

RESEARCH ARTICLE

Locomotion on a slope in leaf-cutter ants: metabolic energy use, behavioural adaptations and the implications for route selection on hilly terrain

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SUMMARY

The metabolic cost of the negotiation of obstacles, and the influence that this has on route selection, are important determinants of an animal's locomotor behaviour. We determined the gross metabolic cost of locomotion on slopes of different gradients, ranging from -90 to $+90$ deg, in leaf-cutter ants (*Acromyrmex octospinosus*) in a closed-circuit respirometry system. Ants were able to select their preferred speed for each gradient. The gross metabolic energy expenditure per unit distance travelled on the slope (C_{path}) was calculated from the rate of CO_2 production and the speed of locomotion. These data were used to predict the optimal slopes for minimising the vertical cost of locomotion and vertical journey time. The gross rate of CO_2 production was approximately constant ($1.7 \text{ ml g}^{-1} \text{ h}^{-1}$) and was not significantly affected by slope. Ants moderated their speed with slope ($P < 0.05$), travelling the fastest during level locomotion ($2.0 \pm 0.1 \text{ cm s}^{-1}$, $N=20$) and increasingly slowly with increased gradient (both on an incline and a decline). C_{path} varied significantly with slope, being lowest during level locomotion ($646.0 \pm 51.2 \text{ J kg}^{-1} \text{ m}^{-1}$) and increasing with increasing gradient. These results suggest that ants adapt their locomotor behaviour to keep metabolic rate constant despite changing mechanical demands. It is predicted that when undertaking a journey involving vertical displacement that ants will select routes with a gradient of between 51 and 57 deg during ascent and with a gradient of between -45 and -51 deg during descent, in order to minimise both vertical journey time and vertical cost of locomotion.

Key words: ant, slope, incline, energetics, respirometry, route, speed, metabolic rate, cost of locomotion.

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INTRODUCTION

The route an animal takes determines the energetic cost of, and the time taken to perform, an activity and, consequently, is an important aspect of animal behaviour. It has been suggested that it is advantageous for animals to use routes that minimise either the time taken or the metabolic energy consumed (Minetti, 1995; Alexander, 2000). The shortest route between two points is a straight line and, on level ground, this is the route that will also incur the minimum time and energetic costs. However, the presence of obstacles, such as hills or soft ground, will change the mechanical demands of a route and consequently incur costs such as increased metabolic energy expenditure and journey time. Such costs may result in the optimal route deviating from a straight line (Minetti, 1995; Alexander, 1998; Alexander, 2000). In a route selection experiment using foraging wood ants, it was shown that route was primarily determined by energetic cost (estimated indirectly from vertical height traversed), although journey time was also a factor (Denny et al., 2001). In addition, paths established by human travellers in mountainous regions over long periods of time have been shown, retrospectively, to correspond to those predicted to minimise metabolic energy expenditure (Minetti, 1995; Alexander, 2000). However, there is little direct, empirical evidence to show that animals select routes that minimise costs. It may therefore be valuable to develop a biological model system in which the relationship between obstacles, such as a slope, and the metabolic cost of locomotion can be measured. Such a system can be used to assess whether animals do indeed use optimal routes, and whether

there are circumstances where factors other than energetic cost or journey time (e.g. predation risk, exploration for resources or environmental conditions) favour a deviation in route from that predicted to be optimal.

The cost of locomotion (C_{path}), defined as the mass-specific metabolic energy expended to move a unit distance on a slope, varies with gradient. C_{path} increases when travelling up an incline compared with level locomotion in a wide range of species, including both vertebrates and invertebrates (Margaria, 1968; Wunder and Morrison, 1974; Armstrong et al., 1983; Full and Tullis, 1990; Minetti et al., 1994; Kram and Dawson, 1998; Wickler et al., 2000; Minetti et al., 2002; Rubenson et al., 2006; Tullis and Andrus, 2011). The cost of locomotion on a decline has been less well studied. Where data exist, C_{path} appears to be lower than that of level locomotion (Margaria, 1968; Armstrong et al., 1983; Minetti et al., 2002; Hoyt et al., 2006) up to a certain gradient beyond which C_{path} increases again (Margaria, 1968; Minetti et al., 2002). For example, in humans, C_{path} is minimal on a slope of approximately -5 deg (-10%) but increases on steeper declines and during level and incline locomotion (Margaria, 1968; Minetti et al., 1994; Minetti et al., 2002). However, some studies suggest that in small species, such as leaf-cutter ants, that the mass-specific metabolic energy consumed per unit distance travelled does not change with incline (Lipp et al., 2005).

