants have to lift fragments from the ground and bring them into an upright position. This handling of the fragments also costs time and energy (Bollazzi and Roces, 2011). It is unknown whether and how fragment properties influence the handling costs and how they contribute to the total foraging costs. An overall cost-benefit analysis of foraging in leaf-cutting ants could help to clarify the energetic costs of a complete harvesting cycle, but such a study would be very difficult to accomplish. Leaf-cutting ants do not eat the harvested leaves themselves, but rear a symbiotic fungus on the plant material in their nest (Weber, 1972). Although the energy content of the leaf fragments could be measured, it is still unknown how much of the delivered energy is consumed by the fungus and how much is actually passed on to the brood or adults (Bass and Cherrett, 1995; Littlelyke and Cherrett, 1976).

Our results suggest that in order to minimize costs of transport, workers should choose shorter fragments, which do not reduce their running speed. In fact, grass-cutting ants avoid the transport of overly long fragments. Given a choice of fragments of different lengths but the same mass, they were more likely to pick up the shorter fragments (Röschard and Roces, 2002). Additionally, the likelihood of ants cutting a fragment into smaller pieces instead of transporting it increased with the original fragment length (Röschard, 2002).

Because grass-cutting ant foragers do not use the reach of their legs to 'measure' fragment size, as is the case in other leaf-cutter ants, most fragments cut are longer than the ants' body length. Workers climb on a grass blade and cut across its width, which results in a longish, more or less rectangular grass fragment. Therefore, cutting effort is directly determined by the width of the grass, which usually does not vary much along a blade except near its tip. Hence, cutting longer fragments implies neither a higher cutting effort nor a longer cutting time. As cutting is an energetically expensive activity, with metabolic rates up to 30 times higher than at rest (Roces and Lighton, 1995), workers may harvest more material per unit cutting effort by simply cutting longer fragments. If only the energy investment during cutting is considered, the ants should prefer very long fragments. However, fragment length has substantial effects on maneuverability (Moll et al., 2010) and speed of transport, as demonstrated in the present study. It is likely that the sizes of the fragments cut and carried by grass-cutting ants under natural conditions represent the outcome of a trade-off between maximising harvesting rate at the cutting site and minimising the effects of fragment size on running speed and transport costs.

The load size selected by individual workers is a crucial variable that ultimately determines the foraging success of a leaf-cutting ant colony. Studies of load size selection in *Atta* have found that loads are mostly smaller than would be predicted to maximize leaf mass transport rate (Burd, 2000; Burd, 2001). It has been argued that the selection of smaller leaf fragments was favored during evolution because of the underground processing rates of the fragments after their delivery, which are maximal for fragments of intermediate size (Burd and Howard, 2005) (but see Roces and Bollazzi, 2009). Load transport rates may also depend on environmental conditions, such as trail gradients (Lewis et al., 2008), height constraints (Dussutour et al., 2009) and fallen branches (Farji-Brener et al., 2007). The relationship between load size and transport rates is likely to be

affected by these factors and hence may influence the ants' decisions. To clarify the process of load size selection, it is very important to consider biomechanical factors that may affect cutting, handling and carrying behavior. The present results underline their importance for the understanding of leaf-cutting ant foraging strategies.

ACKNOWLEDGEMENTS

We thank Pablo Schilman for very helpful comments on an earlier version of this manuscript and Denise Loli for technical support.

FUNDING

Funding for this work was provided by a Dissertation Grant from the UK Biotechnology and Biological Sciences Research Council (BBSRC, BB/D526129/1), the German Academic Exchange Service (DAAD), the Cambridge European Trust, the Balfour Fund of the Department of Zoology (University of Cambridge) and the German Research Foundation (DFG, Sonderforschungsbereich SFB 554).

