

The desert ant odometer: a stride integrator that accounts for stride length and walking speed

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Accepted 8 November 2006

Summary

Desert ants, *Cataglyphis*, use path integration as a major means of navigation. Path integration requires measurement of two parameters, namely, direction and distance of travel. Directional information is provided by a celestial compass, whereas distance measurement is accomplished by a stride integrator, or pedometer. Here we examine the recently demonstrated pedometer function in more detail.

By manipulating leg lengths in foraging desert ants we could also change their stride lengths. Ants with elongated legs ('stilts') or shortened legs ('stumps') take larger or shorter strides, respectively, and misgauge travel distance. Travel distance is overestimated by experimental animals walking on stilts, and underestimated by animals walking on stumps – strongly indicative of stride integrator function in distance measurement.

High-speed video analysis was used to examine the actual changes in stride length, stride frequency and walking speed caused by the manipulations of leg length. Unexpectedly, quantitative characteristics of walking behaviour remained almost unaffected by imposed changes in leg length, demonstrating remarkable robustness of leg coordination and walking performance.

These data further allowed normalisation of homing distances displayed by manipulated animals with regard to scaling and speed effects. The predicted changes in homing distance are in quantitative agreement with the experimental data, further supporting the pedometer hypothesis.

Key words: desert ant, *Cataglyphis*, navigation, stride integration, walking behaviour, odometer.

Introduction

Foraging desert ants *Cataglyphis fortis* cover distances of more than 100 m on a meandering search path in their vast and flat desert habitat. With a prey item in their mandibles they return back home to their nest on a straight trajectory, instead of retracing their circuitous outbound path. The ants achieve this feat by continuously updating their home vector, which is integrated from two parameters, walking direction and walking distance of each path segment. Many central place foragers navigate in this manner, known as path integration (Mittelstaedt and Mittelstaedt, 1980; Wehner and Srinivasan, 1981) (for reviews, see Barth, 2002; Wehner, 1992), which relies on acquisition and summation of these two vector components. In desert ants and honey bees, the estimation of travel direction is based on a well-studied celestial compass, reading the polarised (and spectral) sky light pattern and the sun's azimuth (Wehner and Lafranconi, 1981; Wehner, 1989; Wehner, 1997).

Distance measurement in insect navigation, by contrast, has long remained controversial. Earlier experiments in flying honey bees supported the 'energy hypothesis', initially put

forward by Heran and Wanke (Heran and Wanke, 1952) (see also Heran, 1956; von Frisch, 1965). The energy hypothesis states that travel distance is gauged by the energy expenditure afforded during flight. However, in a series of elegantly designed experiments it was demonstrated that the bees' estimation of travel distance is achieved by the integration of self-induced optic flow experienced *en route* (Esch and Burns, 1995; Srinivasan et al., 1996; Srinivasan et al., 1997; Esch et al., 2001; Tautz et al., 2004). By a similar token, the energy hypothesis is not applicable to desert ant odometry. For instance, desert ants assess their walking distances with amazing accuracy, irrespective of the load they carry (Wehner, 1992). Contrasting with the honeybee odometer, however, the integration of visual flow-field cues ('optic-flow' hypothesis) plays a minor role in the odometer of *Cataglyphis fortis* (Ronacher and Wehner, 1995). This is true for flow-field stimuli presented in the ventral field of vision, while lateral optic flow has no effect at all (Ronacher et al., 2000). In fact, without any visual experience of ongoing movement, in complete darkness or featureless environments (Thiélin-Bescond and Beugnon, 2005), *Cataglyphis* still gauge their

walking distance fairly correctly. Thus, the ants appear to rely on idiothetic cues, derived from the movements of their legs (e.g. Pieron, 1904). That is, they employ a 'stride integrator', as recent experiments have illustrated (Wittlinger et al., 2006).

In this study we investigated how *Cataglyphis* ants measure travel distance, focussing on leg movement during walking. We manipulated leg length, and since stride length, walking speed and leg length are interdependent, we consider all three parameters in our analysis. Leg lengths were altered in ants walking back home from a feeder to their nest, by means of both, truncation and extension, reducing the animals' legs to stumps or providing them with stilts. Manipulation of leg length automatically changed stride length and walking speed, and provided critical parameters for testing the stride integrator hypothesis. We show that experimental ants striding home with manipulated leg lengths, walking on stilts or on stumps, and thus using stride lengths different from their outbound journey, misgauge homing distance in proportion to the imposed changes in (normalised) stride lengths. Nevertheless, characteristics of walking behaviour remained almost unaffected by altered leg length, demonstrating a remarkable robustness of leg coordination and walking performance.

Materials and methods

Experimental situation and procedures

At the field site near Maharès, Tunisia (34°30'N, 19°29'E), large and viable nests of *Cataglyphis fortis* Forel 1902 (Wehner, 1983) were selected for experiments. The experimental season lasted from the middle of June to the start of September in 2004 and 2005. The ants were trained to walk a distance of 10 m in a linear alloy channel from their nest entrance to a feeder established due south (Fig. 1). The channel was 7 cm wide and the walls 7 cm high, such that the ants experienced a strip-like view of the sky of about 45° to almost 60°, depending on the height of the ant's eyes above ground and its actual position on the channel floor, to read the skylight pattern and compass information. To provide traction for walking, the channel floor was coated with fine grey sand. This sand coating reliably prevented slipping of the ants, as judged from high-speed video recordings (see Fig. 3). This held in particular after removal of the ants' tarsi, and the traction devices associated with them, in the course of the operation procedures below. Grain size of the sand ranged between 0.2 and 0.4 mm, to minimise visual orientation cues such as optic flow. The channel walls were painted with matt grey varnish to provide a featureless environment, and avoid distracting reflections.