The primary aim of this study was to determine the effects of slope on the gross metabolic cost of locomotion in leaf-cutter ants. This will clarify whether there is indeed no additional metabolic

cost associated with incline running in this genre. The second aim of this study was to use this information to calculate the effects of slope on the cost of undertaking journeys along routes involving vertical ascent/descent (Minetti et al., 2002), and therefore determine the optimal routes in terms of minimising either gross metabolic energy expenditure or vertical journey time. These predictions will provide the basis for further behavioural studies to investigate whether animals ever use optimal routes, or whether factors other than metabolic energy expenditure or journey time additionally influence route choice. Ants provide an excellent system in which to develop a testable model of foraging strategies as they are central place foragers (Holldobler and Wilson, 1990) and can travel relatively long distances, over complex terrain, between the nest and food resources. Their foraging routes can easily be manipulated experimentally to test predictions about the determinants of foraging behaviour (Denny et al., 2001; Ribeiro et al., 2009).

MATERIALS AND METHODS

Animals

A single colony of leaf-cutter ants [*Acromyrmex octospinosus* (Riech 1793)] was collected in Trinidad. Workers from this colony were used for all experiments (body mass 11.1 ± 1.7 mg, mean \pm s.d., $N=38$). The colony was housed in a glass vivarium, $90 \times 40 \times 38$ cm (length \times width \times height), maintained at $28\text{--}30^\circ\text{C}$ and fed brown rice, fruit or privet leaves every 2–3 days. Worker ants on an outbound foraging route from the nest to a food source were randomly selected for the experiments.

Experimental setup

A modification of the ‘running tube’ technique (Lighton and Feener, 1989) was used to allow measurement of CO_2 production in ants travelling on a range of slopes. The ant was sealed in a respirometry chamber constructed from a Perspex tube (30 cm long, 1.5 cm diameter), with an air inlet and outlet (Fig. 1). The respirometry chamber was attached to a baton marked with a calibration scale, which could be rotated to vary the gradient. The bottom half of the tube was lined with paper to provide a suitable surface to enable the ants to walk on slopes as steep as ± 90 deg. A small magnetic stir bar was placed in the tube and could be moved using a magnet external to the respirometry chamber to encourage the ant to start walking. All experiments were carried out at room temperature ($21\text{--}23^\circ\text{C}$).

Respirometry

The rate of CO_2 production in ants travelling on a range of slopes was determined using a constant-pressure variable-volume closed-loop respirometry system (Ellington et al., 1990). Air was continuously extracted from the chamber at a rate of 200 ml min^{-1} , passed through a drying column to remove water vapour and run through a CO_2 analyser (FoxBox field gas analysis system, Sable Systems, NV, USA) before being returned to the chamber. Therefore, increases in CO_2 were cumulative over the period of locomotion. The drying column contained both Drierite (Acros Organics, Geel, Belgium) and magnesium perchlorate (Alfa Aesar, Ward Hill, MA, USA). New Drierite interacts with CO_2 (White et al., 2006); therefore, predominately exhausted and recharged Drierite was used to remove water vapour from the air, with just a small amount of new Drierite, which had been exposed to room air, to act as an indicator of desiccator exhaustion (White et al., 2006). A semi-inflated balloon attenuated any pressure differences that could otherwise have arisen in the system. The system was calibrated by injecting a known volume of CO_2 into it after each test (Ellington et al., 1990). This procedure results in a slight change of volume of the system; however, as this change represented only 0.2% of the original volume, no correction was performed. Measurements of CO_2 , pressure, temperature and mass flow were logged at 100 Hz from the FoxBox to a PC using a Powerlab data acquisition system (8/30; AD Instruments, Oxford, UK) and Chart software (v. 5.5.6 07; AD Instruments). Analysis of the data was carried out using the program IgorPro (Version 5.01; WaveMetrics, Tigard, OR, USA) and the gross rate of CO_2 production was determined for each trial. The rate of CO_2 production was converted into an estimate of the gross rate of energy consumption assuming a respiratory quotient of 0.71 ($28 \text{ J ml}^{-1} \text{ CO}_2$) based on previous measurements in moving ants (Lighton and Wehner, 1993; Lipp et al., 2005). The gross metabolic energy expenditure per unit distance travelled on the slope (gross C_{path}) was calculated as the ratio between the gross rate of metabolic energy expenditure and average speed [$\text{J kg}^{-1} \text{ m}^{-1}$; following Minetti and Minetti et al. (Minetti, 1995; Minetti et al., 2002)].