REFERENCES

- Bartholomew, G. A. (1988). Energetics of trail running, load carriage and emigration in the column-raiding army ant *Eciton hamatum*. *Physiol. Zool.* **61**, 57-68.
- Bass, M. and Cherrett, J. M. (1995). Fungal hyphae as source of nutrients for the leaf-cutting ant *Atta sexdens*. *Physiol. Entomol.* **20**, 1-6.
- Bollazzi, M. and Roces, F. (2011). Information needs at the beginning of foraging: grass-cutting ants trade off load size for a faster return to the nest. *PLoS ONE* **6**, e17667.
- Brody, S. (1945). *Bioenergetics and Growth*. New York: Reinhold.
- Burd, M. (2000). Foraging behaviour of *Atta cephalotes* (leaf-cutting ants): an examination of two predictions for load selection. *Anim. Behav.* **60**, 781-788.
- Burd, M. (2001). Leaf tissue transport as a function of loading ratio in the leaf-cutting ant *Atta cephalotes*. *Ecol. Entomol.* **26**, 551-556.
- Burd, M. and Howard, J. J. (2005). Central-place foraging continues beyond the nest entrance: the underground performance of leaf-cutting ants. *Anim. Behav.* **70**, 737-744.
- Cherrett, J. M. (1972). Some factors involved in the selection of vegetable substrate by *Atta cephalotes* (L.) (Hymenoptera: Formicidae) in tropical rain forest. *J. Anim. Ecol.* **41**, 660.
- Duncan, F. D. and Lighton, J. R. B. (1994). The burden within: the energy cost of load carriage in the honeypot ant, *Myrmecocystus*. *Physiol. Zool.* **67**, 190-203.
- Dussutour, A., Deneubourg, J. L., Beshers, S. and Fourcassie, V. (2009). Individual and collective problem-solving in a foraging context in the leaf-cutting ant *Atta colombica*. *Anim. Cogn.* **12**, 21-30.
- Farji-Brener, A. G., Barrantes, G., Laverde, O., Fierro-Calderon, K., Bascope, F. and Lopez, A. (2007). Fallen branches as part of leaf-cutting ant trails: their role in resource discovery and leaf transport rates in *Atta cephalotes*. *Biotropica* **39**, 211-215.
- Fewell, J. H. (1988). Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. *Behav. Ecol. Sociobiol.* **22**, 401-408.
- Goldman, R. F. and Iampietro, P. F. (1962). Energy cost of load carriage. *J. Appl. Physiol.* **17**, 675-676.
- Halsey, L. G. and Lighton, J. R. B. (2011). Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. *Comp. Biochem. Physiol.* **158A**, 265-275.
- Herreid, C. F. and Full, R. J. (1984). Cockroaches on a treadmill-aerobic running. *J. Insect Physiol.* **30**, 395-403.
- Herreid, C. F. and Full, R. J. (1986). Energetics of hermit-crabs during locomotion – the cost of carrying a shell. *J. Exp. Biol.* **120**, 297-308.
- Jonkman, J. C. M. (1976). Biology and ecology of the leaf-cutting ant *Atta vollenweideri*. *Z. Angew. Ent.* **81**, 140-148.
- Jonkman, J. C. M. (1979). Distribution and densities of nests of the leaf-cutting ant *Atta vollenweideri* Forel, 1893 in Paraguay. *Z. Angew. Ent.* **88**, 27-43.
- Keren, G., Epstein, Y., Magazani, A. and Sohar, E. (1981). The energy-cost of walking and running with and without a backpack load. *Eur. J. Appl. Physiol.* **46**, 317-324.
- Kram, R. (1996). Inexpensive load carrying by rhinoceros beetles. *J. Exp. Biol.* **199**, 609-612.
- Kram, R. and Taylor, C. R. (1990). Energetics of running – a new perspective. *Nature* **346**, 265-267.
- Lewis, O. T., Martin, M. and Czaczkes, T. J. (2008). Effects of trail gradient on leaf tissue transport and load size selection in leaf-cutter ants. *Behav. Ecol.* **19**, 805-809.