For each test situation, ants were marked with a particular colour. After at least 1 day of training, marked ants were caught at the feeder, manipulated, and then put into a small plastic container together with an assortment of food. After having gathered their booty the ants were transferred to a test channel, aligned in parallel to the training channel and with identical features except for its considerably increased length of 24 m (Fig. 1). The fact that the ants were holding on to a food item

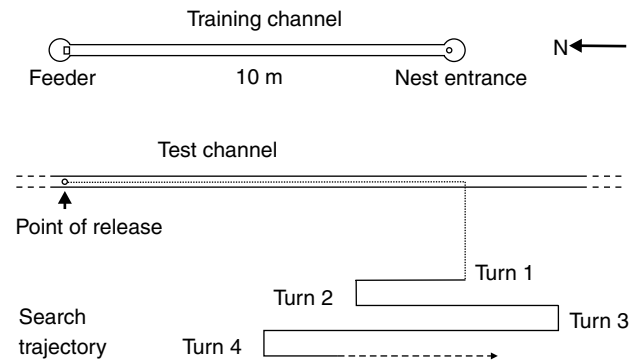


Fig. 1. Experimental situation. Schematic diagram of channel layout; training channel, top; test channel, bottom (broken lines indicate extended length of test channel to 24 m). The search behaviour exhibited by *Cataglyphis* foragers after having run off their home vector is illustrated schematically below the test channel. Not drawn to scale. N, north.

is a sure sign that they were motivated to carry the food back home (Wehner, 1982). Once put into the test channel, the animals immediately took up determined homeward runs that ended when the behaviour switched to nest searching (cf. Müller and Wehner, 1994). This switch is marked by a conspicuous U-turn (Sommer and Wehner, 2004), followed by a run pacing back and forth around the assumed position of the nest entrance. We noted the point of this switch in homing behaviour, as well as the first six consecutive turning points (Fig. 1, search trajectory), for later calculation of the median and construction of search density distributions (below, Fig. 4). Each ant was tested twice, first straight after the manipulation of leg length at the feeding site (Test 1, Fig. 4A); the animal was then put back into the nest and tested a second time after having re-emerged from the nest and travelled to the feeder again (Test 2, Fig. 4B). The marked and manipulated ants were again caught at the feeder and immediately transferred to the test channel. In this situation, leg length, and thus stride length, was the same in both, outbound and homebound runs, although different from normal due to the previous manipulations.

Manipulation of leg length

The ants' legs were shortened either by removing the tarsal segments (stumps I, Fig. 2) or by severing the leg at mid-tibia level (stumps II, Fig. 2) with a pair of small scissors. The animals' legs were splinted and extended with adhered pig bristles, as illustrated in Fig. 2. The pig bristles were glued to the legs with cyanoacrylate glue (Superglue) and extended for about 1 mm beyond the tarsal tips. This procedure extended the effective leg length by about 2.0–3.0 mm since the distal tarsus segments are normally apposed to the substrate for traction (see 'normal' in Fig. 2). Leg length in 'normal' ants was not altered and they served as controls.

Walking speed

The homing walking speeds of ants with extended legs (stilts), shortened legs (stumps) and normal legs were recorded.

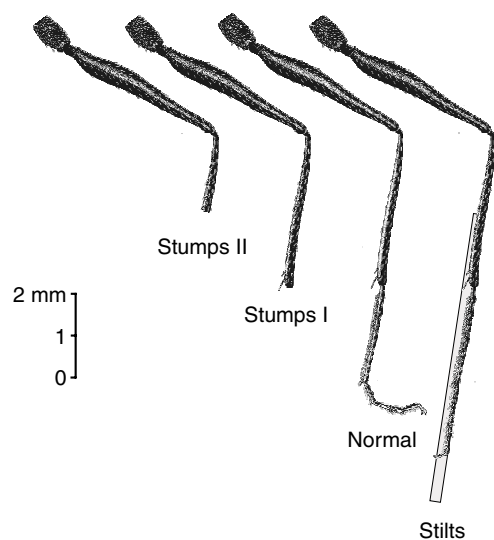


Fig. 2. Manipulation of leg length. The right hind leg of a *Cataglyphis fortis* worker is shown from the anterior [adapted from fig. 1 in Wehner (Wehner, 1983)], and the manipulations performed in the present study are indicated: stumps II and I, the removal of the distal part of the leg from the mid tibia, and removal of the tarsi, respectively; a normal leg; and stilts, the addition of pig bristles to lengthen the leg.

The time required to cover 3 m distance in the test channel with uninterrupted locomotion was determined with a stopwatch to 0.01 s (Oregon Scientific Inc., Portland, USA). In addition, walking speed was analysed from the high-speed video recordings (below) to determine the relationships between walking speed, stride length, and leg length.

Analysis of behavioural data

The test channel was divided into 10 cm bins for distance recording, as outlined above. First, search density distributions (Fig. 4) were evaluated in $N=25$ animals for each experimental situation. The bins of the test channel covered by an ant during its search were noted and cumulated, that is, the more often a channel segment (bin) was visited during the search, the higher its value in the density distribution (see Fig. 4, upper panels). Search density distributions were normalised to their peak values, and their widths at half-maximum height (half widths) were determined. Second, the median values of the initial six turning points of the ants' nest searches were calculated to provide an estimate of the search centre. From the median values of $N=25$ ants per experimental situation, box-and-whisker plots were constructed (see Fig. 4, lower panels). Marked values in the box-and-whisker plots are the centre (median), the spread (interquartile range), and the 10th and 90th percentiles (whiskers) of search centre values.

