Mechanisms of three-dimensional (3D) path integration in the desert ant *Cataglyphis fortis* – odometry and slope detection

Dissertation

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Matthias Wittlinger
aus Kirchheim unter Teck

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Amtierender Dekan: Prof. Dr. Klaus-Dieter Spindler

Erster Gutachter: Prof. Dr. Harald Wolf
Zweiter Gutachter: Prof. Dr. Manfred Ayasse
Dritter Gutachter: Prof. Dr. Rüdiger Wehner

Meinem Herrn und Gott, Jesus Christus, der mir die Liebe und Leidenschaft zur Natur und die Neugier eines Kindes gab
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Summary

General introduction

One of the most eye-catching animals to see at a summer noon in North African salt pans, such as the Chott el Cherid in Tunisia, are the long-legged desert ants, *Cataglyphis fortis* (Forel 1902, Wehner 1983) (Insecta: Hymenoptera: Formicidae) (Fig. 1). They are living in colonies consisting of several hundred individuals whose subterranean nests have only one small entrance to the flat and vast desert floor. Nests are found in salty inundation areas, such as chotts, sebkhas and coastal inundation zones, that are flooded once every year, coinciding with the torpidity of the *Cataglyphis fortis* colonies (Wehner, 1983). Surprisingly, though living in this hot and dry habitat, these ants are diurnal and above all they perform their foraging trips at the hottest times of the day. Desert ants do not lay down or use pheromone trails - the (ground) surface temperature is too hot for volatile chemicals to serve as markers - and they do not show any recruiting behaviour, but rather they forage as individuals. On their foraging trips, that may reach walking distances of several hundred meters, they attain distances to the nest of more than 150 m (Wehner, 1983). On their search for arthropods succumbed to the torridity of the desert, they run over the desert floor with remarkable speeds of up to one meter per second (ca. one hundred body length per second) (Wehner, 1994a), keeping their metasoma (gaster) in a conspicuous upright posture. After finding prey the animals finally start their way back towards the inconspicuous nest entrance - not retracing the circuitous outbound path but in a straightforward way. This remarkable homing ability, a form of dead reckoning or path integration (Mittelstaedt, 1983, Müller and Wehner, 1988; Wehner and

![Fig. 1 Desert ant *Cataglyphis fortis*.](image)
Srinivasan, 2003) is quite similar to that used by mariners at sea before the advent of global positioning system (GPS). The seafarers of the Puluwat atoll in the South Pacific use a dead reckoning system to sail great distances among a chain of islands in the Western and Central Carolines (Gladwin, 1970). This form of navigation has been shown to be employed in many animals, including spiders (Görner and Claas, 1985; Mittelstaedt, 1985; Moller and Görner, 1993), crustaceans (Hoffmann, 1984), several insect species (Frisch, 1965, 1967; Müller and Wehner, 1982; Wehner and Srinivasan, 2003), birds (Mittelstaedt and Mittelstaedt, 1982; Saint Paul, V. v., 1982) and mammals (Mittelstaedt and Mittelstaedt, 1980; Etienne et al., 1985; Séguinot et al., 1993; Etienne and Jeffery, 2004). Path integration, first postulated by Darwin (1873), appears to operate in many and diverse species with a fixed home base. It provides the animal with continuous information about where it is located in its internal representation of space, usually with regard to that home base (Gallistel, 1990). Thus *Cataglyphis fortis*, as a central place forager, may always interrupt its foraging trip to return back home at any place and at any moment of its travel. Wherever it goes, the state of its path integrator connects the ant with its home, or to a place within the foraging area - the vector pointing home is always the inverse of the one pointing towards the present position, or the feeder once it has been reached (Wehner, 2003). The ants achieve this feat by continuously updating their vector, which is defined by two parameters, walking direction and walking distance. Information from both the skylight compass and the odometer, that is, angular and linear information, regarding direction and distance, is simultaneously fed into an (path) integrator.

The estimation of travel direction is based on an external compass. Namely, skylight information is used to measure angles steered. Information about direction is derived from the azimuthal position of the sun as well as from spectral gradients in the sky, but the major and most precise cue is provided by the electric (E-) vector patterns of polarized light in the sky (Wehner and Lafranconi, 1981; Wehner, 1989; Wehner, 1994b; Pomozi et al., 2001).

