Estimation of homing distance in desert ants, *Cataglyphis fortis*, remains unaffected by disturbance of walking behaviour

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SUMMARY

Desert ants, *Cataglyphis fortis*, use a stride integrator as a distance gauge in their well-studied path integration system (while a skylight compass provides the direction gauge). To further scrutinize the mechanisms of the ant odometer, we tried to disturb the stride integrator by interfering with normal walking behaviour. First, legs that contribute to one of the two leg tripods alternately used in normal walking were selectively amputated. This prevented the normal tripod gait and should interfere with both the normal walking programme controlled by the central nervous system, and normal sensory feedback from the legs. Second, manipulation of the walking substrate in the form of regular corrugations was observed to interfere with normal walking behaviour, at least for corrugation wavelengths (12–25 mm) in the range of normal stride lengths. The animals fell and stumbled, or footfall patterns were entrained to the corrugation wavelength. The relationship between stride length and stride frequency was altered in several situations. Surprisingly, distance estimation and homing performance remained virtually unaffected even by the most severe interferences with walking behaviour. This demonstrates a remarkable robustness of walking behaviour and homing, and it suggests that stride length is determined by robust signals of leg sense organs.

Key words: desert ant, *Cataglyphis*, path integration, disturbed walking, odometry.

INTRODUCTION

Desert ants, *Cataglyphis fortis*, are able to use a broad spectrum of cues for orientation, from geocentric features, such as landmarks, to egocentric mechanisms like path integration. The latter is a strategy of primary importance in the often featureless desert habitat of *Cataglyphis* (for reviews, see Wehner, 1996; Wehner, 2003). Path integration requires a compass module and a distance gauge for (approximately trigonometric) summation of covered path segments (Müller and Wehner, 1988). The ants' compass is a well-studied skylight compass that relies on the sun's azimuth and the polarization pattern of sky light to determine the direction of travel (Wehner and Lafranconi, 1981; Wehner, 1989; Wehner, 1997). The distance gauge, or odometer, is a stride integrator [colloquially known as a 'step counter' (Wittlinger et al., 2006)] that considers not just the number of performed strides but also their length – which may vary, depending on walking speed, load and other parameters [(Wittlinger et al., 2007); for recent data on the fiddler crab stride integrator see (Walls and Layne, 2009)].

To further scrutinize the mechanisms of the ant odometer, we tried to disturb the stride integrator by interfering with normal walking behaviour. There are two major, though not mutually exclusive, possibilities of how a stride integrator may work. First, stride length–stride frequency relationships in walking are relatively stable (Bässler, 1983; Bässler and Büschges, 1998), such that stride length might be deduced from stride frequency without the need for sensory measurements. A copy of the central motor command for walking could thus be fed rather directly into a stride integrator. Second, sensory feedback from the legs monitors several aspects of walking behaviour (e.g. Büschges, 2005). It is conceivable that an accurate measure of stride length and stride number may be derived from this sensory input. This would allow a purely proprioceptive measurement of stride length.

Interference with normal walking movements of the legs by disturbing either stride length–stride frequency relationships or sensory feedback, might, in both cases, lead to a misgauging of distance travelled. Two experimental approaches were employed to interfere with normal walking performance. First, amputation of two legs that contribute to a tripod used in normal walking should prevent the normal tripod gait. This should interfere with both, the normal walking programme generated by the central nervous system, and normal sensory feedback from the legs. Second, manipulation of the walking substrate in the form of regular corrugations might interfere with walking movements. Although the desert floor is usually uneven, with diverse smaller and larger obstacles, a regular corrugation with dimensions in the range of normal stride length might, for instance, disallow a regular stride cycle or entrain footfall patterns.

Our observations demonstrate that leg amputations as well as corrugations of the walking substrate may indeed produce severe disturbances of walking behaviour, such as regular stumbling and bumping into the oncoming hill of corrugations. Also, on corrugated walking substrates of a wavelength just exceeding normal stride lengths, footfall patterns were indeed entrained, and stride length–stride frequency relationships were altered in several situations. These results indicate a notable flexibility of walking performance. Surprisingly, distance estimation and homing performance remained unaffected even by the most severe interferences with walking behaviour, suggesting that stride length is determined by robust signals of leg sense organs.

MATERIALS AND METHODS
Experimental situation
Experiments were performed at a field site near the village of Maharès, Tunisia (34°31′46″ north, 10°32′24″ east), between the middle of June and the beginning of September in 2004 to 2007. Large and active nests of *Cataglyphis fortis* Forel 1902 (Wehner, 1983) were selected for experiments.

Training
The ants were trained to walk a distance of 10 m in a linear channel (training channel), connecting the nest entrance to a feeder established due south or due north of the nest, depending on the particular nest chosen (Fig. 1). The channel was U-shaped, 7 cm wide and the walls 7 cm high, such that the ants experienced a strip-like view of the sky of approximately 53 deg. to read skylight pattern and compass information. The channels were made of either aluminium alloy or plywood. The channel walls were painted homogeneously with matt grey varnish or covered with brown adhesive tape to eliminate reflections and optic flow cues. The channel floor was coated with fine grey sand to provide traction for walking. Grain size of the sand ranged between 0.2 and 0.4 mm, again to minimise visual orientation cues such as optic flow. Alternatively, the channel floor consisted of the natural clay substrate of the Maharès chott.

For one set of experiments, the floor of the training channel consisted of sinusoidally corrugated sheets of aluminium alloy, again coated with sand (Fig. 1, inset at top). Wavelengths of the corrugations were 11.6, 14.8 and 24.5 mm (4.8 and 50.2 mm were tested initially to ascertain the useful range of corrugation wavelengths). For easier reference, the three corrugations are referred to by the rounded corrugation periods as P12, P15 and P25 throughout the text. The corresponding corrugation amplitudes were 2.5, 5.0 and 7.0 mm. Owing to the corrugation, the distance measured along the surface of the undulating floor sheets was larger than the base line distance, by factors of 1.19, 1.35 and 1.23, respectively, for the three corrugation wavelengths. Only for the controls walking on level surface was the actual walking distance the same as the base line distance. These factors were also considered for determining walking speed and distance, since the ants had to negotiate the up- and downhill structure of the terrain during locomotion. When travelling at $1\,m\,s^{-1}$ along a channel supplied with a P15 corrugated floor sheet, the actual walking speed of the ant across the sheet surface was thus assumed to be $1.35\,m\,s^{-1}$. The actual walking distance between nest and feeder – 10 m when measured across the desert terrain – was assumed to be 13.5 m. For comparison, walking speed was also calculated with regard to level terrain.