High-speed video films

Film recordings were made with a Redlake MotionScope (Redlake MASD Inc., San Diego, USA) high speed camera at 250 frames s (Fig. 3). Normal and manipulated freely walking

ants were filmed in top view whereas walking in a pen of 10 cm×30 cm or in an alloy channel of 2 m length, both supplied with the sandy walking substrate mentioned above (and a piece of millimetre graph paper for calibration). Since it was not possible to film the complete homebound travel of the above experimental ants, these high-speed video analyses were carried out in a different set of animals. Whereas all other experiments were performed in Maharés, some of the video recordings were made in the laboratory at the University of Ulm to sample at slightly lower temperatures than in the desert (~30–45°C air temperature), and thus broaden the range of walking speeds used to calculate the regression lines in Fig. 6. Each ant performed at least four runs and was subsequently preserved in 70% alcohol for later anatomical inspection and measurement of morphometric data. Stride lengths (s_2) of the left and right middle legs (l_2 , r_2 ; see Fig. 3), as well as walking speeds were analysed from the video films. Only those runs were considered for evaluation that showed straight walking paths without deceleration or abrupt stops.

To derive a prediction of how far the manipulated ants would

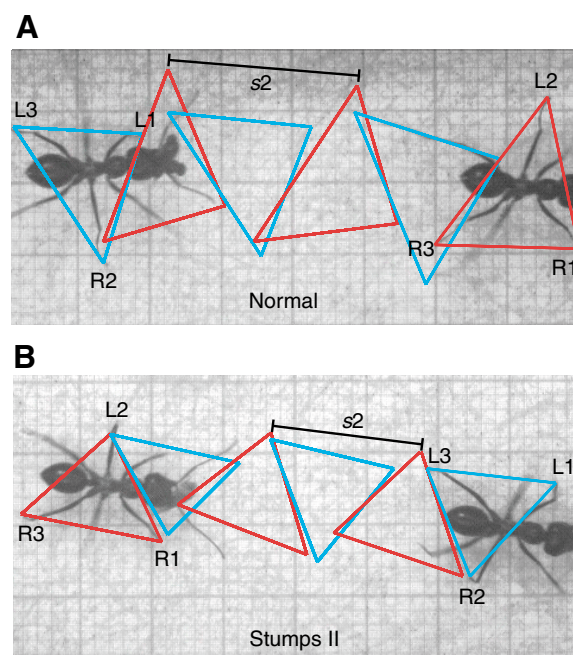


Fig. 3. Tripod gait of a *Cataglyphis* individual before (normal) and after leg shortening (stumps II). The six complete strides captured by the high-speed video are shown, and indicated as the tripods performed by the animal in its normal tripod gate. Tripods formed by the left front and hind (L_1 , L_3) and right middle (R_2) legs are drawn in blue; those formed by the right front and hind (R_1 , R_3) and left middle (L_2) legs are drawn in red. Stride length (s_2) was determined as the distance between two successive middle leg footfalls. (A) Untreated normal situation, showing the typical tripod gait ($s_2=13.3$ mm; $v=0.28$ m s⁻¹). (B) After leg shortening the ant still shows the typical tripod gait but with obviously decreased stride length ($s_2=10.5$ mm; $v=0.27$ m s⁻¹). Single video frames of the ant, taken during the 1st and 6th captured strides, are pasted into the tripod analysis.

have to run on their homebound journeys, based on the pedometer hypothesis, the high-speed video data were normalised. This was first done with regard to body size, that is, relative stride lengths with regard to body dimensions were calculated to eliminate effects of size variation. From the morphometric data, alitrunk length (al) was used for the normalisations (al was measured from the apical edge of the pronotum to the caudal edge of the propodeum). Relative leg length ($l2'$) was calculated from the length of the middle leg ($l2$, measured from the coxo-femoral joint to the tips of the tarsal claws) as $l2' = l2/al$, and relative stride length ($s2'$) was determined from the video data as $s2' = s2/al$.

Second, the video data were normalised with regard to walking speed (v). Stride length (s) and stride frequency (f) are the two determinants of running speed (stride length multiplied by the number of strides per second is locomotor speed), and the relationship of these two parameters was determined for the different experimental situations (Fig. 6) (see also Zollikofer, 1988; Zollikofer, 1994b). This relationship was then used to normalise stride lengths to a stride frequency that occurred in all examined individuals. The relationship between stride length (ordinate in Fig. 6) and stride frequency (abscissa) was described by a regression line ($y = bx + a$) (Fig. 5). The term of the regression line was used to normalise the stride length data to a stride frequency of 20 Hz, that is, the data points were shifted along the respective regression line to 20 Hz on the abscissa: $s2'_{20\text{Hz}} = (b \times 20 \text{ Hz} + a) + [s - (bf + a)]$.

The resulting distributions of stride lengths (on the ordinate) formed the basis for the hatched box plots in Fig. 4 (lower left panel). This normalisation procedure yielded stride lengths that were comparable not only for ants of different body size (first point above) but also for ants travelling at different walking speeds, for instance, because of different prey loads or experimental manipulations. This procedure further allowed a prediction to be made for the respective undershoot or overshoot of manipulated ants, based on the assumption that distance is measured by a stride integrator, and allowing for variability in body size and walking speed (hatched boxes in Fig. 4A, lower panel).

The stride length and stride frequency data were also plotted against walking speed (see Fig. 7) to provide a basis for comparison with other data in the literature (see Discussion).

Statistical tests

Only nonparametric tests were used to compare experimental groups in the field experiments, according to the sample sizes of $N=25$ in each group. In Test 1 and Test 2 the groups that had received different treatments (stilts, normal, stumps I and stumps II) consisted of different sets of animals and thus are independent, which allowed us to use a Kruskal–Wallis ANOVA on ranks to compare them. For pair-wise multiple comparison of the treatment groups Dunn's method was used.

From the high-speed film analysis, linear correlations of the distributions of walking parameters were calculated for each treatment group. Correlation coefficients, regression

coefficients and axis intercepts were tested with student's t -test (for difference from 0, and for differences against each other). Statistical methods are described in detail in Sokal and Rohlf (Sokal and Rohlf, 1995).