Experimental protocol

An ant was introduced to the tube, and the tube resealed and oriented to give the desired slope. Seven slopes between -90 and $+90$ deg, at 30 deg increments, were used. The introduction of the ant into the tube allowed some mixing of room air to occur. Therefore, a brief

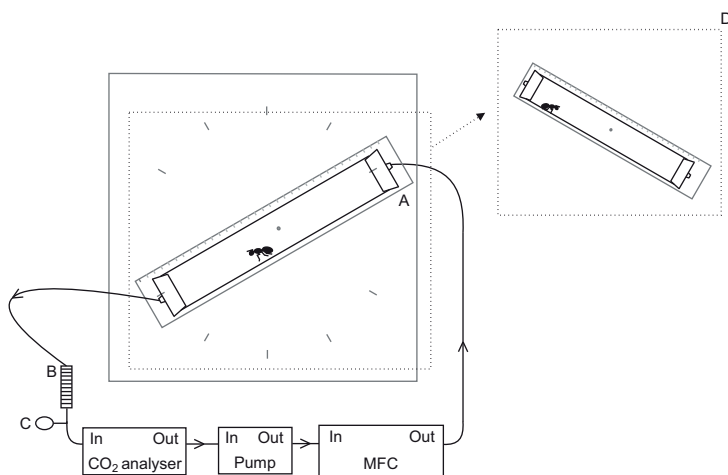


Fig. 1. Schematic representation of the respirometry setup. The ant is contained within the respirometry chamber (A), which is sealed at either end and attached to a scale marker and a board, on which it can be rotated. Air is pumped from the tube through a drying column (B) at a rate of 200 ml min^{-1} , measured and controlled via a mass-flow controller (MFC, FoxBox). The CO_2 concentration of the air circulating within the system is measured by the CO_2 analyser. As the ant reaches the end of the chamber it is rotated so that the ant continues to travel on the same slope; this is illustrated in D. A semi-inflated balloon (C) maintained a constant pressure within the sealed system.

period was allowed for the CO₂ partial pressure reading to stabilise as the air within the closed system was re-mixed. After the stabilisation period, the ant was encouraged to walk by moving the magnetic stir bar in the tube. When the ant reached the opposite end of the tube, the tube was rotated to the same angle so that, despite travelling from end to end in a sealed chamber, the ant travelled continuously at a specified slope (Fig. 1B; note that for level locomotion rotation of the tube was unnecessary). CO₂ was continually monitored and the ant's locomotion within the chamber filmed at 25 frames s⁻¹ using a video camera (GR-DVL 9800; JVC, London, UK) mounted approximately 1.5 m from the tube, perpendicular to the tube and level with its centre point. The video recordings were synchronised with the respirometry measurements and were used to identify a 2 min period where the ant was moving as desired. Because the ant was required to turn at the end of the tube, there was inevitably a short period of time where the ant was not moving on the desired slope. Only periods where the ants turned quickly, thereby moving as desired for at least 75% of the time, were used (actual time spent moving as desired was 78.7±0.47%). The mean rate of CO₂ production and the speed were calculated over this 2 min period. It should be noted that speed was calculated as the linear distance travelled divided by walking duration (excluding the time taken for the ant to turn in the tube). For each ant, measurements were attempted once on each slope; however, ants were not always co-operative and a complete set of data across the whole range of slopes was not always obtained.