The cues by which ants measure travel distance during locomotion, the odometer, have not yet been uncovered. However, there are several promising hypotheses. First, the “energy hypothesis” posits that the (surplus) energy required for locomotion (as opposed to rest) is used to calculate travel distance. This hypothesis has long been hypothesis in arthropod research (Heran and Wanke, 1952; Heran, 1956; von Frisch, 1967), but is not applicable to the problem of the ant odometer. Loading *Cataglyphis*
ants with artificial weights (metal wire pieces) of up to four times of their body weight did not influence gauging of distance travelled. The ants assess their walking distances with remarkable accuracy, irrespective of the load they carry (Schäfer and Wehner, 1993; Wehner, 1992). Second, the “optic flow hypothesis” has been proven in honeybees, which integrate visual flow-field cues during their foraging flights to gauge flight distance (Esch and Burns, 1995; Srinivasan et al., 2000). Certainly, this is an obvious and elegant cue for flying insects to gauge distances flown, but in walking insects this mechanism of detecting visual flow field cues for distance measurement plays indeed a minor role (walking honeybees: Schöne, 1996; walking bumblebees: Chittka et al. 1999). In *Cataglyphis* ants, integration of optic flow presented in the ventral field of vision also plays a minor, if any role (Ronacher and Wehner, 1995). A contribution of less than 8% to the *Cataglyphis* odometer has been observed, but only with strong visual contrast in the ventral visual field, while under low contrast conditions (as in my present experiments) the use of all visual cues is inevitably reduced. Actually, the ants are able to gauge the correct distance without any optical flow cues. Even when walking in complete darkness, on featureless platforms, or with the ventral halves of their eyes covered, the animals are still able to assess travelling distance correctly during their homing runs (Ronacher and Wehner, 1995; Thiélin-Bescond and Beugnon, 2005). And lateral optic flow does not have any influence at all on distance estimation (Ronacher et al., 2000). Third, the step length for a given walking speed is quite constant in desert ants (Zollikofer, 1994a). Considering the relatively constant locomotor speed of desert ants, thus, a time lapse integrator, where distance is measured by time measurement while walking with a constant speed (Ribbands, 1953; Blest, 1960), might function to gauge walking distance. However, this possibility has been refuted for desert ants in slightly different experimental contexts (Wohlgemuth et al., 2001). In summary, then, ants appear to rely primarily on idiothetic cues, most probably derived from the movements of their legs (Mittelstaedt and Mittelstaedt, 1973) Hence, ants seem to be able to measure leg movement or to monitor the output of a locomotor central pattern generator to gauge distances travelled (Wehner; 1992). Although this step integrator or “pedometer hypothesis” was initially proposed as early as 1904 (Pieron, 1904), it has so far remained untested. The walking speed during a particular foraging trip is held relatively constant (Wehner and Srinivasan, 1981), and although stride length can vary by about 20% at a given speed, this variation decreases with higher walking speeds (Zollikofer
1987). This leads to the assumption that the number of steps should be proportional to
the distance travelled, since foraging *C. fortis* ants run with great speeds indeed.
So far, I have considered vector navigation in the two-dimensional plane. Wohlgemuth
and co-workers (2001, 2002) showed in a series of experiments that ants seem to be able
to measure terrain slope, *viz*, also the vertical dimension of travel, and integrate them
into their process of distance estimation. The ants were trained to traverse a linear
‘hilly’ up- and down-slope route to a feeder, simulated by a series of alternately sloping
channel segments (Fig. 2). And they were tested in the flat terrain, simulated by a flat
linear channel. In this situation, the ants just show homing distances that correspond to
the base distance between nest and feeder and not to the effectively walked distance
over the artificial hill segments. Ants trained to walk over a artificial hill set-up with a
distinct 3D-structure, namely an L-shaped hill set-up, also show a homing path when
tested in the open field that corresponds in both distance and direction to the level
ground distance between nest and feeder (Grah et al., 2005). Hence, ants walking over
such hilly terrain determine the ground, or base line, distance irrespective of the actual
shape of the surmounted hills. Ground distance estimation, as opposed to a simple
measurement of walking distances, is a necessary prerequisite for precise path integra-

![Artificial hill set-up](image)

**Fig. 2** Artificial hill set-up near Maharès, Tunisia.
tion in undulating terrain (Grah et al., 2005). This allows accurate return to the nest on a different route than that taken during outbound travel, independent of the substrate structure on these routes. Not only is this feature of the ant odometer surprising but so is its accuracy that is comparable to that achieved on level ground. It is as yet completely unclear how baseline distance is determined by the ants when walking on hilly terrain. It is clear, however, that the ants must be able to measure the slope of their walking substrate quite exactly to achieve this feat. According to Markl (1962), ants determine the relative positions of their body parts, such as head, thorax (or in ants, alitrunk) and abdomen (or in ants, metasoma or gaster), by means of hair fields associated with the joints between these body segments. And since the pull of gravity on these segments is dependent on body posture and inclination, the above hair fields might indeed serve as graviceptors involved in adjusting the odometer module to substrate inclination.

**Thesis objectives, study animal and study area**

The ability to measure distances travelled in the plain as well as in undulated terrain are an obligatory element in navigation of *Cataglyphis* desert ants. As outlined above, the odometer of the desert ant seems likely to be a kind of step integrator, which is implemented into the path integration system. At any point of its travel an ant knows the direction and the distance to the reference point (for instance, the nest or the feeder). This mechanism works both when travelling in a meandering way in the plain and when traversing hills in undulated terrain. In the present thesis I investigate the odometer of the path integration system of the desert ant, *Cataglyphis fortis* (Forel 1902, Wehner 1983). First, I test the step integrator hypothesis by manipulating the leg length, and thus presumably stride length, of foraging ants. Second, this manipulation of leg length presupposed an investigation of stepping patterns in ants walking on shortened or elongated legs to scrutinise and support the results obtained above. Third, I test the hypothesis that terrain slopes are determined by the hair sensillae located between several adjacent body parts of the ant, according to the role Markl (1962) attributed to these hair fields in graviception. This is done by the immobilisation of body parts or by shaving of the respective hair fields, and by assessing the resulting elimination of sensory feedback regarding body posture. Behavioural experiments are employed to examine the conse-
quences of the above interferences, namely, the homing distances of desert ants are measured under a variety of conditions.

These behavioural experiments were carried out in the field seasons 2004 and 2005 in a salt pan (coastal inundation area) near Maharès, Tunisia (34°30’N, 19°29’E). Laboratory work and associated experiments took place in the Department of Neurobiology at the University of Ulm in these same years.

Hypotheses and results

My thesis is structured in three chapters, each representing a separate publication. One chapter has already been accepted for publication in *Science* (chapter 1), the other two have been prepared for submission (chapter 2 and 3) to the Journal of Experimental Biology. The chapters are in this regard independent, and each may be read separately. Naturally, however, the results presented in these three chapters bear on each other and the general problem of navigation mechanisms. This has been alluded to in the Introduction and will also be discussed in the General conclusions.