Results

Homing distances with manipulated leg lengths

As outlined above, *Cataglyphis fortis* ants were trained to visit a feeder 10 m to the south of the nest, to be reached through a narrow channel. Experimental ants were captured at the feeder, had their leg length modified, and were placed in a test channel to record their homing distances (see Fig. 1). One group of experimental ants had their legs extended after visiting the feeding site. That is, they were walking back homeward in the test channel on stilts. These animals clearly overestimated homing distance, in fact by 50% (15.30 m, $N=25$), with regard to control ants with normal legs (10.20 m, $N=25$). Animals with shortened legs, by contrast, clearly undershot. Ants that had half of their tibial segments clipped off (stumps II) searched at 5.75 m ($N=25$), and ants that had just their tarsomeres removed (stumps I) searched at 6.95 m ($N=25$) distance from the release point. These observations are shown in Fig. 4 in the form of search density distributions and median values of the initial six turning points of nest searching. Comparing the treatment groups demonstrated significant difference ($P < 0.001$, Kruskal–Wallis ANOVA). Pair-wise multiple comparison according to Dunn's method, as a *post-hoc* test, revealed significant differences between all treatment groups (normal vs stilts, $P < 0.05$; normal vs stumps I, $P < 0.05$; normal vs stumps II, $P < 0.05$), except the stumps I vs stumps II comparison, where no significant difference was observed ($P > 0.05$).

After testing (Test 1) the ants were put back into their nest, and they were tested again when they turned up at the feeder during one of the following days (Test 2). These animals had thus performed their outbound runs with manipulated leg and stride lengths, and they were caught at the feeder to be tested as further controls (in addition to the unmodified ants, searching around 10.20 m; as described above). We expected them to be 'recalibrated' since they had the same manipulated leg length during the outbound as well as during the homebound runs. They should thus show searches centred on the correct nest–feeder distance of 10 m. And this was indeed what we observed. Each group of experimental ants, whether walking on stilts or on stumps, performed almost identical homing runs, corresponding to that of normal ants (above). Ants on stilts (stilts, $N=25$) searched at 10.55 m, and ants with shortened legs at 9.55 m (stumps I, $N=25$) or at 10.25 m (stumps II, $N=25$). No statistically significant difference was discernible between these groups ($P = 0.502$).

The half widths (see Materials and methods) of the search density distributions, as shown in Fig. 5, reflect the uncertainty of the ants' estimates of their homing distance (Wolf and Wehner, 2005; Merkle et al., 2006). Half width values exhibit a conspicuous relationship to the imposed changes in leg length. In the data from Test 1 (see Fig. 4A), half widths

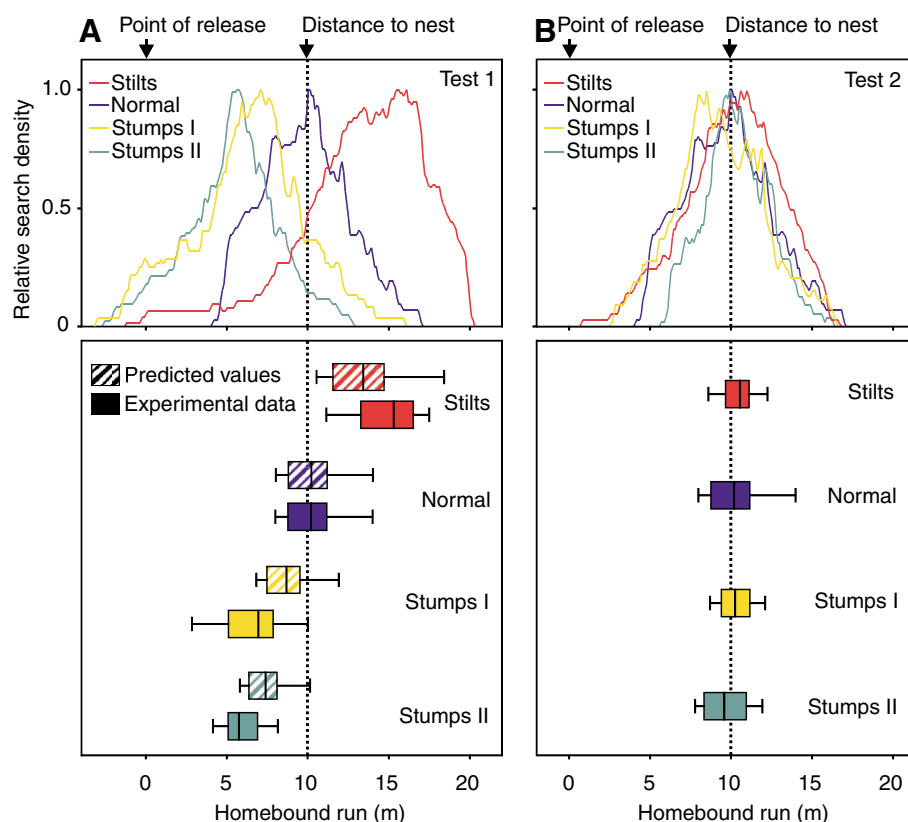


Fig. 4. Homing distances of experimental ants. The top panels show search density plots (abscissae, homing distance; ordinates, cumulated relative search densities between the first and sixth turning points; see Materials and methods), the bottom panels show box-and-whisker plots (medians of the initial six turning points; compare Fig. 1, bottom), derived from the same data sets ($N=25$ ants for each experimental situation). (A) The ants were tested immediately after the lengths of their legs had been modified at the feeding site, that is, leg lengths were normal during the outbound journey but manipulated during the homebound run (Test 1). (B) The ants were tested after re-emerging from the nest after previous manipulation. In this situation leg lengths were equal, though manipulated, during outbound and homebound runs (Test 2). The hatched box plots in the lower panel of A illustrate the search centres as predicted from the high-speed video analyses of stride lengths in normal and manipulated animals; for details see text. Colour code: red, stilts; blue, normal; yellow, stumps I; green, stumps II.

increase with enlarged leg length, and they decrease with shortened legs. This effect is still present, though much reduced, in the Test 2 data.