Statistical analysis and modelling

Statistical analysis and modelling was carried out using the program R (R Development Core Team, 2009). General linear models (GLMs) or generalised additive models (GAMs), as appropriate, were used to model the relationships between slope and speed, rate of CO₂ production and gross C_{path}. They allowed identification of significant relationships between slope and these response variables. GAMs allow exploration of the extent to which the variation in a response variable is associated with variation in the explanatory variable without specifying the shape of the relationship between the variables (Crawley, 2005). This makes them particularly useful when, as here, the explanatory variable goes through zero, therefore making a hump-shaped relationship more likely than a linear one. The outputs of fitted models were used to calculate the predicted slope of the optimal paths to ascend or descend one unit distance in terms of minimising either the time taken or the metabolic energy expenditure.

Vertical cost of locomotion

The vertical cost of locomotion (C_{vert}) has been previously introduced to understand the optimisation of mountain paths (Minetti et al., 2002) and is defined here as the gross energy expenditure per unit vertical displacement (one vertical metre; m_{vert}) ($\text{J kg}^{-1} m_{\text{vert}}^{-1}$). To predict the locomotory behaviour of ants traversing routes involving vertical ascent/descent, we calculated C_{vert} as the product of gross C_{path} and 1/sinθ:

$$C_{\text{vert}} = \frac{C_{\text{path}}}{\sin \theta} \quad (1)$$

We also calculated the vertical journey time (t_{vert} ; $\text{s m}_{\text{vert}}^{-1}$) as:

$$t_{\text{vert}} = \frac{1}{v \sin \theta} \quad (2)$$

where v is speed. Note that paths with a slope of 0 deg were not considered as they are of infinite length.

The optimal slopes that minimised either vertical journey time or the gross vertical cost of locomotion were thus determined.

RESULTS

All results are presented as means ± s.e.m. (number of replicates) unless otherwise stated.

Metabolic cost and speed of locomotion on a slope

The gross rate of CO₂ production was not significantly affected by slope (GLM, adj. $R^2=0.0048$, $P=0.48$; Fig. 2A) and was approximately 1.7 ml g⁻¹ h⁻¹ for all slopes, equivalent to a gross metabolic rate of approximately 47 J g⁻¹ h⁻¹. Speed was significantly affected by slope (GAM, adj. $R^2=0.66$, $P<0.001$). The highest speed, 2.0±0.1 cm s⁻¹ ($N=20$), occurred during level walking, with speed decreasing at steeper gradients on both an incline and a decline, with a minimum value of 0.7±0.06 cm s⁻¹ ($N=17$) while ascending a 90 deg slope (Fig. 2B). Gross C_{path} was significantly affected by slope (GAM, adj. $R^2=0.488$, $P<0.001$). There was an approximately U-shaped relationship between gross C_{path} and slope, with the minimum gross C_{path} (646.0±51.2 J kg⁻¹ m⁻¹, $N=20$) occurring during level locomotion and the maximum gross C_{path} (1961.3±167.3 J kg⁻¹ m⁻¹, $N=17$) occurring during ascent of a 90 deg slope (Fig. 2C).