In chapter 1 I test the “step counter (step integrator) hypothesis”, which posits the use of internal, idiothetic cues derived by the movement of the legs to gauge distances travelled (for a review see Wehner, 1992). Here, I examine whether or not ants with manipulated leg lengths, walking on stilts or on stumps (see Figs 3 and 4), exhibit changes in their stride lengths, and consequently misgauge their travel distance during homebound runs. The ants were trained to walk from their nest entrance to a feeder, over a distance of 10 m and in a linear alloy channel set-up. The animals were caught at the feeding site and transferred to a test channel, aligned parallel to the training channel. Once transferred into this test channel, the ants performed their homebound runs, and I recorded the first six turning points around the anticipated location of the nest entrance to calculate the travelled distance. Ants that had reached the feeder on a foraging trip through the training channel were caught and subjected to experimental manipulation. To increase stride length on the animals’ homebound runs, their legs were splinted and extended with pig bristles glued to the tibia and tarsus. To decrease stride length, the legs were shortened by a cut through the middle of the tibia segment (not considered
here, but see below: “Stumps I”) or by severing the tarsomeres. Operated animals were supplied with a food item and transferred to the test channel, with the food item in their mandibles (“Test 1”). These ants started determined homeward runs, and upon having covered the assumed distance to the nest, they switched to their typical nest searching behaviour. The ants walking back homeward on stilts clearly overshot (15.30 m, interquartile range, IQR = 3.24 m, n = 25), whereas ants with shortened legs undershot (5.75 m, IQR = 1.81 m, n = 25) with regard to their normal homing distance (10.20 m, IQR = 2.40 m, n = 25). There are statistically significant differences (P ≤ 0.001, Kruskal-Wallis Test) among these groups. The modified and tested ants were put back into their nest, and were tested again upon turning up at the feeder during one of the following days (“Test 2”). Having performed already their outbound runs to the feeder on stilts or stumps, these animals exhibited homing runs almost identical to those of normal, unmodified ants. Ants walking on stilts (“stilts”, n = 25) searched for the nest at 10.55 m (IQR = 1.45 m) distance from the release point, and ants with shortened legs at 10.25 m (IQR = 1.76 m) (“stumps”, n = 25). No significant differences were observed between these groups nor with regard to unmodified animals (above, 10.20 m). These results support the hypothesis that desert ants use a step integrator or pedometer for distance measurement (loosely speaking, a step “counter”, although the ants most probably do not literally count (Franks et al., 2005)). According to the pedometer hypothesis, ants that have travelled to the feeder on normal legs, and had

Fig. 3 Cataglyphis fortis on stilts.

Fig. 4 Cataglyphis fortis on stumps.
their leg length modified at the feeder, should cover a different distance on their home-bound journey. This is because the same number of strides made during the outbound travel - as registered by the step integrator - should presumably carry them over longer (“stilts”) or shorter (“stumps”) distances, respectively. 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 (although different from the situation with normal legs in both cases). No such straightforward interpretation is possible for any of the competing hypotheses regarding the desert odometer.

In chapter 2 I analyse and verify the assumptions made in the hypothesis of chapter 1, namely, that shortened or elongated leg length of *Cataglyphis* desert ants leads to shortened or elongated stride length. To analyse stride patterns, and thus stride length, high-speed video recordings were performed. Normal and manipulated freely walking ants were filmed in top view while walking in a channel segment. Video recordings were made both in the torrid field in Maharès, Tunisia, and in the laboratory at the University of Ulm with slightly lower temperatures, to achieve a wide range of walking speeds used to calculate correlations of stepping frequency and stride length (as well as stride frequency versus speed and stride length versus speed). The prediction of the homing distances in manipulated ants was carried out to a more precise and adequate extend than in chapter 1. To derive a prediction of how far the manipulated ants would have to run on their homebound journeys, based on the pedometer hypothesis, the high speed video data were normalised with regard to body size and to walking speed. These video analyses illustrate immediately that the overshoot in ants with stilts, and the undershoot in ants with stumps of different lengths (“Stumps I and Stumps II”), respectively, corresponded to the altered step lengths in a qualitative way. That is, ants on stilts took longer strides and ants on stumps shorter strides than normal animals.

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 step length was eliminated by comparing the animals after normalisation to a certain stride frequency. Ants with elongated legs made longer strides at any given stepping frequency than did normal ants, and ants with shortened legs took shorter strides. According to this normalisation, 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 stepping on stilts. The homing distances actually observed, when converted to percentage values, were in fairly good agreement with these expectations, amounting to -43.6%, -31.9%, and +50.0%, respectively. They thus support the pedometer hypothesis in a qualitative manner. 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 to be expected from the altered stride lengths as recorded in the high speed video analysis. This intriguing feature is discussed in detail at the end of this chapter.

In addition, the shapes of the search density distributions are shown and their half widths (full width at half-maximum height) to point out another piece of evidence for the “step counter (step integrator) hypothesis”. The half widths of the search density distributions in “Test 1” increase with enlarged leg length and thus stride length, and they decrease with shortened legs and strides. This effect clearly decreases in “Test 2”, but does not vanish completely.

I further present an additional data set of ants tested with manipulated (shortened) leg length. Ants that had just their tarsomeres removed ("Stumps I", see above) after being caught at the feeding site underestimated homing distance and searched at 6.95 m (n = 25) distance from the release point. These data are consistent with the results outlined above and thus furnish further support for the pedometer hypothesis.

