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Walking on inclines: energetics of locomotion in the ant *Camponotus*

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Summary

To assess energetic costs during rest and locomotion in a small insect, we measured metabolic rate in freely moving ants *Camponotus* sp. (average body mass 11.9 mg). The animals ran in a straight respirometric chamber in which locomotor speed and CO_2 release were monitored simultaneously using flow-through respirometry and conventional video analysis. In resting intact ants, standard metabolic rate was on average 0.32 ml CO_2 g^{-1} body mass h^{-1}. During walking, the ants breathed continuously and metabolic rate increased between 4.3 times (level walking at 0–5 mm s^{-1}) and 6.9 times (30° ascent at 85–95 mm s^{-1}) over resting rates. Metabolic rate increased linearly with increasing walking speed but superficially leveled off beyond speeds of about 70 mm s^{-1}. Walking on incline (uphill) or decline slopes (downhill) of up to 60° had only a small effect on energy consumption compared to level walking. During slope walking, total metabolic rate averaged over all running speeds ranged from a minimum of 1.55±0.4 (horizontal running) to a maximum of 1.89±0.7 ml CO_2 h^{-1} g^{-1} body mass (30° downhill). The mean cost of transport in *Camponotus* was approximately 130 J g^{-1} km^{-1}. The metabolic requirements in the comparatively small insect *Camponotus* for walking were mostly in the range expected from data obtained from other insects and small poikilotherms, and from allometric scaling laws.

Key words: insect, ant, *Camponotus* sp., metabolic rate, locomotion, slope walking, respirometry.

Introduction

Over the past decades terrestrial locomotion of animals has been intensively investigated, particularly in the areas of circulation, respiration and energetics. Major interest has been devoted to the field of running energetics in vertebrates but, by comparison, few studies have addressed locomotor costs in invertebrates such as insects (e.g. Bartholomew et al., 1985; Herreid and Full, 1984; Lighton et al., 1987, 1993). This is unfortunate since invertebrates are an extremely diverse group exhibiting large differences in the type of locomotion, number of legs, and the respiratory and circulatory apparatus. Notable progress in identifying the dependencies of energetic cost during terrestrial locomotion has been made by comparing metabolic rate with the animal's locomotor gait, running speed or, most prominently, with body mass (Alexander, 1991; Fish et al., 2001; Herr et al., 2002; Kram and Taylor, 1990; Taylor et al., 1970; Taylor, 1985; Walton et al., 1994; Warncke et al., 1988; Wickler et al., 2003). The majority of these studies, however, especially in invertebrates, have focused on level running at different running speeds and have not typically addressed the energetic costs associated with running uphill or downhill. Studies on incline walking in vertebrates have been performed by Raab et al. (1976), Taylor et al. (1972), Wickler et al. (2000), Wunder and Morrison (1974) and in invertebrates by Full and Tullis (1990) and Herreid et al. (1981). Many small animals such as squirrels or leaf cutter ants obviously accomplish extraordinary energetic feats when running on inclined surfaces because they apparently run with similar speed up tree trunks as on level ground. In animals walking on inclines, the total energy required for locomotion depends on three major components: (i) the standard metabolic rate (SMR), which is equal to the energy consumption during rest, (ii) the requirement to move the body a given distance in the horizontal, and (iii) the energetic costs associated to gravitational forces during vertical locomotion. It has been suggested that the major reason for the remarkable ability of small animals to climb up vertical surfaces lies in the small contribution of vertical costs to total energetic expenditure during locomotion (Taylor et al., 1972).

Kleiber's classic equation on the relationship between metabolic rate and body mass states that in mammals, resting metabolism scales in proportion to the size of the animal to the power of three-quarters. It has been shown that this allometric exponent applies to most organisms even including plants, and indicates that resting metabolic rate becomes relatively smaller in larger organisms (Schmidt-Nielsen, 1984; West et al., 1997). Recent studies on scaling laws suggest that the fractal structure of transportation networks supplying the body tissue with nutrients and oxygen is the ultimate reason for this inverse relationship (Banavar et al., 1999; Darveau et al., 2002). During running, the body mass-specific rate of energy

consumption increases linearly with increasing speed in most animals, whereby the amount of energy used to run a certain distance is nearly constant, regardless of the animal's actual running speed. This independence between transport cost and speed was found generally among running animals (for a review, see Full, 1997). Smaller animals have shorter legs and thus need more steps to cover a certain distance. As a consequence, the number of contraction–relaxation cycles of the leg musculature and the associated energy loss, as well as frictional work emitted into the environment, increases with decreasing body size, forcing the cost of transport (COT) to increase likewise (Heglund and Taylor, 1988; Taylor and Heglund, 1982). Most impressively, this relationship between COT and body size spans approximately eight orders of magnitude in body mass and approximately 150 different species, including mammals and insects (Full, 1997). Kram and Taylor (1990) hypothesized that the rate of energy per unit body mass consumed by the leg muscles is inversely proportional to the time for which the foot applies force to the ground during each stride. Since this force-reaction-time depends on step length, which in turn increases with increasing body mass (larger animals typically have longer legs), total energy expenditure may remain nearly constant independently of running speed and body size.

In contrast to SMR and the cost during horizontal locomotion, the relative work required to move a unit of body mass vertically against the force of gravity is the same for large and small animals. For this reason, the absolute cost of vertical locomotion decreases considerably with decreasing body size, resulting in a tedious task for large mammals to scale steep slopes. The scope for energy conservation during downhill walking follows corresponding relationships because in some mammals, such as mice and chimpanzees, part of the potential energy stored during uphill running can be released during downhill running if the incline angle is sufficiently small (15°; Taylor et al., 1972). It has been suggested that gravity may efficiently accelerate swinging limbs and thus lower the work done by the leg muscles during downhill running. Steep declines may be more expensive to negotiate than level terrain or small downhill inclines. This is due to the effort needed for braking in order to avoid excessive acceleration (Taylor et al., 1972).

The above findings are well-documented for mammals and other vertebrates (Schmidt-Nielsen, 1984), but very few studies exist on terrestrial locomotion in small invertebrates such as insects. Energetic costs of level walking have been examined in cockroaches (Full, 1997; Full et al., 1990; Herreid et al., 1981), flies (Berrigan and Lighton, 1994), crickets (Full et al., 1990), beetles (Bartholomew et al., 1985; Full et al., 1990; Lighton, 1985; Rogowitz and Chappell, 2000) and ants (Fewell et al., 1996; Jensen and Holm-Jensen, 1980; Lighton et al., 1993; Lighton and Feener, 1989). The metabolic costs of negotiating ascending or descending slopes has been addressed only in cockroaches (Full and Tullis, 1990; Herreid et al., 1981). Cockroaches are among the largest insect species, yielding body masses of between 1.0 and 6.5 g and thus overlapping with the lower range of vertebrate species (Full and Tu, 1990). However, the results on cockroaches moving on inclines are not consistent, either because the energetic costs of locomotion were unexpectedly large and could not be attributed to the vertical component of transport costs (Full and Tullis, 1990), or because the slope angles investigated were rather small (±5° to ±25°; Herreid et al., 1981), yielding ambiguous results.

Here, we investigate the energetic costs of locomotion in the small ant *Camponotus* on level substrate and on ascending and descending slopes. Ants are abundant and one of the ecologically most relevant groups of insects (Hölldobler and Wilson, 1995). The nest in which a large colony lives and raises its brood is energetically demanding. Thus, the overall fitness of a colony is directly related to the success in locating and transporting food to the nest (foraging efficiency). Foraging efficiency depends on several parameters such as the nutritional content of the food returned to the nest, foraging distance, load carrying costs and the gross cost of locomotion. Since this study aims at an estimation of the relative energy required for vertical transport, the results of the experiments might provide useful information for determining ecological significance when comparing the cost of feeding in different ant species. Examples are ants dwelling in level habitats such as desert ants (Wehner, 1998) and species foraging mainly on vertical structures such as leafcutter ants (Hölldobler and Wilson, 1995). The present study further allows for the extension of the allometric relationships outlined for large mammals regarding metabolic rates and costs of horizontal transport towards the body mass of small insects.