Predicted time and energetic cost of routes on different slopes

The predicted gross vertical cost of locomotion and vertical journey time (Eqns 1, 2) for walking routes using different slopes are shown

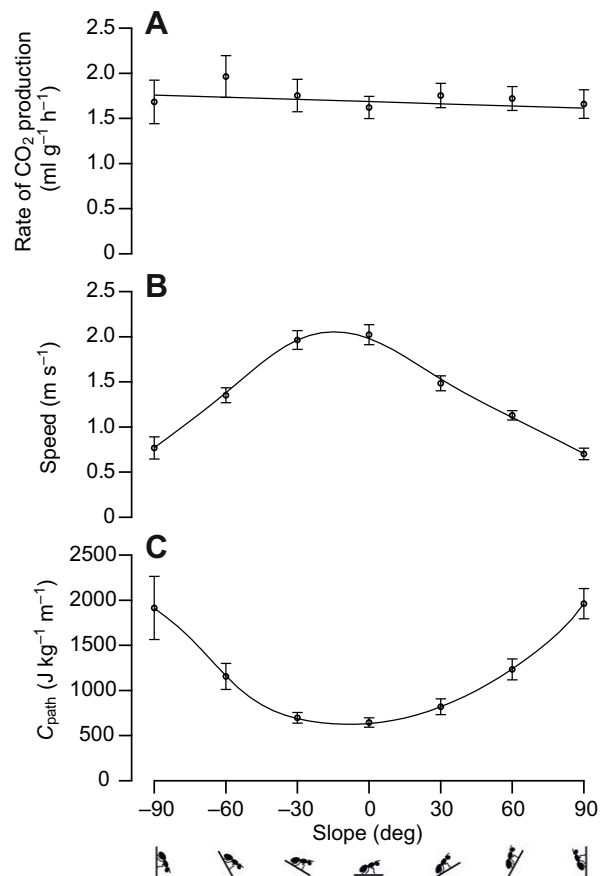


Fig. 2. The effect of slope on (A) the rate of CO₂ production, (B) speed and (C) gross cost of locomotion (gross C_{path}) for ants travelling on slopes ranging from -90 to +90 deg in 30 deg increments ($N=7, 9, 11, 20, 19, 21$ and 17). Predictions of gross C_{path} from general linear models (A) and generalised additive models (B,C) and are shown as solid lines. Data are means ± s.e.m.