Experiments were performed according to established procedures (Wittlinger et al., 2007). For each experimental situation, ants were marked with a particular colour code. After at least one day of training, or at least 10 visits to the feeder, marked ants that had taken up a food morsel were caught at the feeder.

Testing
For the experiments with corrugated sheets in the training channel (or level surface for the control experiment), the animals caught at the feeder were immediately transferred to a test channel. Tests were performed on level walking substrate and thus homing distance was determined without considering any corrugations used in training. The test channel was aligned in parallel to the training channel and had identical features, except for a considerably increased length of 24 m (Fig. 1, bottom). For the leg amputation experiments, the animals were manipulated as described below (or left intact for the control experiment), and then put into a small plastic container, together with an assortment of food morsels. The ants were presently transferred to the test channel once they had gathered a food item. The fact that the ants were holding on to the food indicated that they were motivated to carry the morsel back to the nest (Wehner, 1982). Once put into the test channel, the animals took up determined homeward runs. Homing behaviour switched to nest searching [compare with Müller and Wehner (Müller and Wehner, 1994)] when the ants performed a conspicuous U-turn, followed by a run pacing back and forth around the assumed position of the nest entrance (Sommer and Wehner, 2004). We recorded the point of this switch in homing behaviour, as well as the first six consecutive turning points (Fig. 1, bottom), for later construction of search density distributions and calculation of median values (see data analysis below). Each ant with manipulated legs was tested three times, first, while still intact to serve as control, and second, straight after leg amputation at the feeding site (test 1; Fig. 2). The animal was then put back into the nest and tested a third time after having re-emerged from the nest and travelled to the feeder again (test 2). Similarly, each ant that participated in the corrugated sheet experiments had to complete all corrugation wavelengths and the control (even floor) for its data to be used for evaluations. For each individual, the sequence of corrugation wavelengths and level training was changed randomly to avoid any sequence bias.
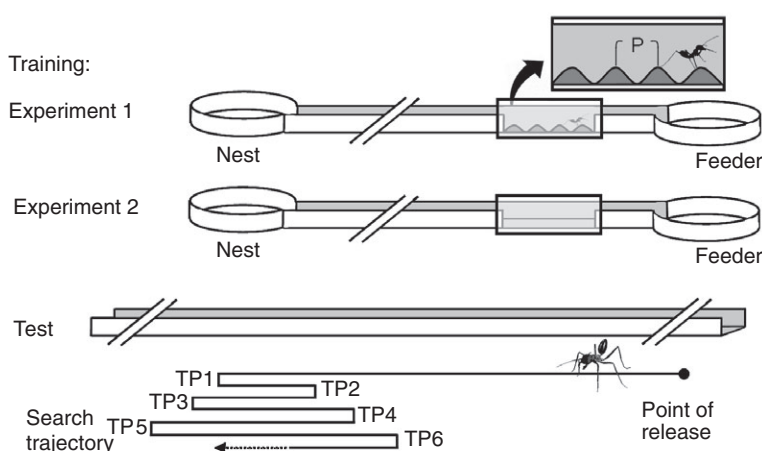

Fig. 1. Training and test paradigms. Top, training situations. The nest of a *Cataglyphis fortis* colony was connected to a feeder established at a distance of 10 m from the nest entrance. A channel connected nest and feeder and guided the ants' foraging excursions, such that a large number of foragers learned the position of the feeding place and visited it repeatedly. Nest and feeder were surrounded by walls connected to the channel to reduce the number of escaping ants. In one set of experiments (1), the channel floor was lined with corrugated metal sheets, with corrugation wavelength, P, ranging from 12 to 25 mm. In another set of experiments (2) the channel floor was level and the ants' legs were manipulated (see Fig. 2). Bottom, test situation. Ants were captured at the feeder as soon as they had taken up a food item in their mandibles, ready to carry the booty to the nest. The animals were transferred to a test channel to examine their homing distances. The test channel was aligned in parallel to the training channel but was considerably longer to allow for search behaviour by the ants around the assumed nest position. Details in text.
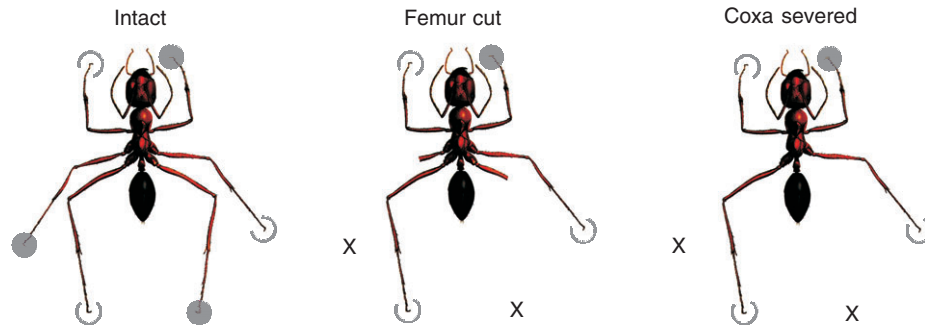
Fig. 2. Leg amputation experiments and disruption of tripod gait. Left, intact *Cataglyphis* ant [adapted after Wehner (Wehner, 1983)], the two leg tripods that alternately support the animal in normal walking (Zollikofer, 1994a; Wittlinger et al., 2007) are indicated by filled and open circles, respectively. Middle, shortening of the left middle and right hind legs at mid-femur level is indicated. The leg stumps were too short to touch the ground and support the animal in walking. Right, amputation, or complete removal, of the left middle and right hind legs in the subcoxal joint is indicated. Missing tripod support points, corresponding to the two amputated legs, are shown as crosses. Body and head together are about 9 mm in length.

Leg amputations

With a pair of minuten scissors two of the ants' legs, the left middle leg and the right hind leg, were manipulated. Both legs were either shortened at the mid-femur level (Fig. 2, middle), or the legs were completely removed at the base of the coxa, including all sensory hair fields (Fig. 2, right). High-speed video analyses (below) showed that the legs that had been shortened at mid-femur level did not touch the ground during walking and thus did not support body weight. The leg stumps moved vigorously in the rhythm of the walking cycle of the other legs, though, and thus the coxal hair fields at least must have provided rhythmic sensory signals to the central nervous system.