Striding on stilts and stumps

High-speed video analysis of running ants was initially performed to examine the actual changes in stride lengths caused by the manipulations of leg length (a linear relationship between leg length and stride length may not be assumed – consider a human balancing on stilts, who takes short strides indeed). These video analyses illustrated immediately that the overshoot in ants with stilts, and the undershoot in ants with stumps of different lengths (stumps I and stumps II), corresponded to the altered stride lengths in a qualitative way. That is, ants on stilts took longer strides and ants on stumps shorter strides than did normal animals.

Stride length in normal *Cataglyphis* depends on two parameters, (i) on leg length and thus body size, as body morphology is isomorphic in *C. fortis* worker ants (Wehner, 1983), and, (ii) on the velocity of locomotion. As is true for all walking animals, the faster *Cataglyphis* run, the larger are the strides they take, and the larger is their stride frequency (Zollikofer, 1994a; Zollikofer, 1994b). To obtain a quantitative assessment of the altered homing distances in manipulated ants, relative stride lengths were thus calculated to eliminate effect of scaling, that is, of variation in body size. The effect of locomotor velocity on stride length was eliminated by comparing the animals after normalisation to a certain stride frequency, as described in the Materials and methods section.

To this end, the relationships between walking speed and its two determinants, stride length and stride frequency, was analysed for the different experimental situations. Stride length is plotted *versus* stride frequency in Fig. 6. It is evident that manipulation of leg length changed the relationship between stride length and stride frequency, as was to be expected. Ants with elongated legs made longer strides at any given stride frequency than did normal ants, and ants with shortened legs took shorter strides. These relationships were used to normalise the stride length data of the experimental animals to a stride frequency of 20 Hz, a frequency observed in all experimental situations. This normalisation allowed direct comparison of ants walking at different speeds, be it due to different prey loads or experimental manipulation.

According to the video analysis and after the above normalisation procedures, the changes in stride length, and thus homing distances, to be expected as a result of the manipulated leg lengths were as follows. Homing distances should have been reduced by 27.7% in ants with the shortest legs, half of their tibiae being clipped (stumps II), by 15.1% in ants with severed tarsal segments (stumps I), and the homing distance would be expected to have increased by 31.5% in ants striding on stilts (Fig. 4A, hatched box-and-whisker plots). The homing distances actually reported above, when converted to percentage values, were in fairly good agreement with these expectations, amounting to 43.6%, 31.9% and 50.0%, respectively. The observed homing distances were, however, consistently farther from the real nest–feeder distance by an average 17.1% (range 15.9–18.5%) than would be expected

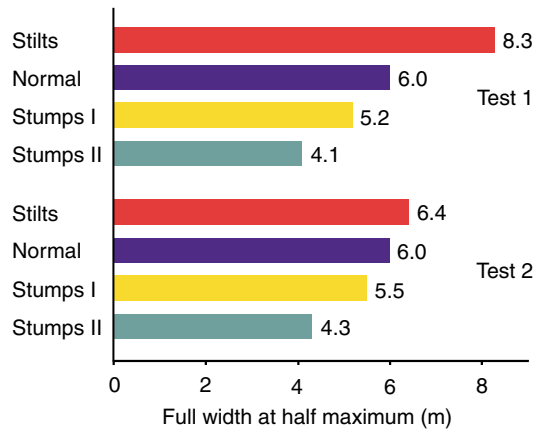


Fig. 5. Widths of search density distributions change with altered leg length. The half widths (width of graph at half-maximum height) of the search density distributions in Fig. 4 were determined. They are shown for the different experimental situations, Test 1, top, Test 2, bottom. Colour code: red, stilts; blue, normal; yellow, stumps I; green, stumps II.

from the altered stride lengths as recorded in the high-speed video analysis.

When the altered homing distances of the ants walking on stilts or stumps are converted into stride numbers (at a normalised 20 Hz and standard body size to make data comparable), the ants walking on stilts, normal legs and stumps I and II took an average 1066, 924, 745 and 727 strides before looking for their nest entrance. According to the altered stride lengths, as determined from the high-speed videos, all groups would have been expected to take 924 strides – the value on which the normalisation procedure was based.

Characteristics of walking behaviour in ants with manipulated leg lengths

Whereas the plot of relative stride length *versus* stride frequency in Fig. 6 served primarily to normalise the behavioural data regarding homing distance, these data may also be used to characterise walking behaviour after the manipulation of leg lengths. Fig. 7 presents plots of stride frequency (Fig. 7A) and stride length (Fig. 4B) against walking speed. These diagrams lend themselves to direct comparison with data from other insects, and indeed other walking animal groups (see Discussion).

The rise in stride frequency (Fig. 7A) and the rise in stride length (Fig. 7B) with increasing walking speed may both be approximated with good accuracy by linear regression lines, values for R^2 ranging from 0.81 to 0.87. Curvilinear (exponential) fits to the data points are just slightly better for the frequency–speed relationship ($R^2=0.92$) and indeed worse for the stride length–speed relationship ($R^2=0.77$). This means that in the recorded speed range no saturation of stride frequency was observed [compare two articles by Full and Tu (Full and Tu, 1990; Full and Tu, 1991)], indicative of the absence of flight phases in the step cycle, or galloping.