In chapter 3 I examine a possible role of the hair sensillae in three-dimensional (3D) path integration of the desert ant, *Cataglyphis fortis*. In formicine ants, the hair fields associated with the neck and the petiole (alitrunk-petiole and petiole-gaster joints) have long been established to function in graviception, and thus presumably could function for slope detection. According to Markl (1962), ants determine the relative positions of their body parts by means of hair fields associated with the joints between these body segments. The pull of gravity on these segments is dependent on body position and inclination, thus these hair fields might indeed serve as graviceptors involved in adjusting the odometer module to sloped terrain. The odometer is able to determine travel distance not just in the typical flat desert pans but also in hilly environment with variable slopes (Wohlgemuth et al., 2001, 2002; Grah et al., 2005, Steck, 2006). Ants walking over such hilly terrain determine the ground, or base line, distance irrespective of the actual shape of the surmounted hills. This allows accurate return to the nest on a different
route than that taken during outbound travel, independent of the substrate structure on these routes. I eliminated the function of these hair sensors in graviception either by shaving the hairs, or by immobilising the joints monitored by the hair plates. With that major component of their sense of graviception eliminated, one would expect the ants to disregard, or at least misgauge, the ascents and descents performed across hills during outbound journey, and consider the (much longer) actual walking trajectory, instead of the base distance, when calculating their homing distance. Apparent underestimation of homing distance was observed in several of our experiments. However, no overestimation of homing distance was observed in any experimental situation. This means that the examine hair fields are dispensable for three-dimensional path integration.

Most notably, fixing the gaster to the alitrunk in an (extremely) upright position (<90°) had the most dramatic effects on homing distance, both in the up- and downhill channel training and in the flat channel training. At the same time, this manipulation had the largest impact on body posture, as the position of an ant’s centre of mass is influenced noticeably by fixing the heavy gaster onto the alitrunk (Wehner, 1983). In the artificial hill training, these animals underestimated their travel distance considerably, by an average 75.6 %, when tested after they emerged with their fixed gaster at the feeding site (1-1). This held true when the manipulation was reversed at the feeder and the animals were tested again, although the undershoot was somewhat smaller (50 %) (1-0). Intriguingly, the ants also misgauged their travel distance in the flat channel (control) experiment, though only when the gaster was fixed during both outbound and homebound runs (1-1). They did not undershoot when the manipulation was reversed before the homebound run was performed (1-0).

**General conclusions**

Desert ants, *Cataglyphis fortis*, use a kind of step integrator (“step counter”) to measure distances travelled. Ants stepping home with manipulated leg lengths, walking on stilts or on stumps, and thus using stride lengths different from the ones on their outbound journey, misgauge homing distance in proportion to the imposed changes in stride lengths. This odometer mechanism relies on a fairly constant stride pattern, namely the alternating tripod gait. The alternate tripod gate is a widespread interleg coordination
Chapter 1

pattern for walking at moderate to high speed in hexapods (for ants see Zollikofer 1988, 1994a; for other insects see Hughes, 1952; Delcomyn, 1971, 2004; Evans, 1977; Kozacik, 1981). Moreover, tripods and thus stride length proved to be highly constant spatial entities, thus, the spatiotemporal constancy of this gait pattern may be a general feature used in fast-running desert ants to gauge distances. This constancy should also be present in ants walking with modified leg length to use the experimental approach in chapter 1 for supporting the “step integrator hypothesis”. Actually, the results presented in chapter 2 provide a good basis for this assumption. The observations indicate that walking behaviour remains largely unchanged by manipulations. In addition a quite good prediction of homing distances of ants with manipulated leg length can be realised. The odometer mechanism that allows the ant to navigate in undulated terrain still remains enigmatic. Presumably the ants use the step integrator for measuring the distance actually walked, but this distance is permanently integrated into the path integrator module, that also works in the vertical dimension as well as in the horizontal plane. Contrary to the well studied compass cue, that provides the path integrator with angles steered in the horizontal dimensions, the cue that provides the path integrator with information about inclinations is still unsolved. Assumptions made, that inclinations are measured by means of detecting the posture of body parts against the gravity vector seem to be most probable, since Markl (1962) found the hair fields located between several body segments to be part of graviception in Formica ants. According to the results in chapter 3 and observations made by Wohlgemuth and co-workers (2002) it appears rather unlikely that inclinations are measured through sensory systems employed in graviception, namely hair fields associated with neck and petiole, but also those associated with the legs. At least these organs are dispensable for 3D path integration and their loss can be compensated.
Publication of the results of this thesis and contributions from other scientists

My thesis is structured in three chapters, each representing a separate publication. One chapter has already been accepted for publication (chapter 1), the other two have been prepared for submission (chapter 2 and 3). The authorship is as following:

Chapter 1: Wittlinger, M., Wehner, R., Wolf, H. accepted for publication in Science

Chapter 2: Wittlinger, M., Wehner, R., Wolf, H. prepared for submission in Journal of Experimental Biology

Chapter 3: Wittlinger, M., Wolf, H., Wehner, R. prepared for submission in Journal of Experimental Biology

Prof. Dr. Rüdiger Wehner and Prof. Dr. Harald Wolf will be co-authors in all three publications (chapter 1, 2 and 3), which will arise from this thesis. They gave many excellent contributions to the manuscripts and shared their extensive experience and knowledge with me. They also provided laboratory, technical and financial support.
Zusammenfassung