Analysis of homing data

The test channel was divided into 10 cm bins for the recording of walking (search) distance. First, search density distributions (Fig. 3) were evaluated for each experimental situation. The bins of the test channel covered by an ant during its search for the nest entrance were noted and cumulated. That is, the more often a channel segment (bin) was visited during the search, the higher was its value in the density distribution. Search density distributions were normalized to their peak values, and the median values of the initial six turning points of the ants' searches were calculated. From these data, box-and-whisker plots were constructed. Marked values in the box-and-whisker plots are the centre (median), the spread (25% and 75% percentile ranges), and the minimum and maximum values (whiskers) of the search centre values. The number of ants evaluated for each experimental situation ranged from $N=21$ to $N=27$. As noted above, each individual was used only once, and for a complete experimental set (e.g. control, leg amputation test 1, and test 2).

High-speed video analysis of walking behaviour

Film recordings were made with a Redlake MotionScope (Redlake MASD, San Diego, CA, USA) high-speed camera using a rate of 250 frames s^{-1}. The camera was equipped with a macro lens of 50 mm focal length (Schneider, Kreuznach, Germany). Intact and manipulated, freely walking ants were filmed in side view while performing their outbound runs (corrugated sheet experiments, Figs 5 and 6) or their homing runs (leg amputation experiments). A 10 cm section of the respective channel had a transparent Perspex wall to allow filming from the side, and the channel diameter was constricted with a piece of cardboard to about 3 cm just opposite the camera lens to lead the ants into its focal plane (Heusser and Wehner, 2002).

A piece of millimetre grid paper served for calibration. After completion of the experiments, the ants were killed and preserved in 70% alcohol for later anatomical inspection and measurement of morphometric data [mainly leg lengths and alitrunk (thorax) lengths]. Qualitative features read from the video recordings included movement patterns of the stumps of amputated legs, stumbling and other peculiarities of leg movements in ants walking on corrugated sheets. Stride lengths of the left and right middle legs, as well as walking speeds were analyzed from the video films.

In ants walking on corrugated substrates, the direct connection between subsequent footfalls was taken as stride length (rather than the distance measured along the surface of the corrugated sheet). By contrast, walking speeds on corrugated substrates were determined in relation to the distance measured along the corrugated surface, rather than the base line distance of corresponding flat terrain (see line of argument above). This was done because the ant's body, i.e. its centre of gravity, followed the undulations of the corrugated surface rather than moving along a level trajectory. Only for the
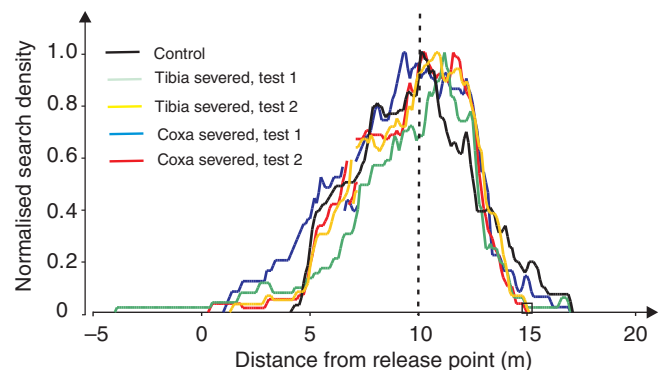

Fig. 3. Homing distances in ants with amputated legs. Search density distributions are shown for intact control ants (black), ants with shortened legs (green; as in Fig. 2, middle) and ants with amputated legs (blue; as in Fig. 2, right; test 1). For the latter two groups, another control (test 2) was performed after the animals had been put back into the nest, re-emerged and reached the feeder again on four legs (shortened, yellow; amputated, red). Abscissa, distance from release point (0) in the direction of the nest (nest to feeder distance, 10 m). Ordinate, normalized search density (cumulated numbers of visits to 10 cm bins of the test channel, recorded for the initial six turning points of nest searching, and normalized to the maximum of the curve; see Fig. 1, bottom diagram).

smallest corrugation wavelengths and amplitudes did the animals' legs average out some of this up-and-down movement of the body. For consistency, we nonetheless always considered the distance measured along the corrugated surface. For comparison, though, walking speed was also calculated with regard to level terrain in the bottom graph of Fig. 7.

Runs that showed abrupt stops, notable acceleration or deceleration, or other irregularities, such as attempts to climb the channel wall, were excluded from the evaluation of stride length and walking speed.

Statistical tests

Only nonparametric tests were used to compare experimental groups in the field experiments, according to the sample sizes of $N=21$ to $N=27$ in each group. All experimental groups consisted of different sets of animals and thus were independent, which allowed us to use a Kruskal–Wallis ANOVA on ranks for comparison. For pair-wise multiple comparison of the treatment groups, Dunn's method was used.

From the high-speed video data, 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); the tests were performed with SigmaStat 2.03 (Systat Software Inc. Point Richmond, CA, USA). The contributions of stride length and stride frequency on walking speed were assessed by multiple regression analysis, performed with STATISTICA software, Rel. 6.1 (StatSoft, Tulsa, OK, USA).

RESULTS
Leg amputations

Our first attempt to interfere with normal walking behaviour was the amputation of a selected pair of legs. The right hind leg and the left middle leg were shortened to the mid-femur level, or completely removed, in ants that had reached the feeder and grabbed a food item, ready to start their homebound journey. The homebound run was thus performed on four, instead of six, legs. The elimination of support from two legs that contribute to one of the two tripods alternately used in normal walking (Fig. 2) severely disrupted walking performance. The animals stumbled on almost every other stride, as revealed by high-speed video recordings. Residual sensory feedback from the operated legs would be expected to be present only in those legs that had been shortened to mid-femur level. This feedback must have been rhythmic in nature, because of the vigorous movement of the leg stumps in the rhythm of the walking movements of the intact legs. Complete removal of the respective legs, including the coxae and the hair fields associated with them, should have completely eliminated sensory feedback from these legs.