Previous research on the subject of metabolic rate during running in ants was pioneered by studies of Jensen and Holm-Jensen (1980) and continued by several others (Fewell, 1988; Fewell et al., 1996; Lighton et al., 1987, 1993; Nielsen and Baroni-Urbani, 1990). The first experiments on the net cost of transport using unloaded leaf cutter ants *Atta colombica* were performed by Lighton et al. (1987) who estimated the relationship between metabolic cost and running speed to yield minimum cost of transport (MCOT) on a treadmill. A major problem in these treadmill experiments, however, was the fact that the animals were forced to run at pre-defined speeds. Although later experiments confirmed the measurements of MCOT made on the treadmill, the treadmill data showed a significant increase in the *y*-intercept of MCOT above SMR. Lighton and Feener (1989) thus proposed a running tube respirometer that allowed metabolic costs to be determined during voluntary locomotion in ants. The data in the present study were obtained using a running tube respirometer that could be tilted by ±60° with respect to the horizontal, allowing us to determine the energetic cost of locomotion in the ant *Camponotus* at five different inclines. In conjunction with a new analytical method to correct for Doppler shift that results from the relative motion of the ant with respect to the air flow inside the 'flow-through' running tube, we determined how metabolic cost changed when the animals varied their running speed with various inclines.

Materials and methods

Animals

For all experiments we used ants *Camponotus* sp. from a colony collected in October 2001 in Uganda. We placed the nest in a 0.4 m×0.3 m×0.2 m plastic container with a substrate layer of gypsum and maintained it at 25–30°C and a 12 h:12 h light:dark cycle. The colony was fed daily with pieces of locust and water *ad libitum*. For our experiments we used 46 intact and 15 decapitated *minor* workers (body mass 11.9±3.5 mg, mean ± S.D.).

Experimental setup

To select active foragers that were prepared to walk in the respirometric chamber, we erected a scaffold of wooden sticks in the plastic container. Individual ants that actively explored the scaffold were then placed in the respirometric running chamber (adapted from Lighton and Feener, 1989). For our initial experiments (N=10 *minor* workers), the chamber was a brass rail (493 mm length, 8 mm width, 9 mm height) covered with a Perspex plate. To increase the visual contrast between ant and rail for video analysis, we later exchanged this chamber for a Perspex running chamber (N=36 *minor* workers; 500 mm length, 10 mm width, 7 mm height) with a white painted floor. We combined data derived from both chambers, because both experimental procedures yielded consistent results. A small steel ball (diameter 2 mm) in the chamber could be swayed by a hand-operated magnet to coax the animal into running (Jensen and Holm-Jensen, 1980). Resting metabolic rates of decapitated workers were determined in a small conventional respirometric chamber (Lighton, 1991). After each test we determined wet body mass of the ants and fixed the animals in ethanol (90%) for further analysis of head size (see paragraph on the estimation of resting metabolic rate).

Video monitoring

We monitored the ant's movement inside the respirometric chamber using a commercial digital video camera (DCR-TRV120E, Sony, Cologne, Germany) mounted approximately 50 cm above the chamber. The entire experimental arrangement could be tilted to adjust the incline angle of the chamber between 60° and –60° with respect to the horizontal. To synchronize respirometric measurements and the position sampling of the ant inside the chamber, we arranged a light-emitting diode next to the chamber. We connected the diode signal to the external input of the CO_2 analyser and flashed the diode at the beginning and the end of each walking sequence in order to these mark these particular points in time. To analyse the costs of incline locomotion we tested 41 ants while they were walking on five different inclines, 0°, ±30° and ±60°. Five ants were tested while running on only one incline (0°, N=3; –60°, N=1) or two inclines (0°, –30°, N=1). Depending on the activity of the individual ant, we recorded on average 1–3 running sequences per incline.

Flow-through respirometry

We recorded CO_2 release of the animals using a computerized flow-through respirometry system. Room air was cleaned, removing water vapour and CO_2 with a Drierite/ascarite column, and pulled through the respirometric chamber and the attached CO_2 analyser (LI-7000, Li-cor, Lincoln, NE, USA) at a flow rate of 1000 ml min^{-1}. Data were recorded at 5 Hz sampling frequency and subsequently converted into body mass-specific CO_2 emissions (ml g^{-1} h^{-1}) for further analysis. We used a respiratory quotient of 0.71 (28.0 J ml^{-1} CO_2; fat metabolism) to calculate body mass-specific metabolic power (Lighton and Wehner, 1993). Baseline corrections were performed before and after each experimental session by recording the gas concentration of the empty chamber for 3 min.

Estimation of resting metabolic rate

To estimate the contribution of SMR to total running metabolic rate, we found it crucial to determine SMR under two established experimental conditions: in intact but motionless ants, and in decapitated ants. Decapitated ants are typically used to address breathing patterns such as the discontinuous gas exchange cycle. Both approaches are associated with potential errors, making a reliable estimate of SMR in ants difficult. Resting metabolic rates in intact animals might be higher than expected due to energetic costs associated with small changes in leg or body position. In contrast, decapitated ants, although typically motionless, might produce an SMR that is too low, because the metabolism of the head (19.6% of the body mass in *Camponotus*) is not considered.

To determine metabolic rate in inactive but intact *minors*, we measured CO_2 emissions after the animal had stopped running and settled into a characteristic resting posture at 0° incline (N=15 ants). To avoid an overestimation of resting CO_2 emission due to processes of paying off any anaerobic debts, or to a delayed release of CO_2 through the tracheal system, we excluded at least the first 5 s from the analysis after the animal had settled down. We determined resting condition as described below, and throughout the experiment, the ants were monitored continuously using conventional video recording. After each experiment, we analysed the video sequences using a motion-tracking program (MaxTRAQ, Innovision, Columbiaville, MI, USA) and subsequently constructed an ethogram of the ants' locomotor behaviour. The computer software allowed us to quantify the movement of antennae, legs and the body of the animal on a frame-by-frame basis. We defined resting condition as the time in which no body, leg or antennal movements were measurable in consecutive video frames. To avoid any confounding effects of costs associated with movement in this analysis, we disregarded time sequences showing even the smallest changes in the orientation of the animal's antennae.

Although all *minor* workers stemmed from the same colony and were selected according to their locomotor activity, we observed two different types of gas exchange in the resting animals: discontinuous and continuous breathing behaviour. A discontinuous gas exchange cycle (DGC) was observed in 11 out of 15 tested decapitated *minor* workers

and in 3 out of 15 intact animals. Fig. 1A–D illustrates this mode of respiration, showing sample recordings from decapitated *minor* workers. Small amounts of CO_2 were released during the flutter phase (F-phase) indicated by the small spikes with gradually increasing amplitude, followed by the open phase (O-phase, large spikes), where most of the gas exchange took place, and a brief period where the tracheal spiracles were completely closed (C-phase, baseline without spikes). Mean DGC frequency in decapitated ants was 0.8±0.3 mHz (N=11, n=86 DGCs) and mean rate of CO_2 release was 0.19±0.05 ml g^{-1} body mass h^{-1} (N=15, DGC and continuous breathing), yielding mass-specific metabolic power of 1.48±0.43 mW g^{-1} body mass. In contrast to decapitated animals, intact ants showed an unusual high DGC frequency that was approximately 15-fold higher and amounted to 12.2±3.6 mHz (N=3; n=52 breathing cycles, Fig. 1E). Intact ants released an average of 0.32±0.10 ml CO_2 g^{-1} body mass h^{-1} (N=15, DGC and continuous breathing), which corresponds to mean metabolic power of 2.51±0.78 mW g^{-1} body mass. Despite the large difference in DGC frequency between both experimental groups, CO_2 release and metabolic power were only 1.7-fold higher in intact ants compared to decapitated workers (ANOVA, P<0.001). SMR during continuous breathing tended to be slightly higher than SMR during DGC in both the decapitated ants (0.24 ±0.04 ml g^{-1} h^{-1} *vs* 0.20±0.05 ml g^{-1} h^{-1}) and the intact animals (0.37±0.12 ml g^{-1} h^{-1} *vs* 0.29±0.05 ml g^{-1} h^{-1}). However, we did not find a significant difference in either case (ANOVA, P>0.05). In sum, the data mentioned above suggest that SMR in *Camponotus* is independent of the breathing pattern but varies slightly between intact and decapitated animals. Since SMR only accounts for approximately 12% (decapitated) and 20% (intact) of total metabolic rate during horizontal running, we did not consider this difference in SMR further.