Die Wüstenameise *Cataglyphis fortis* (Forel, 1902; Wehner, 1983) ist eine der auffälligsten Erscheinungen, die man an einem heißen Sommertag mitten in einer so lebensfeindlichen Umgebung wie einer trockenen Salzpfanne Nordafrikas sehen kann. Man kann sie einzeln mit hohen Geschwindigkeiten von bis zu einem Meter pro Sekunde (ca. hundert Körperlängen pro Sekunde) über den harten und flachen Wüstenboden rennen sehen. Diese Ameisen bewohnen unterirdische Nester mit mehreren hundert Tieren meist am Rande oder mitten in ausgetrockneten Salzseen, wie das bekannte Chott el Cherid in Tunesien. Diese Chotts, Sebkhas und Küstenschwemmflächen stellen einen extrem flachen, salzverkrusteten Lebensraum dar, der meist keine oder nur wenige Orientierungspunkte wie Landmarken bietet. Im Winter, während der Regenzeit, werden diese Flächen oft überschwemmt, dadurch immer wieder eingebeult, und die Kolonien von *Cataglyphis fortis* verbringen unterirdisch ihre Winterruhe. Immer Sommer dagegen fallen diese Schwemmflächen trocken, worauf hin die Kolonien aktiv werden und die Arbeiterinnen auf Nahrungssuche gehen. Die Tiere sind streng tagaktiv und gehen auch während den heißesten Stunden des Tages auf die Suche nach kleinen, dem Hitze- tod erlegenen Arthropoden (Gliedertieren). Das Besondere sind nicht nur ihre Hitze- und Salztoleranz, sondern vor allem die beeindruckenden Navigationsleistungen der Tiere. Die einzeln auf Nahrungssuche gehenden Arbeiterinnen entfernen sich oft mehr als hundert Meter vom Nest und legen dabei Entfernungen von vielen hundert Metern auf ihren mäandrierenden Ausläufen zurück. Wenn sie dann ein Beutestück finden, tragen sie dieses auf direktem, geradlinigem Weg zurück zum Nest mit seinem kleinen und unscheinbaren Nesteingang - sie laufen nicht wieder den gewundenen Weg zurück, der sie zur Beute geführt hat. Dabei sind die Tiere reine Einzelfouragerer; sie legen keine Duftspuren, denn die Oberflächentemperatur ist viel zu heiß für leicht flüchtige Pheromone, und sie rekrutieren auch keine weiteren Arbeiterinnen zu eventuell ergiebigeren Futtermquellen. Diese bemerkenswerten Orientierungsleistungen sind bei den Wüstenameisen der Gattung *Cataglyphis* inzwischen recht gut untersucht. Sie betreiben eine als Pfadintegration bezeichnete Vektornavigation. Es zeigte sich, dass die Tiere während ihres Auslaufs den zurückgelegten Weg integrieren, d. h. sie führen eine (näherungsweise) Vektoraddition der zurückgelegten Wegsegmente durch. Wo immer sich die Ameise befindet, sie ist jederzeit über Richtung und Entfernung zu einem Bezugspunkt - zum Nest oder auch zu einer wiederholt besuchten Futterstelle - exakt informiert. Für eine solche Wegintegration ist sowohl ein Richtungsmesser (Kompass) wie auch ein Entfernungsmesser (Odometer) notwendig. Bei Insekten ist der Richtungsmesser grundsätzlich...
lich als Sonnenkompass realisiert. Dabei werden für die Richtungsbestimmung die Position der Sonne und der spektrale Gradient des Sonnenlichts erfasst, vor allem jedoch als der präziseste und weit wichtigste Sinneseindruck das Polarisationsmuster des Him- melszeltes.


Bedingungen. Diese Voraussagen entsprachen weitgehend den experimentellen Beobachtungen.


Ein generelles leichtes Unterschätzen der Rücklaufdistanz konnte bei Tieren, die über den Berg- und Talaufbau trainiert wurden, festgestellt werden. Allerdings würde man erwarten, dass sich bei einer erfolgreichen Eliminierung der Steigungswahrnehmung (und entsprechend der Messung der Basisdistanz) der Rücklaufweg bei einzelnen Testsituationen verlängern würde. Dies geschah jedoch in keinem einzigen Fall.

Dagegen konnte ich bei Tieren mit steil nach oben festgelegtem Gaster ein deutliches Unterschiessen beim Rücklauf beobachten. Dies war allerdings nur dann der Fall, wenn die Ameisen schon mit festgelegtem Gaster ausgelaufen waren. Dasselbe geschah bei den Kontrollexperimenten mit ebenem Auslauf.

Insgesamt erscheint es aufgrund dieser Daten als unwahrscheinlich, dass Geländeneigungen durch die für die Schwerkraftwahrnehmung (Gravizeption) zuständigen Borsttenfelder auf Hals-, Petiolus- oder Gasterregion gemessen werden. Zumindest sind diese Sinnesorgane für dreidimensionale Wegintegration nicht notwendig und ihr Ausfall kann kompensiert werden.
References


1 The Ant Odometer: Stepping on Stilts and Stumps

Matthias Wittlinger, Department of Neurobiology, University of Ulm, Germany
Rüdiger Wehner, Institute of Zoology, University of Zürich, Switzerland
Harald Wolf, Department of Neurobiology, University of Ulm, Germany

Abstract

Desert ants, *Cataglyphis*, navigate in their vast desert habitat by path integration. They continuously integrate directions steered - as determined by their celestial compass - and distances travelled - gauged by as yet unknown mechanisms. Here we test the hypothesis that navigating ants measure distances travelled by using some kind of step integrator, or “step counter”. We manipulated the lengths of the legs, and hence the stride lengths, in freely walking ants. Animals with elongated (“stilts”) or shortened legs (“stumps”) take larger or shorter strides, respectively, and concomitantly misgauge travel distance. Travel distance is overestimated by experimental animals walking on stilts, and underestimated by animals walking on stumps.