- Lighton, J. R. B. (1988). Discontinuous CO₂ emission in a small insect, the formicine ant *Camponotus vicinus*. *J. Exp. Biol.* **134**, 363-376.
- Lighton, J. R. B. (1989). Individual and whole-colony respiration in an African formicine ant. *Funct. Ecol.* **3**, 523-530.
- Lighton, J. R. B. (1990). Slow discontinuous ventilation in the Namib dune-sea ant *Camponotus detritus* (Hymenoptera, Formicidae). *J. Exp. Biol.* **151**, 71-82.
- Lighton, J. R. B. (2008). *Measuring Metabolic Rates: a Manual for Scientists*. New York: Oxford University Press.
- Lighton, J. R. B. and Bartholomew, G. A. (1988). Standard energy-metabolism of a desert harvester ant, *Pogonomyrmex rugosus* – effects of temperature, body mass, group size, and humidity. *Proc. Natl. Acad. Sci. USA* **85**, 4765-4769.
- Lighton, J. R. B. and 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., Bartholomew, G. A. and Feener, D. H., Jr (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta columbica* Guer. *Physiol. Zool.* **60**, 524-537.
- Lighton, J. R. B., Weier, J. A. and Feener, D. H. (1993). The energetics of locomotion and load carriage in the desert harvester ant *Pogonomyrmex rugosus*. *J. Exp. Biol.* **181**, 49-61.
- Lipp, A., Wolf, H. and Lehmann, F. O. (2005). Walking on inclines: energetics of locomotion in the ant *Camponotus*. *J. Exp. Biol.* **208**, 707-719.
- Littledyke, M. and Cherrett, J. M. (1976). Direct ingestion of plant sap from cut leaves by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Formicidae, Attini). *Bull. Entomol. Res.* **66**, 205-217.
- Lutz, F. E. (1929). Observations on leaf-cutting ants. *Am. Mus. Novitates* **388**, 1-21.
- Maloiy, G. M., Heglund, N. C., Prager, L. M., Cavagna, G. S. and Taylor, R. C. (1986). Energetic cost of carrying loads: have African women discovered an economic way? *Nature* **319**, 668-669.
- Marsh, R. L., Ellerby, D. J., Henry, H. T. and Rubenson, J. (2006). The energetic costs of trunk and distal-limb loading during walking and running in guinea fowl *Numida meleagris*. I. Organismal metabolism and biomechanics. *J. Exp. Biol.* **209**, 2050-2063.
- Moll, K., Roces, F. and Federle, W. (2010). Foraging grass-cutting ants (*Atta vollenweideri*) maintain stability by balancing their loads with controlled head movements. *J. Comp. Physiol. A* **196**, 471-480.
- Nielsen, M. G. and Baroni Urbani, C. (1990). Energetics and foraging behavior of the European seed harvesting ant *Messor capitatus*. I. Respiratory metabolism and energy-consumption of unloaded and loaded workers during locomotion. *Physiol. Entomol.* **15**, 441-448.
- Nielsen, M. G., Jensen, T. F. and Holm-Jensen, I. (1982). Effect of load carriage on the respiratory metabolism of running worker ants of *Camponotus herculeanus* (Formicidae). *Oikos* **39**, 137-142.
- Porter, S. D. and Jorgensen, C. D. (1981). Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? *Behav. Ecol. Sociobiol.* **9**, 247-256.
- Rissing, S. W. (1982). Foraging velocity of seed-harvester ants, *Veremessor pergandei* (Hymenoptera, Formicidae). *Environ. Entomol.* **11**, 905-907.
- Robinson, S. W. and Fowler, H. G. (1982). Foraging and pest potential of Paraguayan grass-cutting ants (*Atta* and *Acromyrmex*) to the cattle industry. *Z. Angew. Ent.* **93**, 42-54.
- Roces, F. (1993). Both evaluation of resource quality and speed of recruited leaf-cutting ants (*Acromyrmex lundii*) depend on their motivational state. *Behav. Ecol. Sociobiol.* **33**, 183-189.