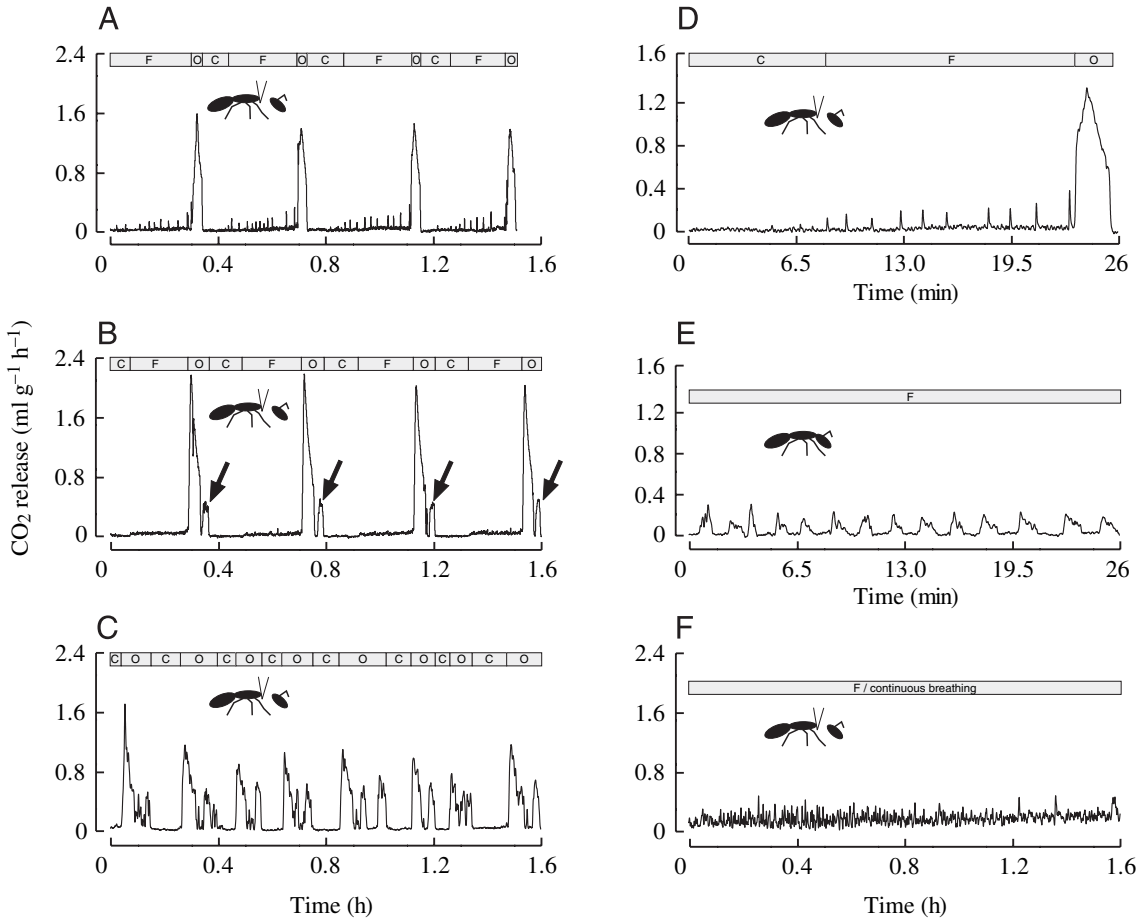

Fig. 1. Different types of breathing behaviour occurred in resting *Camponotus.* (A–D) Various patterns of the discontinuous gas exchange cycle (DGC) in single decapitated *minor* worker ants. CO_2 is released supposedly *via* a single spiracle (A), *via* two spiracles as indicated by the arrows (B), or *via* three spiracles (C). Alternatively, a single spiracle might open repeatedly within one breathing cycle. (D) Time-expanded DGC. Large CO_2 spike represents tracheal opening phase. Small spikes of gradually increasing amplitude signify spiracle flutter phase. (E) DGC in an intact ant during rest. This breathing pattern is characterised by prolonged spiracle opening during flutter phase and no distinct opening phase. (F) Continuous breathing (or higher flutter frequency) behaviour in a decapitated *minor*. Continuous breathing mode was further observed in all ants during locomotion. C, closed phase, F, flutter phase and O, spiracle opening phase. Inset sketches illustrate decapitated and intact situations.

Data evaluation and statistics

The dark ants were clearly visible against the white background in the video recordings, facilitating semi-automatic tracking of ant position in the respirometry chamber (MaxTRAQ). To match video recording to the CO_2 sampling rate, we reduced the video frame rate from 50 Hz half-frame mode to 5 Hz. Running speed was calculated from the ant's positions in adjacent video frames. To analyse the relationship between CO_2 release and running speed, we averaged the CO_2 data of each ant in separate speed bins. Bin width was set to 10 mm s^{-1}. A value of the 20 mm s^{-1} bin, for example, thus represents the mean of all measured CO_2 values while the ant was running at speeds between 15 and 25 mm s^{-1}. Data were analysed using custom-made programming routines developed in LabTalk (Origin 5.0, Microcal, Northampton, MA, USA). Unless stated otherwise, all data are given as means ± S.D. Differences between mean values were assessed by employing the two-sided *t* statistical test. Linear regression, polynomial fit lines and Gaussian curves for normal distributions were calculated using a commercial statistics program (Origin 5.0).

Doppler shift and wash-out time correction

Depending on the relative speeds between the walking ant and the air current through the respirometric chamber, Doppler effects may occur (Berrigan and Lighton, 1994). To correct for possible Doppler shifts, we converted the delay associated with the ant's position in the chamber (transport time for air parcel from and to analyser) into a corresponding time shift of the CO_2 sample point (red curve in Fig. 2A). This shift dated the measured CO_2 back to the time of release by the animal. To stay within the synchronized video frame and gas sample rates of 5 Hz, time-shifted CO_2 values were distributed on two adjacent data points where necessary. For example, when the time shift *i* was 3.4 data points, we distributed 60% of the CO_2 value on data point *i*–3 and 40% on data point *i*–4. All data traces were subsequently filtered with a running average of 1.4 s. The time delay for CO_2 released by the animal was 1.03 and 1.62 s from the inlet to the outlet of the chamber using the brass rail and Perspex chamber, respectively.

To take into account the time lag produced by the temporal low-pass filter characteristics of the chamber wash-out, including possible delays in CO_2 release due to the ant's behaviour, we calculated cross-correlation shifts between the traces of running speed and Doppler-corrected CO_2 release (Fig. 2B,C). Cross-correlations were calculated for a sliding data window of 75 data points, or 15 s, and tested for temporal shifts of up to 10 s between the two curves. The cross-correlation histogram illustrates that the mean time delay