Inspection of high-speed video recordings illustrated in some more detail how walking performance was impaired by leg amputation. Intact ants most often use the tripod gate (e.g. Zollikofer, 1994a). That is, one front leg, the hind leg on the same side of the body, and the middle leg on the contralateral side are moved almost in unison (see tripods in Fig. 2). While one leg tripod supports the animal, the remaining three legs perform their swing movements. Once these legs have touched the substrate at the end of the swing, the previously supporting leg tripod is free to perform the swing. With the middle and hind leg of one tripod amputated, an ant attempting to walk in a normal tripod gait would alternately have a complete tripod for support, and just the front leg remaining after

amputation of the other two tripod legs. And this is indeed more or less the situation that occurred when the ants stumbled during homing. The remaining front leg dragged the animal across the ground while the three other legs performed their swing, even though not in the good synchrony observed in normal walking. Only when the animals walked very slowly a stable gait was achieved by moving just one leg in swing at a time (most often in the sequence left front – right middle – right front – left hind – left front etc., occasionally the right front and left hind leg sequence was reversed). When walking slightly faster, two contralateral legs were moved in swing more or less simultaneously, alternately the right front and left hind leg, and the left front and right middle leg. This pattern is already unstable and cannot keep the ant in balance when carrying a load. Towards higher walking speeds this pattern changed increasingly towards the 'stumbling' pattern noted above, by coupling the left hind leg more strongly to the left front and right middle leg, as in the normal tripod. A more quantitative analysis would exceed the scope of the present report.

Despite the severe impact on walking behaviour, homing performance remained unaffected by leg amputations. Fig. 3 shows the search density distributions of the five animal groups that were tested. First, a level control group was put into the test channel after capture at the feeder and handled for a short time, corresponding to the time taken for the leg amputations performed in the other four groups. Second and third, two animal groups had two of their legs shortened at the mid-femur level, and fourth and fifth, two groups of ants had two of their legs completely removed (see Materials and Methods, and above). The nest searching behaviour of the experimental groups with amputated legs (test 1) was virtually identical to the control group, with regard to both centre (median) and spread (interquartile range, IQR) of the search distributions (test 1: control 10.2 m, IQR 2.4 m; shortened 10.8 m, IQR 1.4 m; removed 10.6 m, IQR 2.3 m). After recording the search behaviour of the operated ants, they were put back into their nest and tested again after they had re-emerged, travelled to the feeder, and grabbed a food item (test 2). The ants had thus reached the feeder on four instead of six legs. This served as a control for possible unspecific changes caused by the operation, for instance, reduced homing motivation. However, no such changes were observed and the search density distributions were again virtually identical to the control data (test 2; same control data 10.2 m, IQR 2.4 m; shortened 10.4 m, IQR 2.9 m; removed 10.9 m, IQR 3.1 m).

To scrutinise in more detail possible changes in homing behaviour, apart from homing distance, we further analyzed the initial reversal points in the ants' homing runs (Fig. 4) (see Materials and Methods), and the coefficients of variation of the different experimental groups. These parameters might reveal possible changes in homing accuracy (in addition to the standard deviations of the search density distributions noted above). The coefficient of variation provides a measure of homing accuracy independent of homing distance (Cheng et al., 2006; Narendra et al., 2007; Wolf, 2007) and should reveal such changes. However, the coefficient of variation was not significantly different between the experimental groups ($P=0.627$), ranging around 0.28. The distribution of the initial reversal points of homing ants is shown in Fig. 4 (five distributions on the left). Here, too, no significant differences were observed ($P=0.069$).

In summary, distance estimation in *Cataglyphis fortis* appears to remain completely unaffected by leg amputations during foraging excursions, regardless of whether or not residual sensory feedback from leg stumps is present.
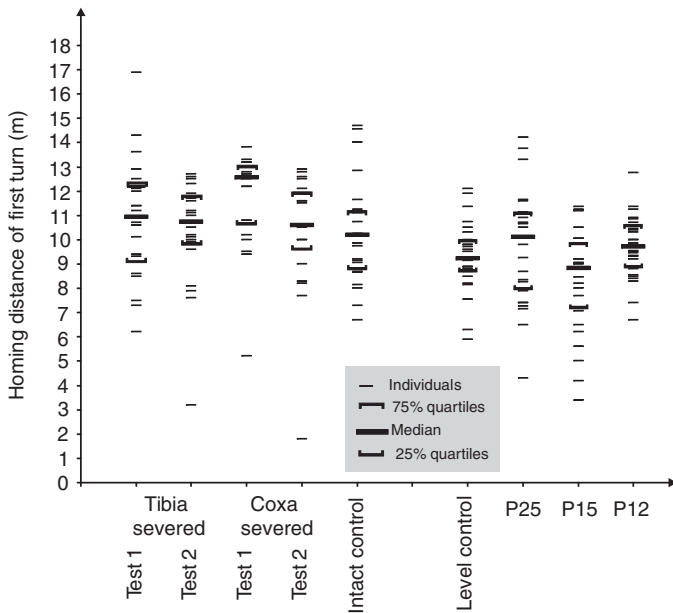
Fig. 4. Initial reversal points (TP 1 in bottom diagram of Fig. 1) of homing ants in the different experimental situations of this study. Ordinate, homing distance of first turn; abscissa, experimental situation. Labels on the abscissa correspond to labels in Fig. 3 (five distributions on the left, leg amputation experiments) and Fig. 7 (four distributions on the right, corrugated floor experiments). Each data point (small lines) represents the initial reversal point of one individual in the respective experimental situation, means (broad lines) and 25% and 75% quartiles (brackets) are indicated. Statistical analysis revealed no differences among the experimental manipulations of one experimental set, that is, amputation or corrugated sheet experiments (*P*=0.627 and *P*=0.621, respectively).

Walking across corrugated substrates

Our second set of experiments interfered with normal walking behaviour through the introduction of corrugated walking substrates during the outbound journey, from nest to feeder. The corrugations were in the range of the ants' body size and stride length, and tests of homing distance were performed on level walking substrates (see Materials and methods).