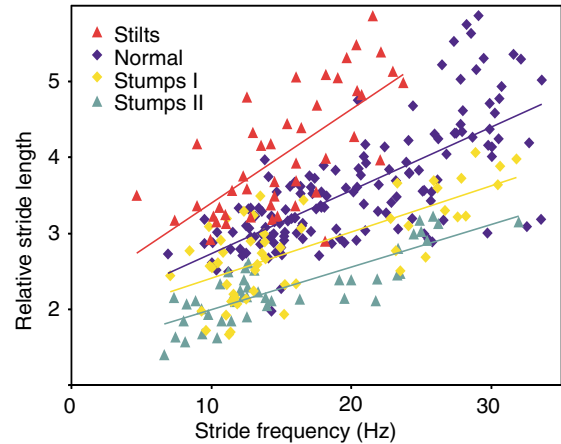


Fig. 6. Relationship between (relative) stride length and stride frequency in normal ants and after manipulation of leg length. Stride length was normalised with regard to body size (alitrunk length, see Materials and methods), and is shown on the ordinate; the abscissa gives stride frequency. Each data point represents one individual. Best fit regression lines are indicated; the respective equations are, from top to bottom: stilts, stride length = $0.124 \times \text{stride frequency} + 2.14$; normal, stride length = $0.084 \times \text{stride frequency} + 1.89$; stumps I, stride length = $0.061 \times \text{stride frequency} + 1.788$; stumps II, stride length = $0.057 \times \text{stride frequency} + 1.42$ (stride frequency = Hz). Colour code: red, stilts; blue, normal; yellow, stumps I; green, stumps II.

Considering the different manipulations of leg length, it was to be expected that, at any given walking speed, stride frequency is higher in the animals walking on shortened legs than it was in normal ants. And animals walking on stilts exhibit even lower stride frequencies at that given stride frequency. The slopes of the regression lines show corresponding changes, the slope being highest for the animals with the shortest legs and lowest for the ants walking on stilts. The frequency range is slightly reduced at the high end in all manipulated animals (to 95% in ants with stumps), most notably in the ants walking on stilts (to 71%). The associated reduction in maximum walking speed is less pronounced (to just 82%), because of these animals' elongated legs. By contrast, maximum walking speed is clearly reduced in the ants with the shortest stumps (to 56%, and to just 80% in stumps I animals).

By the same line of argument, it was to be expected that, at any given walking speed, stride length is larger in ants walking on stilts and smaller in ants walking on stumps than it is in normal animals. The slopes of the regression lines are in the same range for all groups of animals. Maximum stride lengths are, of course, clearly reduced in ants with shortened legs (to 75% and 65%, respectively). Unexpectedly, ants walking on stilts exhibit maximum stride lengths that are just marginally larger (104%) than those of normal ants, instead of clearly exceeding the normal range. This is indicative of some impairment resulting from the operation, most probably caused by the added load of the stilts and glue on the legs. In fact, stilts and glue added about 56% to the legs mass, and in a distal

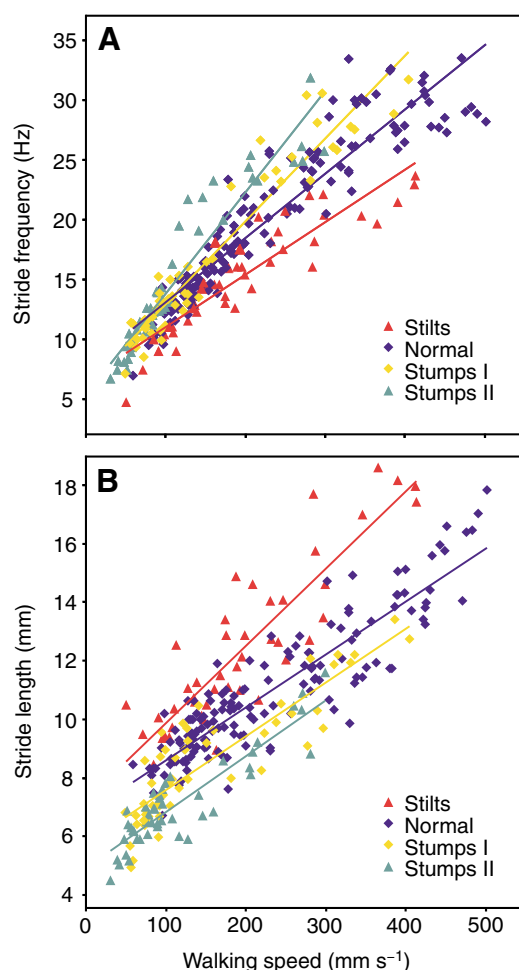


Fig. 7. The relationship between walking speed and (A) stride frequency, (B) (relative) stride length. The different experimental situations are colour-coded as in the previous figures: red, stilts; blue, normal; yellow, stumps I; green, stumps II. Linear regression lines are indicated. The regression lines for the normal ants: stride frequency = $0.054 \times \text{walking speed} + 7.72$ ($R^2 = 0.87$) in A; stride length = $0.018 \times \text{walking speed} + 6.80$ ($R^2 = 0.81$) in B (stride frequency = Hz, stride length = mm and walking speed = mm s^{-1}).

position, producing disproportionately high inertial momentum. Considering this added mass it is indeed surprising that maximum stride frequency, and to a minor extent maximum stride length, are the only parameters of walking behaviour that deviated from expectations.

Discussion

The stride integrator, or pedometer, hypothesis

Two major hypotheses regarding the nature of the odometer in desert ants have been convincingly refuted in previous studies (see also Introduction). The 'energy hypothesis' originally put forward for honeybee navigation by Heran and Wanke (Heran and Wanke, 1952) is in fact neither applicable to bees (Esch and Burns, 1995), nor to desert ants (Wehner,