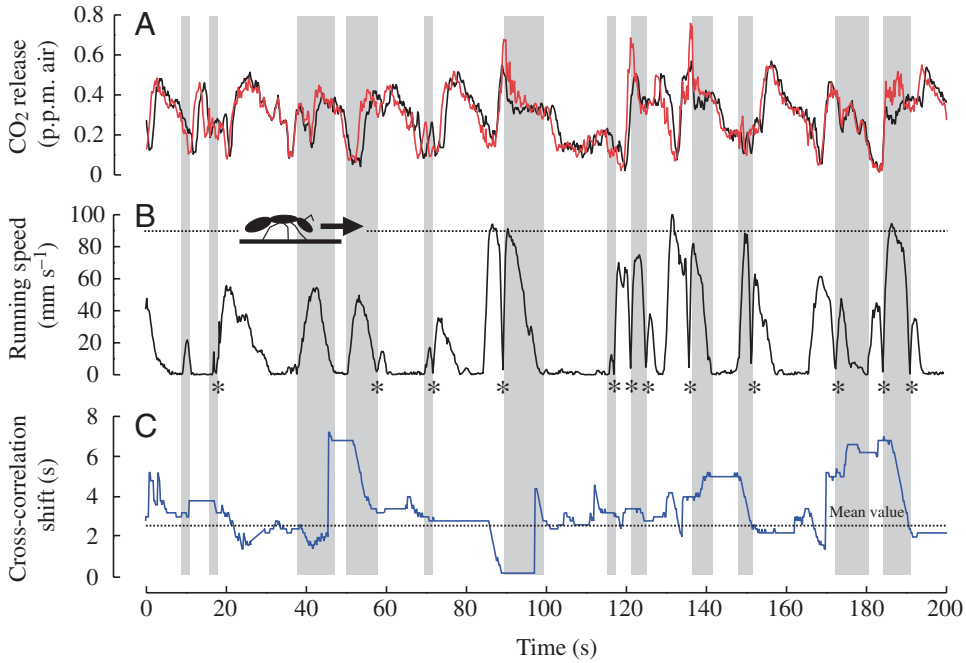

Fig. 2. CO_2 release during locomotor activity in a single ant. (A) The measured amounts of CO_2 (black line) are confounded by the Doppler effect, due to the ant's movement in the air current of the respirometric chamber. Doppler time shifts were corrected according to the momentary position of the ant in the chamber (red line; details in Materials and methods). The red line is also shifted by a fixed offset, compensating for a small constant delay produced by the tubing which connected respirometric chamber and gas analyser. (B) Running speed of the ant, as calculated from the position (video) recording above. Shaded areas indicate that the ant was running against the direction of air flow during flow-through respirometry. Asterisks mark turns of the animal at the ends of the respirometric chamber. The broken line indicates the maximum running speed considered for linear regression in Fig. 4. (C) To correct time delays produced by chamber washout, and possible behavioural delays in CO_2 release, we estimated the time shift between the CO_2 release and the speed traces using cross-correlation. The cross-correlation shift was calculated in a sliding data window (75 data points, or 15 s), and tested for temporal shifts of up to 10 s between the two traces. Mean value for cross correlation is 2.4 s (broken line); see Fig. 3A.

according to a Gaussian data fit was 2.4 s (Fig. 3A). In the analysis we disregarded values below 1.2 s time shift, because they were usually produced by small chance fluctuations. The correction of wash-out time yielded almost simultaneous increases in walking speed and CO_2 release of the animal (Δt, Fig. 3B).

Results

Metabolic rate at various walking speeds

Fig. 2 suggests that the magnitude of CO_2 release during walking depends on the speed at which the animal moves back and forth inside the respirometric chamber. Walking sequences were typically interrupted by brief decelerations and accelerations, corresponding to turns at the ends of the respirometric chamber, as indicated by the asterisks in Fig. 2B. We evaluated the relationship between metabolic rate and walking speed by binning respiratory rates for 13 speed categories and sorting them into bins of ±5 mm s^{-1} walking speed. This was done for the five different slopes: –60°, –30°, 0°, 30° and 60°. The data show that locomotion at the lowest speeds of 0–5 mm s^{-1} is approximately 4 times more energy consuming than rest in intact animals (–60°: 4.4; –30°: 4.8; 0°: 3.9; 30°: 4.3 and 60°: 3.7-fold; Fig. 4). The broken lines in Fig. 4 indicate SMR in intact (a) and decapitated (b) resting ants. Metabolic rate increases with increasing walking speed of the animals on all slopes and apparently levels off beyond approximately 70 mm s^{-1} walking speed.

The statistical analysis suggests that the increase in metabolic rate is linearly correlated with speed for the two downhill inclines –60° and –30°, and level walking ($\hat{F}$-test, $P<0.05$, linear fit, –60°: $y=1.69–7.9\times10^{-3}x$, $r^2=0.85$; –30°: $y=1.73–10.2\times10^{-3}x$, $r^2=0.96$; horizontal: $y=1.57–9.2\times10^{-3}x$, $r^2=0.92$, 13 speed bins; Fig. 4; Sachs, 1992). In contrast, the cost of running on uphill slopes (30° and 60° inclines) appears not to be linearly correlated with running speed ($\hat{F}$-test, $P>0.05$). However, because only a small number of animals achieved running speeds above 90 mm s^{-1} (mean $N=25$ ants for speeds below 100 mm s^{-1} *vs* mean $N=12$ ants for speeds above 90 mm s^{-1}), in conjunction with the short running time at higher speeds (see Discussion), we tested the data on linearity using the 10 lower speed bins only. In this case, metabolic data appear to be linearly correlated on all tested inclines ($\hat{F}$-test, $P<0.05$) and all slopes were statistically identical ($\hat{t}$-test on slope, $P>0.05$). Mean slope for the lower speed range, averaged over the five inclines, was approximately 0.010 ($y=1.59+0.010x$, $r^2=0.93$, $P<0.0001$, $N=10$, red dots, Fig. 4C). Statistical analysis of possible differences in metabolic rate between the individual speed bins of each respective incline demonstrated that in none of the speed ranges was there a significant difference between the population means (ANOVA, d.f.=4; $P>0.05$ for all 13 speed bins) and the population variances (ANOVA, Levene's test, d.f.=4; $P>0.05$).

Metabolic rate and direction of air flow

To avoid extensive Doppler shifts of the CO_2 signal in flow-through respirometry measurements, we favoured high air flow speeds of approximately 232 mm s^{-1} (brass rail) and 238 mm s^{-1} (Perspex rail). These values are approximately twice the maximum running speed we measured in single walking ants (approximately 170 mm s^{-1}, Fig. 2B). As a consequence, an animal always faced head wind when it ran against the direction of air flow and always tail wind when running with the direction of air flow. The relative flow speeds

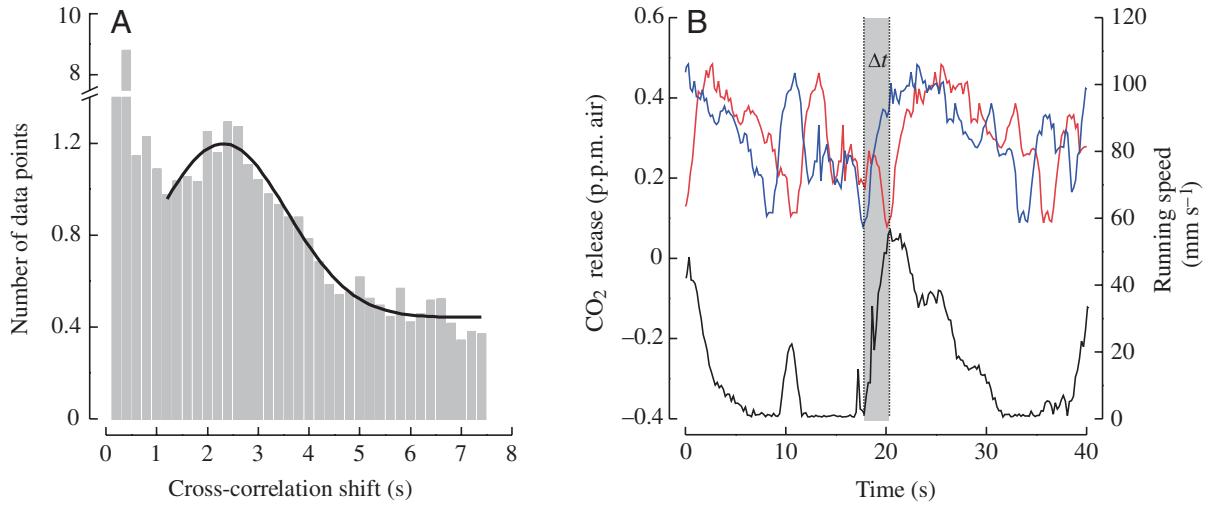

Fig. 3. Cross-correlation time shift between walking speed and CO_2 release, due to both wash-out of the respirometric chamber and possible behavioural delays in gas exchange during breathing. (A) Histogram of cross-correlation time shifts calculated from 46 animals. Mean temporal shift between speed and metabolic rate traces was 2.4 s according to the *Gaussian* fit curve (black line). Values below 1.2 s shift were disregarded because they were mostly produced by small chance fluctuations. (B) Sample trace illustrating the shift of the CO_2 release trace (red, Doppler-corrected trace) by the time delay (Δt, shaded area) obtained with the cross-correlation method (blue). The time correction results in approximately simultaneous fluctuations of CO_2 release and running speed.