In previous experiments, Wohlgemuth and co-workers (Wohlgemuth et al., 2001; Wohlgemuth et al., 2002) had trained *Cataglyphis* ants across hills to investigate their capability of path integration in the vertical dimension. And indeed, ants that had covered a walking distance of 10 m across the surface of a series of steep hills searched for the nest entrance in a level test channel after they had covered the corresponding base line distance of only 6 m. This demonstrates that *Cataglyphis* ants are able to judge (base line) distances correctly even when travelling across hilly terrain. One would expect, therefore, that the ants reaching a feeder across corrugated substrates in the present experiments should be able to assess the nest–feeder distance correctly, irrespective of the structure of the walking substrate in the training and test channels. This expectation turned out to be correct. Homing distances of the ants walking across the different corrugation wavelengths of 12, 15 and 25 mm, respectively, on their outbound journeys searched for the nest during homebound travel across level terrain at distances that closely agreed with the control situation of a level training channel [control 8.75 m, IQR 2.93 m; corrugation period 12 mm (P12), 9.14 m, IQR 2.28 m; corrugation period 15 mm (P15), 8.54 m, IQR 3.01 m; corrugation period 25 mm (P25), 9.09 m, IQR 2.39 m;

distances not significantly different (*P*=0.30) according to repeated-measures ANOVA]. However, the ants consistently underestimated their distances from home, by about 1 to 1.5 m, in all experimental situations, including the level control training.

This latter observation is not unusual since actual homing performance depends, to some extent, on the experimental situation. The apparent reason is a different *external* situation in training and test channels. The outbound journey leads the ants across corrugated terrain that is familiar from several previous foraging excursions, whereas the flat test channel is unfamiliar. Differences in the environment during out- and inbound travel apparently trigger a safety strategy that centres the search somewhat into the direction of the outbound trip (Sommer and Wehner, 2004). Under normal circumstances, this would lead the animal into familiar terrain in the nest vicinity since *Cataglyphis* individuals usually forage into roughly the same direction throughout their lifetime, depending on foraging success (Wehner and Srinivasan, 1981; Wehner et al., 1983; Schmid-Hempel, 1984; Buchkremer and Reinhold, 2008). The side of the nest opposite the outbound journey is unfamiliar, by contrast. The ants were indeed trained and tested in different channels.

This line of argument appears not to hold for the control ants since these did not experience different substrate structures in the training and test channels. However, training and test channels were indeed different since, for reasons of consistency, the control animals were trained across level aluminium floors (rather than corrugated aluminium sheets) coated with sand, whereas the floor of the test channel was always the desert clay.

Again, we scrutinized in more detail possible changes in homing behaviour, with particular regard to homing accuracy. As for the leg amputation experiments, however, the coefficient of variation was not significantly different between the experimental groups (*P*=0.621), ranging around 0.27. The distribution of the initial reversal points of homing ants is shown in Fig. 4 (four distributions on the right). No significant differences were observed here either (*P*=0.089).

The corrugations in our experiments were much smaller than the hills used in the experiments of Wohlgemuth and co-workers (Wohlgemuth et al., 2002); however, our goal was not the study of path integration in the vertical dimension but rather the interference with the ants' normal walking behaviour. The 'corrugation period' of Wohlgemuth and co-workers (Wohlgemuth et al., 2002) was about 1 m, whereas in the present experiments it was between 12 and 25 mm. Initial experiments with larger wavelengths of 50 mm had demonstrated that the animals' walking behaviour remained unaffected, with the ants walking up and down the corrugation slopes, the situation thus corresponding to that in Wohlgemuth and co-workers (Wohlgemuth et al., 2002). This was a major reason why we determined walking speeds on corrugated substrates usually in relation to the distance measured along the corrugated surface, rather than the base line distance of a corresponding flat terrain (except bottom graph in Fig. 7). Initial experiments with shorter wavelengths (5 mm or below) showed that these were treated by the ants in much the same way as level substrates, with their legs largely evening out changes in substrate height, and their bodies moving on an almost level trajectory.

The intermediate corrugation periods of 12 to 25 mm were roughly in the range of the animals' stride lengths, by contrast, and interfered with walking behaviour in several ways. Samples are shown in Fig. 5 and Fig. 6 in the form of selected frames taken from high-speed video films (see Materials and methods). On corrugated sheets with periods P25 and P15, the most significant characteristic of the ants' locomotor behaviour was an unsteady walk. The ants' legs often

missed ground contact when stepping into a trough (right front legs in top half of Fig. 5), at least initially, resulting in omitted or delayed touch down of the tarsus. In addition, the legs were often raised much higher than in normal walking during their swing phases (right middle legs in top half of Fig. 5). The latter behaviour was probably used to avoid touching a corrugation peak during swing. More dramatic disturbances were frequent falls or bumping into the oncoming hill (lower half of Fig. 5). The percentage of the animals that fell or stumbled on corrugated substrates P25 and P15 was 86% and 82%, respectively. By comparison, on P12 only 15% of the animals exhibited such behaviour. On P12 intriguing regularities could be observed, however. The ants often 'jumped' from one corrugation peak to the next. That is, the same leg was regularly used to step near the corrugation peak, usually the middle leg (Fig. 6), and all legs tended to avoid the corrugation troughs. This resulted in an enforcement of stride lengths that agreed with the corrugation wavelength of 12 mm, a condition termed 'entrainment'.

A quantitative analysis of walking behaviour, particularly of stride frequency, stride length and walking speed, and of the relationships between these parameters revealed further important features.

Stride length

Stride length (Fig. 7, top), perhaps not surprisingly, was significantly shorter on P15 and P25 than during level walking (control, $N=21$; P15, $P<0.05$, $N=22$; P25, $P<0.001$, $N=27$; no significant differences between P15 and P25, $P>0.05$). The frequent stumbling and falling necessitated corrective leg placements, which resulted in reduced average stride lengths. Stride length on P12 was not significantly different from the control situation ($P>0.05$, $N=26$), indicating that average stride length of *Cataglyphis* ants is in the range of 12 mm, a fact well documented in the literature (Zollikofer, 1994a; Zollikofer, 1994b).

Stride frequency

Stride frequency (Fig. 7, upper middle) was lower on P15 than during level walking (P15, $P<0.05$; no significant differences between level walking and P25, $P>0.05$). This may also be interpreted as a consequence of the disturbed walking behaviour and the resulting corrective actions, disrupting regular walking and leg movements at normal stride frequency. Stride frequency on P12 was significantly higher than on P15 and P25 ($P<0.001$) but not different from level walking controls. A trend towards higher stride frequencies is apparent, however, and stride frequencies were significantly higher on P12 than on the level in some individuals.