Text

Foraging Saharan desert ants, *Cataglyphis fortis*, use a mode of dead reckoning known as path integration (1, 2) to monitor their current position relative to the nest and to find their way home (3). This enables them to return on a direct route, rather than retracing the tortuous outbound journey performed when searching for food items in their flat desert habitat, which is often completely devoid of landmarks. The path integrator requires two kinds of input information: (i) about directions steered, as obtained via the ant’s celestial compass (4), and (ii) about distance travelled, as gauged by the ant’s odometer. The cues by which ants measure travel distance during locomotion have not yet been revealed. However, there are several promising hypotheses. The “energy hypothesis” posits that the (surplus) energy required for locomotion (as opposed to rest) is used to calculate travel distance. This hypothesis is of long standing in arthropod research (5),
but is not applicable to the problem of the ant odometer because ants assess their walking distances with great accuracy, irrespective of the load they carry (6). The “optic flow hypothesis” has been proven in honeybees, which integrate visual flow-field cues during their foraging flights to gauge flight distance (7, 8). In Cataglyphis ants, this mechanism plays a minor, if any role (9) (detailed discussion of the “optic flow hypothesis” see SOM). Even in complete darkness, on featureless platforms, or with the ventral halves of their eyes covered, the animals are still able to assess travelling distance correctly during their homing runs (9, 10). And lateral optic flow does not have any influence at all on distance estimation (11). Considering the relatively constant locomotor speed of desert ants, a time lapse integrator might function to measure walking distance – although this possibility has been refuted in slightly different experimental contexts (12). Thus, ants appear to rely primarily on idiothetic cues, most probably derived from the movements of their legs (13). Although this step integrator or “pedometer” hypothesis was initially proposed as early as 1904 (14), it has remained untested. Here, we examine whether or not ants with manipulated leg lengths, walking on stilts or on stumps, exhibit changes in their stride lengths, and consequently misgauge their travel distance during homebound runs.

The ants were trained to walk from their nest entrance to a feeder, over a distance of 10 m and in a linear alloy channel (Fig. 1, top). After at least one day of training, the animals were caught at the feeding site and transferred to a test channel, aligned parallel to the training channel (Fig. 1, bottom). Once transferred into this test channel, the ants performed their homebound runs, and we recorded the point at which the ants switched from their straight and steady return path to their characteristic nest searching behaviour. This point is marked by a 180° U-turn (15), followed by a run pacing back and forth around the anticipated location of the nest entrance. Details of the experimental procedure are given in SOM.

Ants that had reached the feeder on a foraging trip through the training channel were caught and subjected to experimental manipulation. To increase stride length on the animals’ homebound runs, their legs were splinted and extended with pig bristles glued to the tibia and tarsus, as illustrated in Fig. 2 (“stilts”). To decrease stride length, the legs were shortened by severing the tarsomeres in the middle of the tibia segment (Fig. 2, “stumps”). Operated animals were supplied with a food item and transferred to the test channel, with the food item in their mandibles (“test 1”). These ants started determined homeward runs, and upon having covered the assumed distance to the nest, they
switched to nest searching behaviour outlined above. The ants walking back homeward on stilts clearly overshot (15.30 m, interquartile range, IQR = 3.24 m, n = 25), whereas ants with shortened legs undershot (5.75 m, IQR = 1.81 m, n = 25) with regard to their normal homing distance (10.20 m, IQR = 2.40 m, n = 25) (Fig. 3, A). There are statistically significant differences ($P \leq 0.001$, Kruskal-Wallis Test) among these groups.

The modified and tested ants were put back into their nest, and were tested again upon turning up at the feeder during one of the following days (“test 2”). Having performed already their outbound runs to the feeder on stilts or stumps, these animals exhibited homing runs almost identical to those of normal, unmodified ants (Fig. 3, B). Ants walking on stilts (“stilts”, n = 25) searched for the nest at 10.55 m (IQR = 1.45 m) distance from the release point, and ants with shortened legs at 10.25 m (IQR = 1.76 m) (“stumps”, n = 25). No significant differences were observed between these groups nor with regard to unmodified animals (above, 10.20 m).

These results support the hypothesis that desert ants use a pedometer for distance measurement, or a step integrator (loosely speaking, a step “counter”, although the ants most probably do not literally count (16)). 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 - as registered by the step integrator - should presumably carry them over longer (“stilts”) or shorter (“stumps”) distances, respectively. 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 (although different from the situation with normal legs in both cases). No such straightforward interpretation is possible for any of the competing hypotheses.

This interpretation rests on the assumption, however, that stride length is indeed altered by the manipulation of leg length, roughly in proportion to the imposed length changes. Thus, a quantitative assessment of the (manipulated) ants’ stride lengths and their relation to walking distances is also necessary. It was impossible to count the ants’ strides during experiments (for instance, by filming the complete outbound and homebound journeys on high-speed video). Instead, stride lengths were determined in a different set of animals, both normal and manipulated, in a small pen erected on the desert floor. As in most animals, stride length in (“normal”) *Cataglyphis* depends on at least two parameters. The first is leg length, and thus body size, with body morphology being isomorphic in *Cataglyphis fortis* workers (17). That is, larger ants take correspondingly
longer strides. The second is walking speed. The faster an ant runs, the larger are the strides it takes (18, 19, 20). Normalisation of homebound travel distances with regard to both body size and walking speed is thus required for a quantitative interpretation of the above experiments. High speed video recordings of running ants were analysed to determine the actual changes in stride lengths caused by the “stilts” and “stumps” manipulations described above. Walking speeds were determined in the above experimental animals by the time required for uninterrupted straight segments of homebound travel (and confirmed on the high-speed movies in a different set of animals).