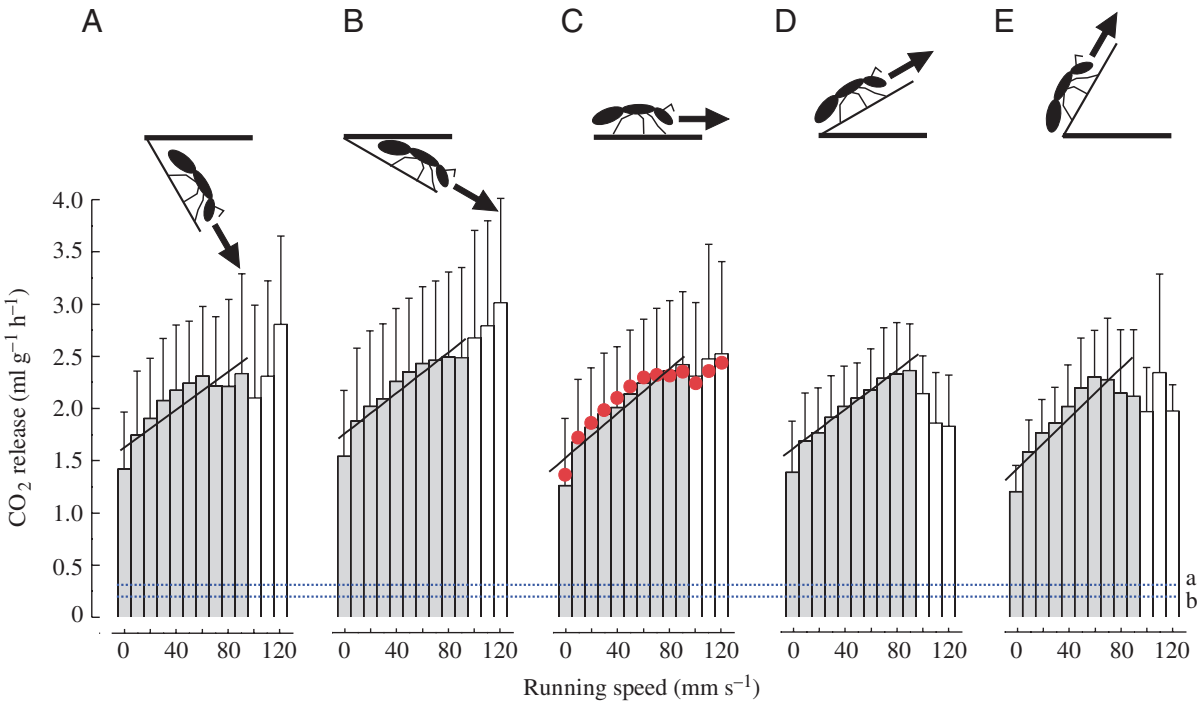

Fig. 4. Relationship between CO_2 release and running speed. Data are shown for the five different slopes of the respirometric chamber examined: (A) –60° (steep descent), (B) –30°, (C) horizontal, (D) 30°, and (E) 60° (steep ascent). See also inset sketches. Red data points in C give mean values obtained from all five inclines. Black lines represent linear fit regressions to the data up to 90 mm s^{-1} speed (A) $y=1.64+9.7\times10^{-3}x$, $r^2=0.71$; (B) $y=1.73+10.5\times10^{-3}x$, $r^2=0.88$; (C) $y=1.50+11.4\times10^{-3}x$, $r^2=0.91$; (D) $y=1.55+10.1\times10^{-3}x$, $r^2=0.94$; (E) $y=1.37+13.0\times10^{-3}x$, $r^2=0.82$. Numbers of ants in the speed bins were 26 in each of the 6 lower bins (0–5 mm s^{-1} to 45–55 mm s^{-1}), 25 (55–65 mm s^{-1}), 25 (65–75 mm s^{-1}), 24 (75–85 mm s^{-1}), 22 (85–95 mm s^{-1}), 18 (95–105 mm s^{-1}), 12 (105–115 mm s^{-1}) and 7 (115–125 mm s^{-1}). Estimates for resting metabolic rates of intact (a) and decapitated (b) ants are indicated as blue dotted lines. The speed range up to 90 mms^{-1} is shaded (see Discussion for explanation). Values are means ± S.D.

of head and tail wind with regard to the animal body, in turn, depended on the ant's own running speed. An increase in running speed resulted in an increased head-wind speed when the animal moved against the direction of flow, and in a decreased tail-wind speed when the animal moved with the direction of air flow. Since the running speed was always smaller than the air speed inside the tube, this relationship was maintained throughout the entire range of running speeds.

In Fig. 4 we sorted both walking conditions (head and tail wind, respectively) into the same bins, ignoring the energetic costs that might be associated with the difference between walking with strong head and tail winds. To evaluate the relationship between relative air speed and energetic costs in the running ant, we estimated the difference in metabolic rate (a–b) for time periods when the ant walked against (a) and with (b) the direction of air flow, and subsequently pooled these data over all five inclines of the chamber. The relative difference between head and tail wind speed (relative running speed) is thus twice the running speed of the animal over ground (Fig. 5A). For example, a *relative* running speed of 80 mm s^{-1} indicates that the animal faced an increase of 40 mm s^{-1} in head wind while walking against the air flow, and a 40 mm s^{-1} decrease in tail wind while walking with the direction of the air flow. The data suggest that the relative difference in costs associated with different strength of head and tail wind increased with increasing running speed and followed quite accurately a 'speed-squared' relationship (polynomial fit, $y=0.016+2.3\times10^{-5}x^2$, $r^2=0.98$, $\chi^2/\text{d.f.}=9.5\times10^{-4}$; Fig. 5A). Similar coefficients were found for a polynomial fit to data determined during horizontal walking only ($y=-0.03+2.0\times10^{-5}x^2$, $r^2=0.97$, $\chi^2/\text{d.f.}=2.3\times10^{-3}$).

Walking on inclines

The main objective of the present study was to examine the energy consumption during walking on declining and ascending slopes in small ants. Thus, in contrast to Fig. 5A, where data from all inclines were pooled to examine the relationship between metabolic rate and relative running speed, we pooled metabolic rates at all walking speeds in order to study the effect of slope on energy consumption in the small insect (Fig. 5B). As suggested previously by the statistics given above, in most cases we did not find significant differences between the energy requirements for walking on different slope angles (ANOVA, $P>0.05$), except for the difference between the 30° decline slope and level walking (0° inclination, ANOVA, $P<0.05$, Fig. 5B). Pooling data across all speeds, however, might have obscured small but significant effects of slope on the speed-dependency of locomotion, even though the