Walking speed

Walking speed (Fig. 7, lower middle) was slower on both larger profiles, P15 and P25, than on P12 and the flat control (significant differences only for P15 *versus* P12, $P<0.001$; P25 *versus* P12, $P<0.001$; P15 *versus* control, $P<0.001$). This is in agreement with the disturbed walking behaviour, and reduced stride lengths and stride frequencies, as noted above. On P12, walking speed was highest, although not significantly different from the controls (again, individual ants exhibited significant differences here). This must have been brought about by the increased stride frequency shown in the middle part of Fig. 7.

For comparison, walking speed was also calculated with regard to base line distance, rather than the distance measured along the corrugated substrate surface (Fig. 7, bottom). After all, the animals did not completely follow the surface of the corrugations in their up-and-down walking trajectory. On P12, in particular, the undulations in body movement appeared smaller than corrugation
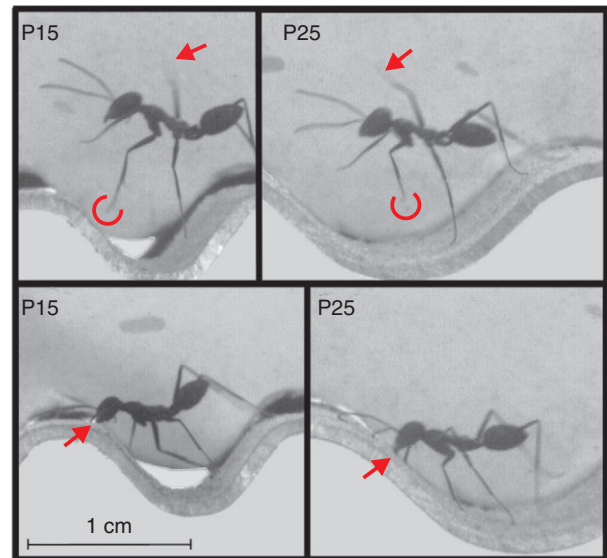

Fig. 5. Missing of ground contact (top) and stumbling (bottom). Sample frames of high-speed video recordings illustrate disturbed walking behaviour on substrates with corrugation periods of 15 mm (left) and 25 mm (right). Note the following details. (1) The right front leg normally forms a tripod with the left middle and right hind leg, both of which are on the ground in the images; in the top two examples, however, the right front legs apparently miss ground contact (encircled tarsi). (ii) In the top two examples, the right middle legs are raised very high above the ground, well above body level, (arrows). (iii) In the bottom two examples, the animals bumped into the oncoming hill (arrows). Scale bar, 1 cm.

amplitude. It is evident in Fig. 7 (bottom) that this evaluation did not change the main observations reported above. Walking speed relative to level ground was slower on the two larger profiles, P15 and P25, than on P12 and the flat control. And again, walking speed was highest on P12, on average, although this was not statistically significant.

The above observations suggest that the relationships between stride frequency and stride length may differ among the different experimental groups. Stride frequency appeared to increase on P12, whereas both stride frequency and stride length decreased on P15 and P25, with corresponding changes in walking speed. Fig. 8 demonstrates that the relationship between stride frequency and stride length was indeed different for the control ants, those walking
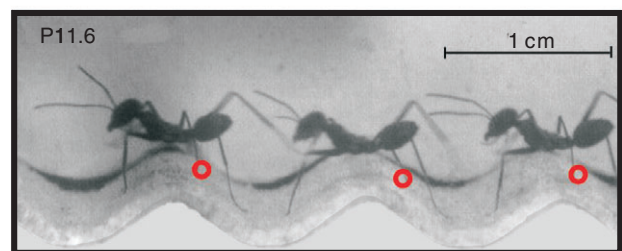

Fig. 6. Entrainment of footfall patterns. Three superimposed sample frames of a high-speed video recording are shown. Touch-down positions of the left middle leg during three successive strides are indicated by red circles. The ant was captured just before lift-off of the right middle tarsus in all three superimposed video frames. Note that the tarsus touched the substrate just to the right of each corrugation peak. Corrugation period is 11.6 mm; scale bar, 1 cm.
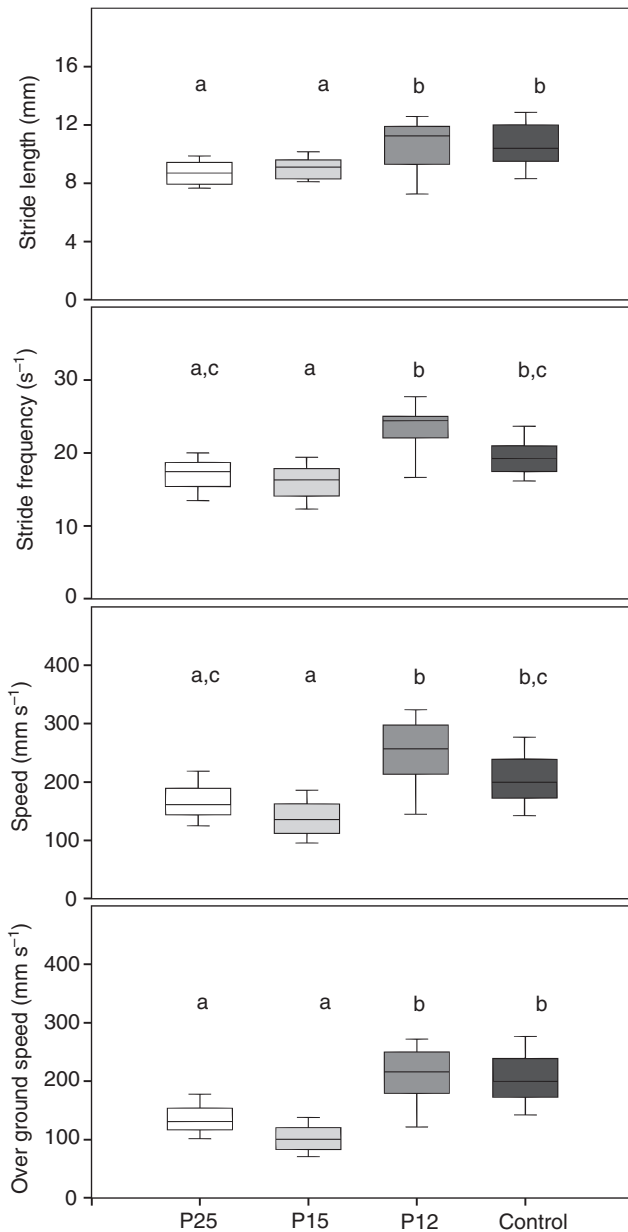
Fig. 7. Stride length (top), stride frequency (upper middle), walking speed (lower middle), and over ground speed (bottom) on corrugated substrates. The three box-and-whisker plots show the median values, 25% and 75% percentiles (box margins) and maximum and minimum values (whiskers) for the different experimental situations noted on the abscissa of each diagram. Results of statistical evaluations are indicated above the box-and-whisker plots by letters; data sets with the same letters are not significantly different from each other, different letters indicate significant differences: $P<0.05$ (for details see text).