- Roces, F. and Bollazzi, M. (2009). Information transfer and the organization of foraging in grass- and leaf-cutting ants. In *Food Exploitation by Social Insects: Ecological, Behavioral, and Theoretical Approaches* (ed. S. Jarau and M. Hrcir), pp. 261-275. Boca Raton, FL: CRC Press.
- Roces, F. and Lighton, J. R. B. (1995). Larger bites of leaf-cutting ants. *Nature* **373**, 392-393.
- Roces, F. and Núñez, J. A. (1993). Information about food quality influences load-size selection in recruited leaf-cutting ants. *Anim. Behav.* **45**, 135-143.
- Röschard, J. (2002). Cutter, carriers and bucket brigades... Foraging decisions in the grass-cutting ant *Atta vollenweideri*. PhD thesis, Universität Würzburg, Würzburg, Germany.
- Röschard, J. and Roces, F. (2002). The effect of load length, width and mass on transport rate in the grass-cutting ant *Atta vollenweideri*. *Oecologia* **131**, 319-324.
- Röschard, J. and Roces, F. (2003a). Cutters, carriers and transport chains: Distance-dependent foraging strategies in the grass-cutting ant *Atta vollenweideri*. *Insectes Soc.* **50**, 237-244.
- Röschard, J. and Roces, F. (2003b). Fragment-size determination and size-matching in the grass-cutting ant *Atta vollenweideri* depend on the distance from the nest. *J. Trop. Ecol.* **19**, 647-653.
- Röschard, J. and Roces, F. (2011). Sequential load transport in grass-cutting ants (*Atta vollenweideri*): maximization of plant delivery rate or improved information transfer? *Psyche* **2011**, ID643127.
- Rudolph, S. G. and Loudon, C. (1986). Load size selection by foraging leaf-cutter ants (*Atta cephalotes*). *Ecol. Entomol.* **11**, 401-410.
- Schilman, P. E. and Roces, F. (2005). Energetics of locomotion and load carriage in the nectar feeding ant, *Camponotus rufipes*. *Physiol. Entomol.* **30**, 332-337.
- Schilman, P. E. and Roces, F. (2006). Foraging energetics of a nectar-feeding ant: metabolic expenditure as a function of food-source profitability. *J. Exp. Biol.* **209**, 4091-4101.
- Schmidt-Nielsen, K. (1972). Locomotion: energy cost of swimming, flying, and running. *Science* **177**, 222-228.
- Soule, R. G., Pandolf, K. B. and Goldman, R. F. (1978). Energy expenditure of heavy load carriage. *Ergonomics* **21**, 373-381.
- Taylor, C. R., Schmidt-Nielsen, K. and Raab, J. L. (1970). Scaling of energetic cost of running to body size in mammals. *Am. J. Physiol.* **219**, 1104-1107.
- Taylor, C. R., Heglund, N. C., McMahon, T. A. and Looney, T. R. (1980). Energetic cost of generating muscular force during running – a comparison of large and small animals. *J. Exp. Biol.* **86**, 9-18.
- Taylor, C. R., Heglund, N. C. and Maloiy, G. M. O. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 1-21.
- Ting, L. H., Blickhan, R. and Full, R. J. (1994). Dynamic and static stability in hexapedal runners. *J. Exp. Biol.* **197**, 251-269.
- Traniello, J. F. A. (1989). Foraging strategies of ants. *Annu. Rev. Entomol.* **34**, 191-210.
- Weber, N. A. (1972). *Gardening Ants – the Attines*. Philadelphia, PA: The American Philosophical Society.
- Wetterer, J. K. (1990). Load-size determination in the leaf-cutting ant, *Atta cephalotes*. *Behav. Ecol.* **11**, 95-101.
- Zollikofer, C. P. E. (1994). Stepping patterns in ants. III. Influence of load. *J. Exp. Biol.* **192**, 119-127.