1992; Schäfer and Wehner, 1993). And the 'optic flow hypothesis', although elegantly proven for flying bees (Esch and Burns, 1995; Srinivasan et al., 2000), plays only a minor role, if any, in desert ant navigation [a minor role was implicated for optic flow in the ventral visual field (Ronacher and Wehner, 1995) (below); no contribution at all for the lateral visual field (Ronacher et al., 2000)]. The remaining, and actually most straightforward, idea is the 'step counter hypothesis' proposed by Pieron (Pieron, 1904). More correctly, this hypothesis should be termed 'stride integrator hypothesis' since insects most probably do not literally count but rather integrate some parameter associated with rhythmic leg movement in walking (below) (Franks et al., 2006). We thus use that latter term in the present study. The 'stride integrator hypothesis' has been confirmed in principle recently by studying the homing behaviour of desert ants with imposed changes in leg length (Wittlinger et al., 2006). However, detailed analysis of walking behaviour in ants with manipulated leg lengths was not performed in that study. Such analysis is indispensable for a quantitative assessment of the pedometer hypothesis, though, due to possible changes in walking behaviour brought about by the manipulations. This has been pointed out in a recent review on distance estimation in flying and walking insects (Collett et al., 2006). In a tentative calculation, because of the lack of appropriate data such as those presented here, the authors conclude that desert ants might indeed systematically change their walking behaviour in response to the stilts and stumps manipulations, for example, regarding the swing phase of leg movement and thus stride length. This possibility is clearly ruled out by the present data set (see below: possible physiological bases of the stride integrator). In fact, our present results are in unequivocal agreement with the above hypothesis – that desert ants employ a stride integrator, or pedometer, for distance measurement in navigation. And our data demonstrate a remarkable robustness of walking behaviour, since all major characteristics remained within expectations, despite the often severe manipulation of leg length.

According to the pedometer hypothesis, ants that have travelled to the feeder on normal legs, and had their leg length modified at the feeder, should cover a different distance on their homebound journey. This is because the same number of strides made during the outbound travel, and registered by the stride integrator, will presumably carry them over a different homebound distance after modification of leg length at the feeder. Animals walking on stilts would be expected to cover longer distances with the same number of strides, and animals walking on stumps, shorter distances. And later, upon re-emerging from the nest with their legs still modified, the number of strides will be the same again for outbound and inbound travel (even though different from the situation with normal legs). No such straightforward interpretation is possible for the energy hypothesis or the optic flow hypothesis.

For a quantitative assessment of the pedometer hypothesis it was necessary to determine the actual changes in stride lengths brought about by the manipulations of leg length. This was

achieved by the evaluation of high-speed video recordings of ants walking on modified legs (Fig. 6). For a useful comparison, the resulting data of stride lengths had to be normalised with regard to both body size and walking speed, since these two parameters influence stride length independently of the experimental manipulations (Fig. 6; details see Materials and methods). The predictions derived from the observed changes in stride length are in good agreement with the experimental data of the homing experiments (Fig. 4A, lower panel), providing further quantitative support for the stride integrator hypothesis.

There are, however, small but consistent differences between the observed and the predicted homebound travel distances (compare filled and hatched box plots in Fig. 4A, lower panel). These differences are in the range of 17% (or roughly 1.75 m) of the total homing distance, and intriguingly they always point away from the correct nest–feeder distance. That is, the animals do not just run too short, for instance, because of possible impairment by the manipulation procedures. Rather, ants on stilts run too far and ants on stumps, too short, all groups searching a bit farther from the true nest–feeder distance than would have been expected from the imposed change in stride length. This consistent observation rules out a number of immediate explanations, since these should always have the same effect, that is, bias the data either towards too short or too far distance estimates. For example, any serious impairment, such as slipping as a result of removal of the tarsal segments, would tend to shorten the ants' homebound runs, independent of the preparation procedure. The animals walking on stilts clearly travelled for much longer distances than the normal controls, however, and they did so with apparently normal vigour and motivation. Indeed, experimentally modified ants were observed to stilt or stump through their habitat on successful foraging trips several times a day, and for many days.

The small but consistent shift of the search centres away from the correct nest–feeder distance might most easily be explained by the contribution of another odometer mechanisms. A candidate mechanism is optic flow in the ventral visual field, which has been demonstrated to make a small contribution to the *Cataglyphis* odometer (Ronacher and Wehner, 1995). Indeed, ants walking on stumps will experience a higher optic flow than normal ants since their eyes are closer to the ground (and the reverse is true for animals walking on stilts). And that increased optic flow might prompt the ants to search for the nest earlier than expected from the manipulations in leg length alone. However, a significant contribution of optic flow would appear unlikely since this has already been ruled out (Ronacher and Wehner, 1995). A contribution of not quite 8% may be calculated from their data. Further, we strove to minimise visual cues in our experiments, mainly through reducing any visual contrast in the animals' field of view, through grey paint on the channel walls and even coating of the channel floor with fine-grained sand. The grain size was selected to be well below the visual resolution of the *Cataglyphis* eye at the given eye–substrate distances (Zollikofer et al., 1995) (see also Materials and methods). Nevertheless, it remains to be determined whether or not the

relatively small differences between predicted and observed homing distance may indeed be due to another odometer mechanism, notably optic flow perceived in the ventral visual field.

The time-lapse integrator hypothesis may be regarded as a variant of the step counter hypothesis. Considering the rather constant walking speed of *Cataglyphis* ants under normal conditions (Zollikofer, 1988), integration of travel time might function as the odometer in vector navigation. However, the time-lapse integrator hypothesis is readily refuted by the observation that homing performance remains unaffected by changes in walking speed, for instance, due to heavy (prey) loads (Zollikofer, 1994c). In the present experiments homing performance did not conform to this hypothesis either, in particular, since the ants on stilts often walked more slowly than normal ants, their maximum speed being reduced to about 82% of expectations. Nevertheless, these animals overestimated homing distance by about 50%.

Possible physiological bases of the stride integrator

Only speculations are possible with regard to the possible physiological bases of the desert ant stride integrator. The fact that the animals account for changes in stride length associated with changes in stride frequency and walking speed in their estimation of travel distance would appear to imply the contribution of sensory feedback from leg receptors. Candidate receptors are campaniform sensillae that monitor cuticular stress and deformation, and coxal and trochanteral hair fields that signal joint angles and movements (see Markl, 1962). Internal muscle receptors and chordotonal organs are also candidates (for a review, see Bässler, 1983), but these possibilities can only be addressed by future experiments. Considering the relatively close coupling of the major parameters of leg movement in walking, namely, stride length and stride frequency, a central nervous integrator of walking pattern generation is a distinct alternative, however.