Table 1. Multiple linear regression: effects of stride frequency and stride length on speed

Condition	Coefficient of correlation	β stride frequency	β stride length
P25 (*N*=29)	R^2=0.79; *P*<0.01	**0.70**; *P*<0.01	**0.52**; *P*<0.01
P15 (*N*=22)	R^2=0.81; *P*<0.01	**0.60**; *P*<0.01	**0.54**; *P*<0.01
P12 (*N*=26)	R^2=0.89; *P*<0.01	0.13; *P*=0.24	**0.85**; *P*<0.01
Control (*N*=21)	R^2=0.92; *P*<0.01	**0.32**; *P*<0.01	**0.70**; *P*<0.01

Stride frequency and stride length were taken as the independent variables, walking speed as the dependent variable.

β stride frequency and β stride length are the standardized coefficient of correlation for stride frequency and stride length, respectively. Significant values are in bold print.

slopes of the regression lines decreased to 0.33 for P12, 0.08 for P15 and 0.15 for P25. Intercepts followed an opposite trend (see Fig. 8, regression terms in insets). In the graph for P12, there is a notable concentration of data points around a stride length of 11.6 mm (indicated in Fig. 8 by stippled line and shaded area; 15 out of 26 data points are between 11 and 13 mm), reflecting the entrainment of the footfall pattern by the corrugation wavelength noted above.

The influence of stride length and stride frequency on locomotor speed was assessed by multiple linear regressions (Table 1). Pronounced and highly significant correlations were observed between speed, stride length and frequency, respectively, for all the corrugated profiles (second column in Table 1). However, the relative impacts of stride length and stride frequency on walking speed differed between the profiles (third and forth columns in Table 1). Changes in walking speed were brought about predominantly by altered stride lengths in control animals, and even more so in ants walking on P12. The latter observation may again be related to the entrainment of footfall patterns by the corrugated substrate. By contrast, changes in walking speed were brought about almost equally by altered stride frequency and stride length on corrugated sheets with wavelengths P15 and P25.

These results demonstrate that basic properties of walking behaviour, such as the relationship between walking speed, stride length and stride frequency, may be altered by manipulation of the substrate. This occurs without noticeable deterioration in homing performance.

DISCUSSION

These results demonstrate that homing performance of desert ants, *Cataglyphis fortis*, is remarkably resistant to disturbances of walking behaviour, at least with regard to distance measurements, even though distance estimation is probably that component of the ants' path integration system which is most prone to errors. This finding is independent of whether or not residual sensory feedback from leg stumps is present. In *Cataglyphis* directional fixes are provided more or less continuously and with good precision by the skylight compass (reviewed by Wehner, 1989; Wehner, 1997; Wehner, 2003). However, the distance component of the path integration system is provided by a stride integrator (Wittlinger et al., 2006; Wittlinger et al., 2007), that would have to rely on proprioceptive feedback from leg mechanoreceptors or on central leg motor commands as representations of stride length (see Introduction). Such measurements of stride length would appear to suffer from the variability of both stride length itself and parameters of leg movement related to stride length, such as stride frequency and walking speed (e.g. Wittlinger et al., 2006), or prey load (Zollikofer, 1994c).

on P12, and those walking on P15 or P25 (statistical data and equations for the respective regression lines see insets in Fig. 8). The slope of the regression lines was significantly different from zero for the control ants, walking on level terrain, the P12 and the P25 animals. Data for the control ants agree with previous reports (Zollikofer 1994a; Zollikofer, 1994b; Wittlinger et al., 2007), both stride frequency and stride length increasing considerably with increasing walking speed. The slope of the regression line was 0.55 in the present relationship. Towards longer corrugation periods the
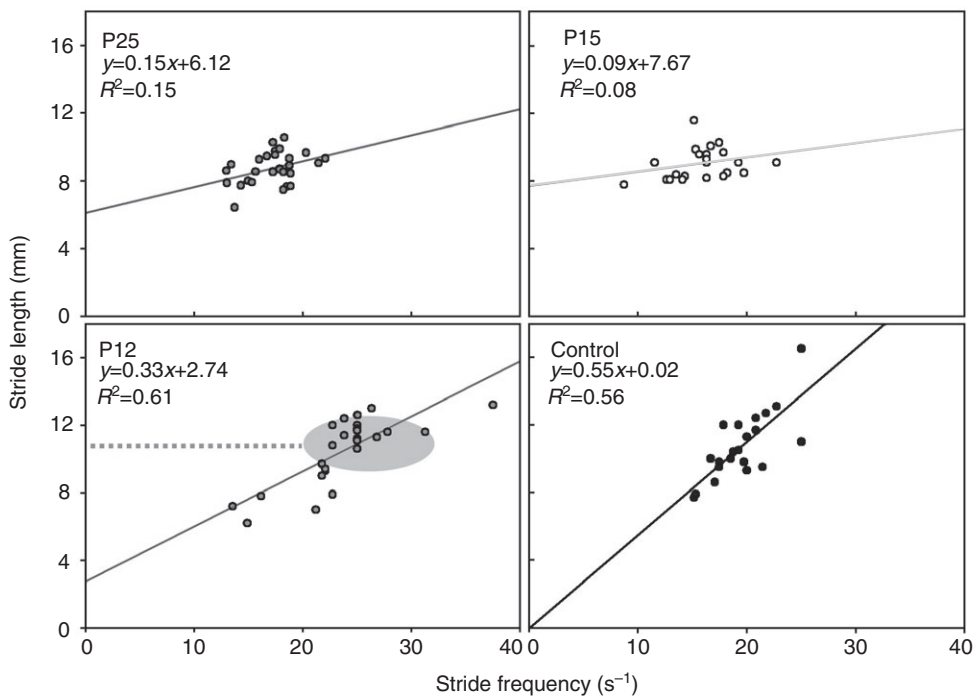
Fig. 8. Stride length–stride frequency relationships on corrugated walking substrates. All diagrams show stride lengths on the ordinates and stride frequencies on the abscissae. Each point represents the median of the data from one individual ant. Substrates: corrugation wavelengths of P25 (top left), P15 (top right), P12 (bottom left), and level control (bottom right). A concentration of data points (shaded area) close to 11.6 mm (dashed line) is indicated for P12.