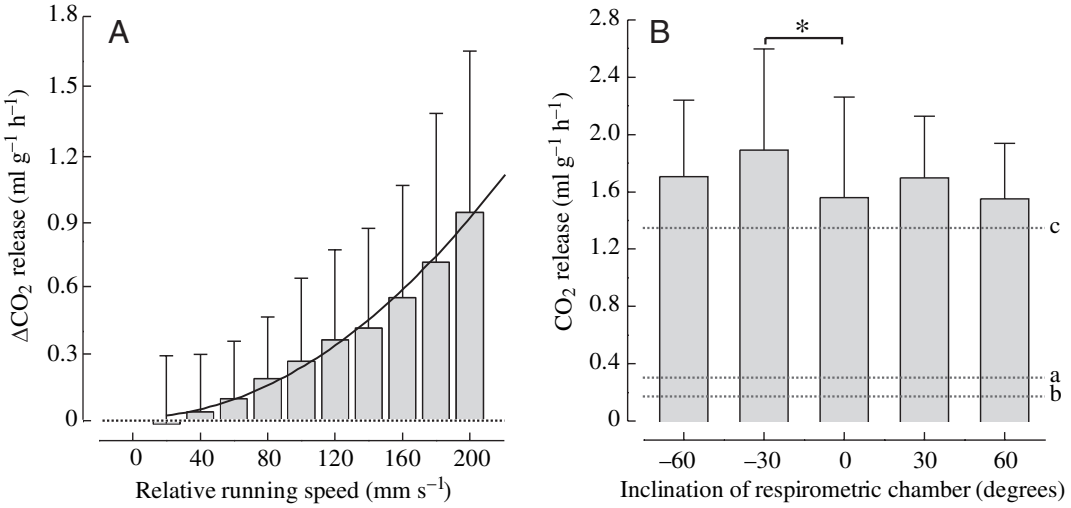

Fig. 5. Metabolic costs calculated from pooled data (five inclines in Fig. 4), as dependent on relative running speed (A) and incline of the walking substrate (B). (A) Differences (a–b) in metabolic rate between locomotion against (a) and with (b) the direction of air flow (ΔCO_2 release) are plotted against relative running speed (that is twice the running speed over ground). Airflow speed in the running tube was approximately 235 mm s^{-1}. At low walking speeds the ants experience similar head and tail wind speeds, whereas at high running speeds head wind becomes progressively faster than tail wind (see text for details). A polynomial fit line approximates the data points following a 'speed-squared' relationship. *N*=26, 26, 26, 26, 26, 25, 24, 22, 18, 13 ants from left to right. (B) Metabolic rate of *Camponotus* at the five different inclines of the running chamber, averaged for all running speeds. Data are weighted by the duration of the mean running period in each ant. *N*=30 (–60°), 30 (–30°), 45 (0°), 12 (30°), 12 ants (60° inclination). None of the values are significantly different from each other, except metabolic rates at 0° and –30° ($P<0.05$). Broken lines indicate metabolic rates of resting intact (a) and decapitated (b) ants, and during locomotion at walking speeds below 5 mm s^{-1} (c).

general relationship appears similar for all slope angles (Fig. 4). Thus, to evaluate the differences in energy consumption between level and slope walking for every single speed bin, we calculated the differences by subtracting mean metabolic rates at horizontal walking from the metabolic rate during slope walking and plotted the differences for each speed bin ranging from 0 to 120 mm s^{-1} (Fig. 6).

Superficially there was little difference in energy consumption when comparing incline running with horizontal running. Up to running speeds of 90 mm s^{-1}, the differences were small, reaching a maximum value of ±0.4 ml g^{-1} h^{-1}, which is a value close to SMR in these ants. A consistent feature of the data sets obtained from the four inclines is their negative gradient up to running speeds of 90 mm s^{-1} (linear regression between 0 and 90 mm s^{-1} speed: –60°: $y=0.19–0.003x$, $r^2=0.62$, $P=0.007$; –30°: $y=0.26–0.002x$, $r^2=0.59$, $P=0.009$; 30°: $y=0.05–0.001x$, $r^2=0.42$, $P=0.04$; 60°: $y=–0.004–0.002x$, $r^2=0.16$, $P=0.25$; Fig. 6). None of the regressions slopes were different when tested against each other (7 combinations, ANOVA, $P>0.05$), except for the comparison between –60° and 30° inclines (ANOVA, $P<0.05$). Thus, with increasing walking speed the difference in energy consumption between moving on level and inclined surfaces decreased and even resulted in negative differences in some cases. Although most of the linear regression lines suggest a significant decrease in the relative change of metabolic rate with increasing running speed, the changes are small and do not yield more than 0.28 ml g^{-1} h^{-1} (18% of the 1.5 ml g^{-1} h^{-1} mean metabolic rate at 0° slope) difference in metabolic rate below 100 mm s^{-1} walking speed.

Above 90 mm s^{-1} running speed the differences between level and slope walking became more pronounced. Uphill walking (positive incline angles) with maximum speed (120 mm s^{-1}) appeared to be approximately 35% (60°) and 44% (30°) less expensive than level walking (Fig. 6C,D). In contrast, downhill walking (negative incline angles) with maximum speed was more costly by 17% (–60°) and 32% (–30°) than walking on a horizontal surface (Fig. 6A,B). However, none of these values were statistically different from each other (6 combinations, ANOVA, $P>0.05$). In sum, it is surprising that at high running speeds, uphill running tended to be energetically more favourable than level running, while downhill running caused an increase in metabolic rate compared to running in the horizontal.

Discussion

The experiments on the energetics of slope walking have provided several new insights into the cost of terrestrial locomotion in the small ant *Camponotus*. By means of flow-through respirometry, we found that resting ants may breathe continuously and discontinuously, and measured a notable difference in metabolic rate between decapitated and intact animals. During walking behaviour, discontinuous gas exchange cycle typically ceased in all tested ants. The data demonstrate that during walking, metabolic rate increases

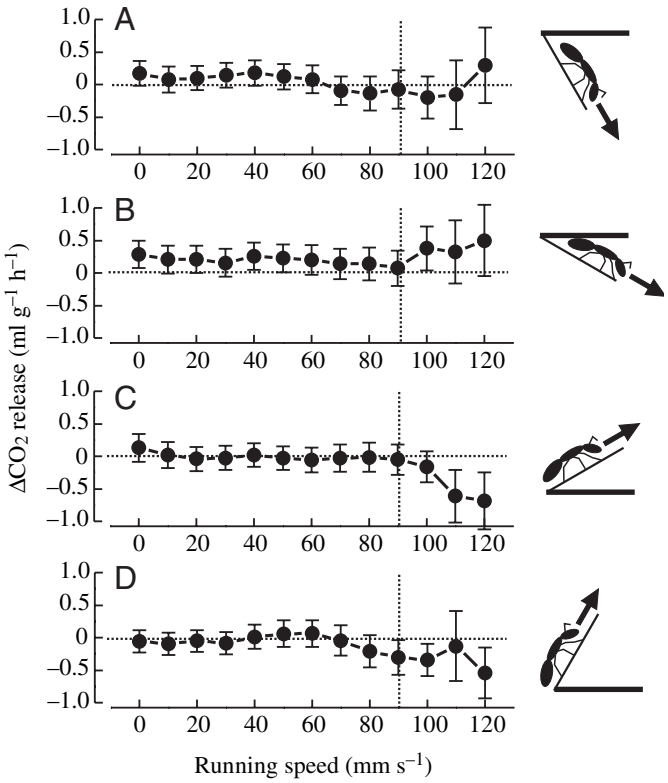

Fig. 6. Differences (a–b) in metabolic rate (ΔCO_2 release) between running on (a) inclines of –60° (A), –30° (B), 30° (C), 60° (D) and (b) level walking. Differences are plotted as a function of running speed of the animal over ground and derived from the mean values for each speed bin (see Fig. 4). Dotted lines indicate both equal metabolic costs between inclined and level walking (horizontal line), and 90 mm s^{-1} walking speed (vertical line), which was the maximum speed used for linear regression fits. Values are means ± S.E.M.

between approximately 4.3 and 6.9 times over resting rates, illustrating the increased demands for oxygen during locomotion. Energetic cost for walking linearly increased with increasing walking speed (–60°, –30° and 0° inclination) but superficially levelled off beyond approximately 70 mm s^{-1} walking speed. Metabolic rates during locomotion appeared to be related to the relative difference between the ant's walking speed and the air flow speed inside the respirometric chamber. The additional energetic requirements of the animals to walk on inclines or declines was small and did not result in a significant increase in energy consumption during locomotion in most experiments.