To derive a quantitative prediction of how far the manipulated ants would have to run on their homebound journey, based on the pedometer hypothesis, the experimental data were first normalised with regard to body size. That is, relative stride lengths with regard to body dimensions were calculated to eliminate effects of size variation. Second, the actual stride lengths were determined in normal and manipulated ants. Ants walking on stilts took significantly longer strides ($P < 0.01, t$-test; 14.8 mm, SD = ± 2.5 mm, or +13.9 %) than did normal animals (13.0 mm, SD = ± 1.98 mm), and ants on stumps made significantly shorter strides ($P < 0.001; 8.6$ mm, SD = ± 1.73 mm, or –33.2 %).

These values were then used to derive a prediction for the respective undershoot or overshoot of manipulated ants, based on the assumption that distance is measured by a step integrator, that is, that the ants took the same number of strides during their outbound journey to the feeder, and when returning to the nest on manipulated legs (hatched boxes in Fig. 3 A). The predicted values show that there is general agreement between the predicted and observed homebound travel distances in manipulated ants, further corroborating the step integrator hypothesis.

However, there are some differences (significant in “stilts”, $P = 0.015$; not significant in “stumps”, $P = 0.125$; Mann-Whitney Rank Sum Test) between the observed and the predicted homebound travel distances. Impairment of the ants by the preparation procedures cannot account for these effects. Any serious impairment would tend to shorten the ants’ homebound runs. The animals walking on stilts clearly travelled for much longer distances than the normal controls, however, and they did so with apparently normal vigour. 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 (see movie S1 in SOM).

The differences between observed and predicted homing distances may be attributable, though, to altered walking speeds in the manipulated ants. In fact, normal ants travelled
at an average 0.31 m/s, and ants walking on stumps averaged 0.14 m/s, a value in good agreement with their shortened legs and stride lengths (see similarity of predicted and experimentally determined homing distances in Fig. 3, A). Ants on stilts however, rather than walking at increased speeds, were also slightly slower than normal animals, walking at an average 0.29 m/s. This was presumably due to the added load of glue and pig bristles on their legs. As noted above, changed walking speeds are associated with correspondingly altered stride lengths, which would appear to explain the unexpectedly short prediction value in Fig. 3 (A, top hatched box). We thus corrected this prediction with the established relationships between walking speed and stride length (18, 19). We assumed as a first approximation (and conservatively, when considering the actually imposed changes in leg length) that ants might run by as much faster on stilts as they ran slower on stumps (0.48 m/s, a value regularly observed in highly motivated normal ants, and almost reached by the fastest ants on stilts). This procedure indeed yields a value that is not significantly different from the observed homing distances in ants on stilts (open box in Fig. 3, A; 14.25 m, IQR = 3.35 m), thus confirming the consistency of our data with the step integrator hypothesis.

The slower speeds of the ants walking on stilts further rule out the only alternative explanation of our homing distance data (Fig. 3 A; solid boxes). In principle, a step integrator and a time lapse integrator would both yield the same homing distances, even in ants with manipulated leg and stride lengths, if only the ants kept their stride frequencies constant (or in normal ants, walking speed - which in fact they almost do under normal conditions (18, 19)). Constant stride frequency would result in walking speed being changed in proportion to altered stride length, and a resulting difference in homing distance during a set (outbound) travel time. This assumption is evidently not correct, though, given the walking speeds of the experimental animals.

Future studies will have to address the mechanism the proposed step integrator, for example, whether it actually registers steps by means of proprioreceptors, or whether it integrates activity of a walking pattern generator, and to what extent sensory feedback regarding stride length and walking performance is considered.
Fig. 1. Schematic diagram of channel layout, as well as training and testing procedures applied in *Cataglyphis* foragers; not drawn to scale

Fig. 2. Manipulation of ant legs as performed in the present study. In “stilts” attached pig bristles elongated the legs; unmodified legs in “normal”, with approximate range of tarsus movement indicated; shortened legs in “stumps”. The right hind leg is shown from *anterior* (see Fig. 1 in (16))
Fig. 3. Homing distances of experimental ants, tested immediately after the lengths of their legs had been modified at the feeding site. (A) Leg lengths were normal during the outbound journey but manipulated during the homebound run, resulting in different homing distances. (B) Ants tested after re-emerging from the nest after previous manipulation. In this situation leg lengths were equal, though manipulated, during outbound and homebound runs.

Box plots show median values of the homing distances recorded in n = 25 ants per experiment (as well as interquartile ranges, box margins, and 5th and 95th percentiles, whiskers). Median values of the initial six turning points of an ant’s nest search behaviour were considered as the centres of search, indicating homing distance. The hatched box plots in (A) illustrate the centres of search as predicted from the high-speed video analyses of stride lengths in normal and manipulated animals. The open box represents the prediction corrected for slow walking speed. Details in text.
References and Notes

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Supporting online materials (SOM)

Methods details

Both training and test channels were 7 cm wide, and channel walls were 7 cm high, such that the ants experienced a restricted, strip-like view of the sky of approximately 53° to read the skylight pattern and compass information. Channel walls were painted with matt grey varnish to provide a featureless and even environment, and the channel floor was coated with fine grey sand to provide traction for walking.

We had carefully chosen the fine grey sand that covered the floor of the test and training channels explicitly to avoid optic flow cues. Sandblasting sand with grain sizes ranging from 0.2 to 0.4mm was used. Assuming an optical divergence angle of 5° in the ventral hemisphere of the Cataglyphis eye (S1), and an eye-substrate distance of 5mm for the smallest normal ants, the largest sand grains are still well below the ant’s optical resolution (about 0.9 mm; note that according to Shannon’s Information Theory criterion one needs one receptor in between two resolvable points; see, e.g. review in S2). This is a worst-case scenario since most of the ant’s ventral visual field is much farther away from the floor than the eye-substrate distance (compare S3). Finally, the contrast provided by the below-resolution-threshold ground floor (i.e., the light-reflection difference between the centres of the grain particles and the boundaries between adjacent particles) was so low that even an above-resolution-threshold graininess would certainly not have provided the ants with sufficient optical ground heterogeneity.