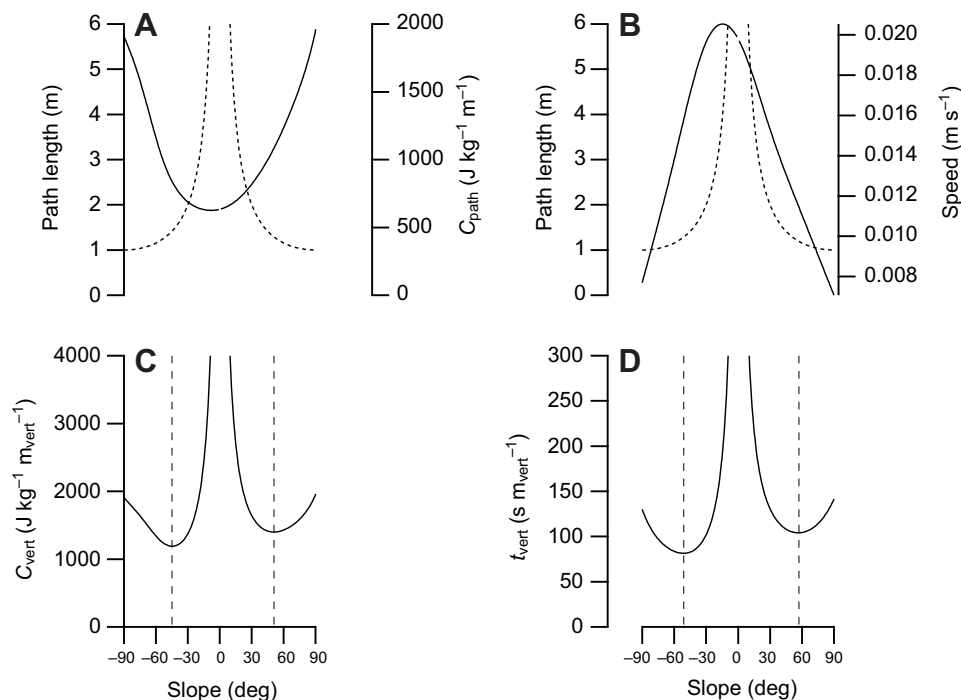


Fig. 3. Predictions for (A) C_{path} (solid line) and (B) speed (solid line) are shown along with calculated path length (dashed lines) for slopes ranging from -90 to $+90$ deg. The predicted vertical gross cost of locomotion (C_{vert}) (C), and vertical journey time (t_{vert}) (D) for a route at each slope are shown. Note that values are only shown for angles from 10 to 90 deg and -10 to -90 deg because path length tends to infinity at a slope of 0 deg. Angles of slopes giving minimum vertical cost of locomotion and vertical journey time are denoted by vertical dashed lines (C,D).

in Fig. 3. The path length to cover m_{vert} necessarily increases as slope angle decreases; path length tends to infinity as the slope approaches zero (i.e. level locomotion) (Fig. 3A,B). The gross metabolic cost of following a path of a given slope was calculated using Eqn 1 (Fig. 3A,C). Predicted minimum metabolic cost paths occur at -45 deg for a vertical descent of 1 m and 51 deg for a vertical ascent of 1 m (Fig. 3C). Time taken to cover a path on a given slope is given by Eqn 2 using the predicted speed for that slope (Fig. 3B). Predicted minimum time paths occur at -51 deg for a vertical descent of 1 m and 57 deg for a vertical ascent of 1 m (Fig. 3D).

DISCUSSION

Metabolic rate and speed of locomotion on a slope

The gross metabolic rate of leaf-cutter ants moving at their preferred speed is independent of slope (Fig. 2A). This finding is in contrast to studies in other species where metabolic rate has been found to increase with incline; however, in these previous studies, speed was kept constant for all gradients (Wunder and Morrison, 1974; Full and Tullis, 1990; Snyder and Carello, 2008). In our experiments, leaf-cutter ants were able to freely select their speed of locomotion and, rather than maintaining a constant speed, ants moderated speed, travelling fastest on the shallowest slopes and more slowly as slope increased (Fig. 2B). During level locomotion, gross metabolic rate increases with speed (Hoyt and Taylor, 1981; Taylor et al., 1982; Full and Tullis, 1990; Full et al., 1990); therefore, the modulation of speed with slope in ants (Fig. 2B) may be a strategy to maintain a constant metabolic rate.

To our knowledge, this study is the first to demonstrate an animal modulating speed to maintain a constant metabolic rate with changing slope. This behavioural adaptation suggests that the maintenance of a constant metabolic rate may confer some advantage to the animal as its preservation appears to be prioritised over the minimisation of the time taken to complete a journey. Maintenance of a constant metabolic rate represents a constant effort by the ants and it is possible that this allows them to sustain locomotion over long foraging periods. The moderation of speed with incline has also been noted in a variety of species under experimental (Wickler et al., 2000; Denny et al.,

2001) and natural (Jayne and Irschick, 2000; Mogollon and Farji-Brener, 2009) conditions. In some of these species, the moderation of speed could – at least in theory – allow a constant metabolic rate to be maintained at different inclines [e.g. cockroaches (Full and Tullis, 1990); ghost crabs (Tullis and Andrus, 2011); red squirrels (Wunder and Morrison, 1974); guinea fowl (Rubenson et al., 2006); and horses (Wickler et al., 2000)]. However, in previous studies where the preferred speed has been measured [e.g. horses (Wickler et al., 2000)], animals were not found not to moderate speed to maintain a constant metabolic rate. This suggests that, in some species, factors other than endurance [e.g. minimisation of the time taken to complete a journey; reduction of the risk of navigational error (Denny et al., 2001)] are important, there is a difference depending on gait or there may be an effect of body mass (Taylor et al., 1972; Full and Tullis, 1990; Wickler et al., 2000).

The lack of difference in metabolic rate with varying slope could potentially have arisen because of a bias in the proportion of time spent running on each slope. However, there was no systematic variation in the proportion of running time across slopes suggesting that the constancy of gross metabolic rate was not due to ants spending more time resting on steeper slopes.