Respiratory pattern during running

The spiracles of the tracheal system in many insects function as barriers that control gas exchange between the tracheae and tracheoles, and the outer environment. It has been shown previously that in the ant *Camponotus*, water leaves the tracheal system when the spiracles open for gas exchange (Lighton, 1992; Lighton and Garrigan, 1995). Recordings of breathing behaviour in various insects support this observation, which has led to the assumption that DGC evolved as a mechanism for reducing rates of respiratory water loss (Snyder et al., 1995; see reviews by Miller, 1981; Kestler, 1985; Slama, 1994). During locomotion in *Camponotus*, DGC completely ceased, and all animals breathed continuously during walking (Fig. 2). This finding is consistent with previous studies on breathing behaviour in insects during locomotion (for reviews, see Lighton, 1994, 1996) and probably reflects the increased oxygen requirements during locomotion. However, in the desert ant *Pogonomyrmex rugosus*, Lighton and Feener (1989) reported a discontinuous breathing pattern while the animal was walking with constant speed within a respirometric chamber. This behaviour was interpreted as an example where the environment has constrained breathing behaviour to avoid high water loss under xeric conditions (Lighton, 1996). An alternative explanation of this finding is that the respiratory spikes were artifacts produced by Doppler shifts due to flow-through respirometry. As outlined in the Materials and methods section, an ant walking exactly with the speed of air flow in the respirometric chamber would exchange O_2 and CO_2 only with the air parcel it is travelling in. It appears evident that under such conditions the ant produces 'pseudo' spikes of CO_2 release once it stops or reverses running direction at the end of the chamber. At this moment the accumulated CO_2 is shed toward the gas analyser, which results in large CO_2 peaks similar to those produced by blowflies *Protophormia terraenovae* walking in a similar respirometric chamber (Berrigan and Lighton, 1994). Under these experimental conditions, Doppler correction of the CO_2 samples appears to be necessary. This can only be achieved if the speed of air flow inside the respirometric chamber exceeds forward speed of the walking animal. The disadvantage associated with high air speeds inside the chamber, however, is a reduction in signal-to-noise ratio of gas samples, potentially limiting the size of the animal that can be tested using flow-through respirometry. For example, in the present study maximum CO_2 concentrations in the sampled gas amounted to just about 1.0 p.p.m. air, with a flow speed of 235 mm s^{-1} inside the running tube (1000 ml min^{-1} flow rate). In the case of the walking desert ant *Pogonomyrmex*, the flow speed inside the chamber was 21 mm s^{-1}, and the authors supposedly did not correct for Doppler effects. A simple analytical model shows that, at an average walking speed of 42 mm s^{-1} and a chamber length of 1 m, the ants would have had to run back and forth approximately 6 times within the 5 min measurement period to produce Doppler artefacts corresponding to the measured data. Surprisingly, this number of runs is similar to the 6 CO_2 spikes per 5 min observed by Lighton and Feener (1989), suggesting that Doppler effects might have shaped the recorded signal.

Metabolic cost of locomotion

The cost for locomotion on inclines may be split into at least four components: (i) resting metabolism of the animal, (ii) the energetic cost for locomotion in the horizontal plane, (iii) metabolic cost to transport body mass vertically, and (iv) (potentially) energy needed to overcome viscous air friction.

The examination of the costs of vertical transport was the initial and main objective of the present study.

Horizontal walking

Fig. 4C shows that metabolic rate in ants walking in the horizontal is at least approximately fourfold higher than resting metabolism. This finding is consistent with previous studies on the costs of walking that have demonstrated a twofold increase during intermittent activity of an ant (Lighton and Wehner, 1993) and a 6- to 11-fold increase during level locomotion in cockroaches (Herreid et al., 1981; Herreid and Full, 1984; Full and Tullis, 1990). Limiting our analysis to walking speeds below 100 mm s^{-1}, we found a linear relationship between energy consumption and speed at all inclines. A linear relationship between metabolic rate and walking speed has also been demonstrated consistently in mammals (Taylor et al., 1982; reviewed in Heglund and Taylor, 1988) and insects (Full, 1997). As a consequence of a linear relationship, the cost of horizontal transport (COT) is constant and independent of walking speed. In our 12 mg *Camponotus*, transport costs were about 130 J g^{-1} km^{-1}. The COT for *Camponotus* is similar to that reported for other insects with similar body mass, varying approximately between 150–200 J g^{-1} km^{-1} (Berrigan and Lighton, 1994; Lighton and Feener, 1989; Lighton et al., 1993).

In the few experimental studies where energy consumption saturated towards higher locomotor speeds, similar to the present data set, researchers attributed their findings to gait switching between lower and higher walking speeds (Bartholomew et al., 1985; Hoyt and Taylor, 1981). In the present study we did not observe indications for use of different gaits in *Camponotus*, such as different preferred speeds or even discontinuous speed distributions (Fig. 7A). Instead, the data were scattered around small walking speeds and a simple Gaussian fit seems to describe the speed histograms sufficiently. However, it has been shown previously that the desert ant *Cataglyphis bombycina* changes gait, from the tripod gait commonly employed at lower speeds to the tetrapod gait at higher speeds (Zollikofer, 1994). A possible explanation for this difference in the use of gaits between the two ant species might be that *Cataglyphis* reaches much higher running speeds of up to 1 m s^{-1} compared to *Camponotus*, which reaches a maximum speed in the running tube of approximately 0.17 m s^{-1}. The much higher running speed of *Cataglyphis* even holds when considering running speed in terms of body lengths per unit time, since both species are approximately the same size (Lighton and Wehner, 1993).

However, a change in gait might not be the only reason for energy consumption levelling off towards higher walking speeds in *Camponotus*. An alternative explanation is that brief running sequences at high walking speeds may favour misassignment of CO_2 release. With brief and rapid walking bouts, the probability is high that significant CO_2 release occurs after the ant has already decelerated to lower speeds. Likewise, the ants may have employed anaerobic metabolic pathways during locomotion that could have influenced the respirometric recordings (Hoback and Stanley, 2001). It has been shown, for example, that during jumping in locusts and grasshoppers phosphate stores are depleted up to 70% in conjunction with accelerated glycolysis (30%). This was

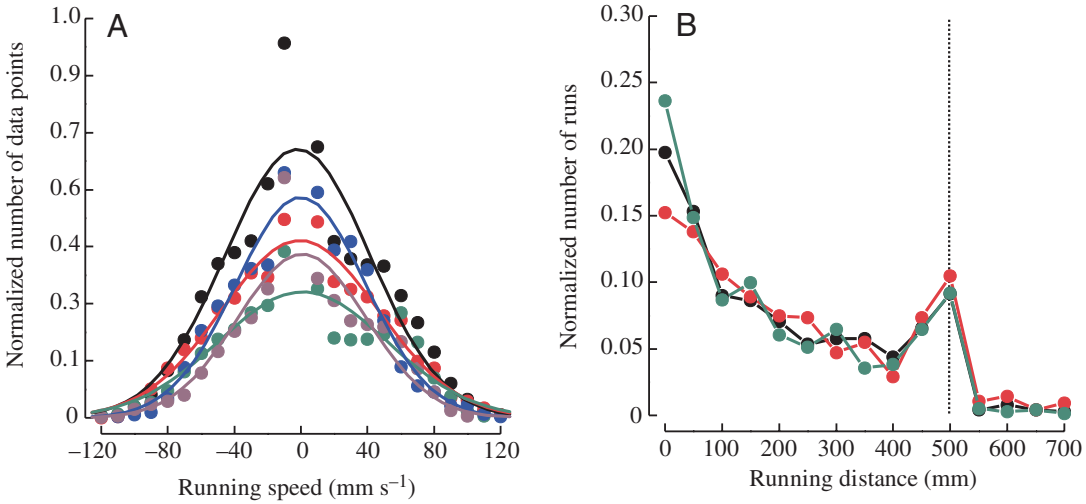

Fig. 7. Frequency distributions of running speed (A) and continuous running distance (B) in the respirometric chamber. (A) Histograms of running speeds measured in all 46 ants at the five different inclines: –60° (purple data points), –30° (green), 0° (black), 30° (red), 60° (blue). Speeds from 0 to ±5 mm s^{-1} were disregarded to avoid disruption of curves by resting periods. Positive and negative running speeds indicate locomotion against and with the direction of airflow, respectively. Speed distributions were approximated by *Gaussian* fits (curves with respective colours). Peak locations and standard deviations (0.85 times half width) of the *Gaussian* curves are (in mm s^{-1}) 0.7, 78 (–60°); 1.1, 101 (–30°); 2.0, 87 (0°); –0.6, 94 (30°); –0.1, 78 (60°). None of the values are significantly different from each other (P>0.05). (B) Histograms of walking distances covered in continuous bouts by ants in the respirometric chamber (time segments >0.4 s during which the ant maintained running speeds >5 mm s^{-1}; N=42, n=4038 running segments). Level walking, black; ±30° incline, red; ±60° incline, green. Length of respirometric chamber was 500 mm (vertical dotted line).