Optical flow cues are unlikely to contribute to this resistance of the ant odometer against disturbances of walking behaviour. First, optic flow cues are known to contribute little, if anything, to the desert ant odometer (Ronacher and Wehner, 1995; Ronacher et al., 2000), and second, we minimised optic flow by coating the surfaces of the training and test channels with homogenous substrates (see Materials and methods).

Distance estimation is resistant to severe disturbances in walking behaviour

In retrospect, the ability to cope with even severe disturbances of walking performance is perhaps not too surprising, considering our knowledge of *Cataglyphis*' navigation skills. It agrees well with common, though anecdotal, observations that *Cataglyphis* ants return successfully to their nest after major injuries. Such injuries may include leg loss inflicted by predators, such as asilid flies, or by conspecifics from other colonies which often fiercely defend resources, such as plentiful feeding sites, against ants from other nests. Uneven walking substrates are not uncommon either, mostly in the form of small pebbles or rippled sand terrain produced by wind and water waves. These structures are usually much less regular in appearance, and even in the case of rippled terrain, the ripple wavelengths are much larger. Nevertheless, the robustness of homing performance is noteworthy, and the underlying sensory and neuronal mechanisms remain unknown, particularly in view of the very different types of disturbances that may need to be dealt with.

Estimation of homing distance in the experimental situations was in fact statistically (Fig. 4; and by visual inspection, see e.g. Fig. 3) indistinguishable from that in normal foraging situations. This held true even when considering the coefficient of variation that reliably reflects navigation accuracy (Cheng et al., 2006; Narendra et al., 2007; Wolf, 2007). This robustness implies utilization of sensory feedback about leg movements as a major input to the distance module of the path integrator. In normal walking conditions it is conceivable that the relatively fixed relationship between walking speed, stride length and stride frequency allows the (centrally

generated) motor command to the leg muscles to be used as a measure of walking distance or stride length. After all, a given stride length (to be used as input to the desert ant path integrator) is usually associated with a particular stride frequency and walking speed [figures 6 and 7 in Wittlinger et al. (Wittlinger et al., 2007)], and these parameters are generated by the central nervous system (it is irrelevant here, whether or not the generation of this motor command relies on sensory feedback for patterning purposes). This would allow a purely central nervous determination of walking distance. The severe disturbances of walking performance observed in our present experiments should mean that the consequences of a given motor command cannot be interpreted reliably in terms of covered distance. This is true in particular for the altered relationships between stride frequency, stride length and walking speed in some of the experiments involving corrugated substrates (Fig. 8). Even if sophisticated evaluation of corrective actions in the motor programme should still provide information about covered distance, at least the accuracy of distance estimation should be compromised. Therefore, the resistance of the odometer module against the disturbance of walking behaviour observed here strongly suggests that walking distance is measured by a reliable sensory monitor of actual leg movement during ground contact.

General aspects of walking control

An intriguing observation was the altered stride length–stride frequency relationship on all corrugated walking substrates (Fig. 8). A corrugation period of 11.6 mm just exceeds average normal stride length in desert ants, and thus apparently leads to an accelerated walk of the animals in their effort to adjust stride length to substrate structure, associated with a temporary entrainment of footfall patterns. Locomotor speed is increased since the entrained stride width is some 10–15% larger than normal stride length, with stride frequencies raised even further – perhaps as a result of the high reliability of walking conditions. The clustering of data points in Fig. 8 between 10 and 13 mm stride length and 21 to 28 Hz stride frequency supports this view. This entrainment also alters the stride

length–stride frequency relationship, which thus appears to be flexible indeed. Inspection of the high-speed video recordings shows that the entrainment is only temporary, though. The animals apparently 'fall out of step' once in a while, resulting in a noticeable change of walking speed and stride frequency until the entrainment is resumed. The averaged data in Fig. 8 thus do not show the maximum effects of entrainment.

From a physiological perspective, entrainment of walking motor patterns is not a novel observation. It has been reported in physiological preparations (Elson et al., 1992; Akay et al., 2007), and several lines of evidence indicate that load signals, in particular, are able to entrain the step cycle (for a review, see Duysens et al., 2000).

Corrugation periods of 15 and 25 mm well exceed stride lengths in desert ants. Here, the frequent irregularities in walking performance, most notably stumbling, loss of ground contact, and substrate impact, slowed the ants' locomotion considerably. Their apparent strategy to cope with these impediments consisted of a reduction in stride length and stride frequency (Fig. 7), and use of stride length and stride frequency about equally for speed variation (Table 1). This strategy clearly increases the safety margin of walking with regard to unpredictable walking conditions and obstacle avoidance. It is reminiscent of the strategy used by human cross country runners, as compared with sprinters on tarmac.

Previous analyses of relationships between walking speed, stride length and stride frequency (Lipp et al., 2005; Bässler, 1983; Full and Tu, 1990; Full and Tu, 1991) reported near stable relationships. Locomotor speed is increased by employing both useful parameters of leg movement, that is, stride length is extended and stride frequency is increased, with stride length making a somewhat larger contribution to speed increase [control situation in Table 1; other examples in insects (Full and Tu, 1990; Full and Tu, 1991; Zollikofer, 1994a; Zollikofer, 1994b); a review for quadruped vertebrates (e.g. Taylor and Heglund, 1982)]. Once the maximum sustainable stride length is reached, the animals switch to a different gait, trot or gallop. It is an intriguing observation, that stride length and stride frequency can be coupled in a rather flexible manner, depending on walking conditions. Such flexibility is well known from humans and some skilful and trainable mammals [e.g. human competition walkers or show horses (e.g. Schmidt-Nielsen, 1984)]. It is certainly unexpected for the seemingly less flexible insects.

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