Metabolic cost of locomotion on a slope

The result of the moderation of speed and the maintenance of gross metabolic rate is a U-shaped relationship between gross C_{path} and slope (Fig. 2C): gross C_{path} is lowest for ants travelling on the level and on shallow declines but increases on both inclines and steeper declines. This is broadly in line with previous findings in larger animals (Margaria, 1968; Full and Tullis, 1990; Wickler et al., 2000) but contradicts previous findings showing only small effects of slope on metabolic cost per unit distance travelled in another species of leaf-cutter ant (Lipp et al., 2005). The reasons for this difference are uncertain, but may be related to the much higher airflow rates used in the earlier study [resulting in air velocities of approximately 24 cm s^{-1} (Lipp et al., 2005), compared with approximately 1.9 cm s^{-1} in our study]. Gross C_{path} measured here for level locomotion lies within the range previously measured in *Atta colombica* (Lighton et al., 1987).

Locomotion on an incline as opposed to on the level is likely to incur a higher cost of locomotion as an increase in muscular work is required to raise the centre of mass; this is metabolically expensive (Fenn, 1923; Kushmerick and Davies, 1969; Bigland-Ritchie and Woods, 1976; Beltman et al., 2004). In addition, increasing muscular work must occur through changes in force generation and/or increased muscular shortening. Increased force could occur through the recruitment of a larger muscle volume; increased muscle shortening may also (though not necessarily, depending on whether muscle shortening velocity changes) require the recruitment of a greater muscle volume to compensate for a reduction in force because of the force–velocity relationship. Recruiting more muscle for either of these two reasons may further increase metabolic cost (Bigland-Ritchie and Woods, 1976; Roberts et al., 1997). However, the case for the increased cost of locomotion on a decline is less clear. During descent, muscles must absorb and dissipate as heat the potential energy lost by the centre of mass. This is achieved by active lengthening of the muscles, which is metabolically inexpensive (Curtin and Davies, 1973; Beltman et al., 2004). In addition, because actively lengthening muscles generate high forces (the negative part of the force–velocity relationship), a smaller volume of muscle may need to be recruited to absorb the potential energy, which is expected to further lower the metabolic costs during descent. However, the finding that gross C_{path} begins to increase beyond a certain gradient has been reported in other species (Margaria, 1968; Minetti et al., 2002). It has been suggested that, because of muscle fatigue and damage, energy consumption may increase during sustained downhill locomotion as more muscle fibres are recruited (Dick and Cavanagh, 1987). However, it is unlikely that either fatigue or muscle damage would be issues for ants exercising over the durations studied here. Hence, the underlying cause(s) for the decline locomotion being energetically expensive is currently unclear.

Predicting optimal routes in ants

The predictions of gross vertical cost of locomotion and vertical journey time using paths of different gradients (Fig. 3) suggest that there are minima for both C_{vert} (Fig. 3C) and journey time (Fig. 3D). However, the effect of modifying speed means that the routes that minimise C_{vert} and t_{vert} are very similar (Fig. 3). The route that minimises vertical journey time incurs a 0.1% increase in the gross vertical cost of locomotion compared with the route that minimises C_{vert} , and the route that minimises C_{vert} incurs a 2% increase in vertical journey time compared with the route that minimises t_{vert} . Therefore, the optimal route for ants undertaking a journey involving vertical displacement is one following a path with a slope of between 51 and 57 deg on an incline and between –45 and –51 deg on a decline in order to keep both vertical journey time and vertical cost of locomotion close to their respective minima. The application of these findings in future behavioural studies will allow us to explore the strategies used by foraging ants to negotiate slopes and to determine whether routes that minimise either energetic or time costs of a journey are preferentially used or whether other factors result in a deviation from these predicted routes.

LIST OF SYMBOLS AND ABBREVIATIONS

C_{path}	metabolic energy expenditure per unit distance travelled on the slope
C_{vert}	vertical metabolic cost of locomotion
GAM	generalised additive model
GLM	general linear model
m_{vert}	vertical metre
t_{vert}	vertical journey time
v	speed

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