measured by an increase in L-lactate (Hitzemann, 1979). In the desert harvester ant *Pogonomyrmex rugosus*, Lighton and Bartholomew (1988) measured a mean respiratory quotient (RQ) of 0.796 that changed with ambient temperature (10–45°C). Although this change was small and not significantly temperature dependent, it might indicate that ants could potentially employ anaerobic metabolism during locomotor behaviour. To achieve the best correlation between locomotor activity and CO_2 release in our experiments, we corrected our CO_2 traces with the mean delay of 2.4 s, as derived from cross correlation analysis (see Materials and methods). Nevertheless, longer delays may exist between walking activity and CO_2 release, which would be especially critical for the evaluation of short time intervals. This assumption is supported by the data presented in Fig. 7 illustrating the general preference of the animals for short walking distances (Fig. 7B), in conjunction with low walking speeds (Fig. 7A). In summary, the effects mentioned above might lead to an underestimation of CO_2 release at high running speeds and, less pronounced, an overestimation of metabolic rate at lower walking speeds. The observation that the increase in metabolic rate with increasing locomotor speed apparently levels off beyond approximately 70 mm s^{-1} may thus partly be attributed to the much rarer occurrence of higher walking speeds. As a consequence, we disregarded walking speeds above 90 mm s^{-1} in the evaluation and further discussion of our data (open bars in Fig. 4 representing less than 3 s total recording time).

Walking on inclines

The finding that the metabolic rate of walking ants is rather independent from the slope of the substrate appears surprising when considering the vastly different energy requirements for level walking, ascent and descent in large vertebrates (Taylor et al., 1972; reviewed in Schmidt-Nielsen, 1984). Even considering the sparse existing data on insects, we expected some change in the locomotor costs of small *Camponotus* walking on ascending or descending slopes (see Full and Tullis, 1990; Herreid et al., 1981). A probable explanation for our result is that the relative cost of vertical locomotion, caused by the gain in potential energy, becomes progressively smaller in smaller animals, reflecting the relatively larger cost of basic metabolism (Banavar et al., 1999). For example, a *Camponotus minor* worker with an average body mass of 12 mg requires 5.89 μW or 0.49 mW g^{-1} body mass of mechanical power for the vertical transport component when scaling a 60° ascent at a speed of 100 mm s^{-1} (Fig. 4). Assuming that the muscular system of the ant converts metabolic energy into mechanical power with an efficiency of 20%, that is about the upper limit to be expected for extra loads (Taylor et al., 1980), this value results in metabolic power requirements for vertical transport of approximately 30 μW or 2.5 mW g^{-1} body mass. In comparison, energy consumption measured during horizontal walking at similar speed amounts to 20 mW g^{-1} and is approximately eightfold higher. In this perspective, and considering the variation inherent in CO_2 measurements during walking, it is not surprising that walking on different inclines did not produce noticeable changes in energy consumption (Fig. 5B).

Eventually, this result might stimulate an ongoing discussion regarding the capability of ants to gauge the incline of foraging paths in uneven terrain (Wohlgemuth et al., 2001). Ants do not use optical flow for distance measurements (Ronacher et al., 2000), and the results of this study appear to refute an energy-based mechanism for slope angle measurements in small ants such as *Camponotus.*

Metabolic rate and flow speed

One of the most puzzling results in this study is the dependency of metabolic rate on the head wind that the animal experiences during walking (Fig. 5A). The data demonstrate that there is little difference in energetic cost associated with head or tail wind at low running speeds. In other words, when the animals experienced almost equal strengths of head or tail wind while walking back and forth inside the respirometric chamber at low speeds, we only measured an insignificant difference in metabolic rate. With increasing running speed, the difference between head and tail wind speeds changed and resulted in a 'speed-squared' increase in metabolic rate with increasing speed. At a running speed of 100 mm s^{-1} (=335 mm s^{-1} head wind *vs* 135 mm s^{-1} tail wind) the ants released up to 0.93 ml g^{-1} h^{-1} more CO_2 while running against a head wind than while running with a tail wind. This value compares to a mean value of total metabolic activity of approximately 2.5 ml CO_2 g^{-1} h^{-1} (Fig. 4C). Under our limited experimental conditions, it thus seems that at a walking speed of 100 mm s^{-1}, wind direction and strength may account for up to 36% difference in metabolic rate. It is difficult to assess the ecological significance of that finding but we believe that our results might have some importance for animals foraging in windy environments.

We considered body friction as the most likely explanation for the observed increase in energetic cost with increasing head wind, assuming that *Camponotus* move in a domain of low Reynolds numbers. We estimated Reynolds number, *Re*, for motion of the ant's body using the following equation:

$$Re = uc\rho\nu^{-1}, \quad (1)$$

where u is walking speed, c is the characteristic length of the animal (body length), ρ is air density, and ν is kinematic viscosity of air. For *Camponotus minors* with a body length of approximately 10 mm, *Re* ranges from approximately 7 at a walking speed of 10 mm s^{-1}, to approximately 110 at the maximum running speed measured in a single animal of 170 mm s^{-1}. The low *Re* suggest that viscous drag increases, as does energy expenditure, when running speed increases. We derived a rough estimate of viscous drag, D, according to Stoke's Law for laminar flow around a sphere with radius r moving at low *Re*:

$$D = 6\pi\mu ru, \quad (2)$$

where μ is dynamic viscosity of air. For animals that face a

head wind of 335 mm s^{-1} at a walking speed of 100 mm s^{-1}, for example, the cost to overcome viscous drag amounts to approximately 0.19 μW (0.016 W kg^{-1} body mass) of metabolic power. This surprising result is obtained when assuming 20% muscle efficiency and a radius of 1 mm for the ant's head. In comparison, the measured metabolic power for running is 86 μW, as noted above, and a value of 0.19 μW alone accounts for approximately 3% of the vertical transport cost during 60° uphill walking in *Camponotus*. In sum, the prediction derived from the simple analytical model is far too low and cannot explain the increase in metabolic rate associated with an increase in head-wind speed. An alternative explanation, though vague and able to account for only a fraction of the high power requirements, might be viscous drag on the legs, which is potentially able to outscore drag on head, thorax and gaster. Since energetic cost due to air friction (Stoke's friction) depends on the running speed squared, even small variations in leg movements might affect the power requirements for walking. In conclusion, we cannot offer a reasonable explanation for the finding presented in Fig. 5A, because the quantitative reasons for the observed relationship between metabolic rate and increasing running speed remain unclear.

Conclusions

The results of this study demonstrate that the energetic costs associated with vertical transport in the comparatively small insect *Camponotus* are small and do not significantly change the cost of transport within a range of slope angles varying between ±60° (–30° appears to be an exception). However, since the contribution of vertical transport cost to the total cost of locomotion scales with the body mass of an animal, body mass should constrain walking behaviour in larger insect species or when the ant is heavily loaded with food or prey. Our results show that the metabolic requirements for walking in unloaded *Camponotus* are within the range expected from data obtained with other insects and small poikilotherms, and these data are consistent with allometric scaling laws.

Nevertheless, the ability of an animal to walk on inclines might not depend exclusively on the maximum mechanical power produced by the leg muscles but also on other factors, such as the general biomechanics of the locomotor system and the animal's ability to stay attached to the ground during slope walking. The walking chamber in this study had a smooth surface and may thus have allowed the ants to employ 'wet adhesion' due to a liquid film secreted by the pads of the ants' tarsi (Federle et al., 2004). Desert ants such as *Cataglyphis*, in contrast, move in rough terrain and loose sand (Wehner, 1998), and walking is assumed to be more challenging. As a consequence, future research on the evaluation of the significance of substrate structure to the costs of both horizontal and vertical locomotion should allow us to draw a more comprehensive picture of the overall energy budget during walking of small and medium sized insects such as ants.

List of symbols and abbreviations

c	body length
COT	cost of transport
D	viscous drag
DGC	discontinuous gas exchange cycle
i	time shift
MCOT	minimum cost of transport
r	mean radius of the ant body
Re	Reynolds number
RQ	respiratory quotient
SMR	standard metabolic rate
u	walking speed
μ	dynamic viscosity of air
ν	kinematic viscosity of air
ρ	air density

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