There exists a wealth of literature on kinaesthetic orientation in other animals, and in arthropods (for a review, see Wehner, 1992), and spiders in particular (e.g. Görner and Claas, 1985; Seyfarth and Barth, 1972; Seyfarth et al., 1982) (for a review, see Barth, 2002). Many spider species are able to navigate in complete darkness, employing leg mechanoreceptors as an important means of path integration (e.g. Seyfarth et al., 1982). Slit sense organs and lyriform organs are used to determine both, direction and distance of travel (Seyfarth and Barth, 1972). This makes a comparison with desert ants difficult since those animals rely on different sensory modalities for determining these two parameters. Nonetheless, it is evident that leg mechanoreceptors, and perhaps indeed campaniform sensillae, may suffice in principle to provide input to a pedometer and determine walking distance.

The width of search density distribution reflects navigation uncertainty

One may interpret the widths of the search density distributions (Fig. 4) as signs of the animals' navigation

uncertainty (Wolf and Wehner, 2005; Merkle et al., 2006), an uncertainty that increases with increasing nest–feeder distance in a more or less linear fashion. With regard to the distance component of this uncertainty, one would expect that it is related to the mechanisms of distance measurement, that is, to the stride integrator. The widths of search density distributions should accordingly reflect the (relative) error per stride and thus depend on both, the number of strides, or nest–feeder distance, and stride length, that is kept more or less constant by the ants under normal circumstances (Zollikofer, 1988). However, in the present experiments stride length was manipulated, affording a unique opportunity to examine not only the stride integrator hypothesis but also uncertainty of distance measurement. And indeed the half widths of the search density distributions were clearly related to stride length (Fig. 5, Test 1). In fact, an average 24.8% change in stride length produced an average 27.8% change in distribution width, in the same direction (the differences between experimental groups decreased in Test 2 since the numbers of strides were again the same for out- and inbound travel, and thus also relative and absolute errors of distance estimation). This result is in clear agreement with the above interpretation regarding navigation uncertainty. The data further suggest that the half width of the uncertainty distribution per step cycle is in the range of 25%, at least under the present experimental conditions.

Walking behaviour is robust with regard to imposed changes in leg length

The plots of stride frequency and stride length vs walking speed (in normal ants) follow the general observations made in other animals, and in insects in particular (e.g. Full and Tu, 1990). As noted above, there were no indications of galloping in the present experimental conditions, although in principle, *Cataglyphis* may gallop (Zollikofer, 1988; Zollikofer, 1994b). The agreement of our data with previous results (Zollikofer, 1988; Zollikofer, 1994a; Zollikofer, 1994b) corroborates and extends the rules governing walking behaviour (Full and Tu, 1990; Full and Tu, 1991) to a very small insect, the desert ant of about 10 mg body mass.

All else being equal, a linear relationship between stride frequency and walking speed (Fig. 7A) would be expected. Any increase in the number of strides per second yields a corresponding increase in speed, as long as stride length stays constant. The fact that stride length increases in conjunction with stride frequency, though usually less pronounced (in normal ants, a doubling in stride frequency is associated with an increase in stride length of just about 130%), will just increase the slope of the graph as long as the latter relationship is linear. And the intercept of this curve should be zero. Non-linearity is introduced into the frequency–speed relationship by the fact that there is a maximum sustainable stride frequency (Full and Tu, 1990). Beyond that, frequency stride length is further increased by adding flight phases into the step cycle, yielding a horizontal graph beyond maximum sustainable stride frequency (Full and Tu, 1990). As noted above, though,

maximum sustainable stride frequency was apparently not reached by our experimental animals and thus a linear fit to the data points is almost as good as an exponential fit. Finally, the ‘stilts’ and ‘stumps’ operations should yield curves with different slopes, if indeed the manipulations in leg lengths resulted in consistent increases in stride length. The differences in slopes represent the relative increases or decreases in stride length brought about by the respective manipulations.

It is evident from the graphs in Fig. 7A that they conform to the above expectation almost perfectly. The only notable feature is the reduced maximum stride frequency in ants with stilts, as noted in the Results section. This conformity indicates that walking behaviour remains largely unaffected by careful manipulation of leg length. This is remarkable considering the actual impediments brought about by the operations. The stumps II animals carry their body close to substrate, and among other things, increasing the risk of stumbling and getting caught on uneven structures. The ants on stilts carry ~56% excess mass on their legs. Both groups have poorer ground contact because of the missing tarsal pads.

By a similar line of argument, a linear relationship would be expected between stride length and walking speed (Fig. 7B). At any given stride frequency, an increase in stride length yields a corresponding increase in speed. And as noted above, stride frequency increases in conjunction with stride length, producing a steeper slope of the relationship. A curvilinear relationship should occur beyond stride length at maximum sustainable stride frequency – a situation not relevant here. The stilts and stumps operations should yield curves with similar slopes, because of basically unchanged stride width–walking speed relationships. The intercepts should differ, though, and reflect the changes in stride length imposed by the altered leg lengths: at any given speed, the stride lengths would be expected to differ by the same amount (and *vice versa*, at any given stride length, stride frequency will differ depending on the manipulations, resulting in different speeds).

Again, the graphs in Fig. 7B conform to expectation almost perfectly. And as noted above, this observation indicates that walking behaviour remains remarkably unchanged by the manipulations.

We thank Stefan Sommer, Tobias Seidl and Markus Knaden for much help during the field work and many stimulating discussions. This study was funded by the Volkswagen Stiftung (I/78 580 to R.W. and H.W.) and the Swiss National Science Foundation (3100-61844 to R.W.), and by the Universities of Ulm and Zurich.

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