For each experimental situation – stilts, stumps, and normal controls - the respective ants were marked with a particular colour, such that every animal participated in only one experiment, and each individual was successively run through control, test 1, and test 2 (below).

To extend the legs, pig bristles were glued to the legs with cyanoacrylate adhesive (superglue) and extended beyond the tarsal claws for about another 1 mm. Already the leg splint itself would have increased stride length by causing the animals to “tiptoe” on their tarsi. This is, since the tarsal segments, except the most proximal one (the basitarsus), are quite flexible and normally touch the substrate during walking (compare
“Normal” in Fig. 2). Legs were shortened by simply clipping the tibiae at the desired place with a pair of minute scissors.

Each manipulated ant was put into a small container, together with an assortment of food items. Only when the ant accepted one of the food items (usually a biscuit crumb) - indicative of a predominant motivation to carry the booty home (S4) - was it placed into the test channel. Having covered their assumed homing distance, the ants switched to a search pacing up and down the test channel, with the search centred on the assumed nest position. We took the initial six turns of each search to calculate homing distance, as the median value. Each ant was tested twice, first, right after the experimental manipulation (test 1), and second, after having re-emerged from the nest and visited the feeder again (test 2).

High-speed video recordings were made with a Redlake Motionscope, with a frame rate of 250 per second. Stride lengths were evaluated in frame-by-frame analysis on a computer monitor, aided by Redlake software. Stride lengths were determined for left and right middle legs as the distances between subsequent foot falls (S5).

**Discussion of optic flow cues**

As mentioned in the main text, it has been established previously that optic flow also plays a role in ant odometry, at least flow in the ventral visual field. However, this contribution is small indeed. The data provided by Ronacher and Wehner (S6) allow a quantitative judgment of this contribution of optic flow. The training distance in these experiments was 10 m. In one particular experimental situation, 26 m homing distance would have been expected if optic flow were the only mechanism used in ant odometry. Actual homing distance was, however, only 11.24 m. The overshoot was thus 1.24 m, compared to 16 m expectation. This amounts to a little less than 8% contribution of the optic flow mechanism to the *Cataglyphis* odometer. And this contribution was observed with strong visual contrast in the ventral visual field, while under low contrast conditions (as in our experiments) the use of all visual cues is inevitably reduced.
References


Video S1

An experimental ant walking on stilts filmed in its typical desert habitat to demonstrate how accurate and trouble-free the animals are able to walk with these modified legs (walking speed ca. 0.25 m/s). The reproduction is in slow motion, x0.5.
Chapter 2

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

Matthias Wittlinger, Department of Neurobiology, University of Ulm, Germany
Rüdiger Wehner, Institute of Zoology, University of Zürich, Switzerland
Harald Wolf, Department of Neurobiology, University of Ulm, Germany

Abstract

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, while 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 were also able to 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.
Introduction

Foraging desert ants, *Cataglyphis fortis*, cover distances of more than 100 meters 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), 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 (1952; see also Heran (1956), and 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, 1997; Esch et al., 2001). 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. That is, they employ a “stride integrator”, as recent experiments have illustrated (Wittlinger et al., in press).

In this study we investigate how *Cataglyphis* ants measure travel distance, focussing on leg movement during walking. We manipulate 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 mid 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 approximately 53° to read the skylight pattern and compass information. To provide traction for walking, the channel floor was coated with fine grey sand, the grain size ranging 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, to avoid distracting reflections.

For each test situation, ants were marked with a particular colour. After at least one 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 presently 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 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 recently ended when the behaviour switched to nest searching (compare Müller and Wehner,
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. 3). Each ant was tested twice, first straight after the manipulation of leg length at the feeding site (Test 1; Fig. 3A). 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. 3B). 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 small pair of 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 cyanacrylate glue (superglue) and extended of 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). Normal ants were left unaffected and served as controls.

**Walking speed**

The walking speeds of homing ants were recorded for ants with extended legs (Stilts), normal ants, and ants walking on shortened legs (Stumps). 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, U. S. A.). In addition, walking speed was analysed from the high speed video material (below) to determine the relationships between walking speed, stride length, and leg length, respectively.

**Analysis of behavioural data**

The test channel was divided into 10 cm bins for distance recording, as outlined above. First, search density distributions (Fig. 3) 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. 3, 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. 3, 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, U.S.A.) high speed camera using a frame rate of 1/250 s. Normal and manipulated freely walking ants were filmed in top view while walking in a pen of 10 cm x 30 cm or in an alloy channel of 2 m length, both supplied with the sandy walking substrate mentioned above (and a piece of mm grid 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). While all other experiments were performed in Maharés, some of the video recordings were made in the laboratory at the University of Ulm to sample slightly lower temperatures than in the desert (ca. 30 - 45°C air temperature), and thus a broaden the range of walking speeds used to calculate the regression lines in Fig. 5. 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 of the left and right middle legs (L2, R2), 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 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 \(l2' = \frac{l2}{al}\), and relative stride length \((s2')\) was determined from the video data as \(s2' = \frac{s2}{al}\).

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. 5; see also Zollikofer (1988), (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. 5) 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 23 Hz, that is, the data points were shifted along the respective regression line to 23 Hz on the abscissa: \(s2'_{23Hz} = \frac{(b \cdot 23Hz + a) + (s - (b \cdot f + a))}{(b \cdot f + a)}\).

The resulting distributions of stride lengths (on the ordinate) formed the basis for the hatched box plots in Fig. 3 (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, due to different prey loads or experimental manipulations. This procedure further allowed to derive a prediction 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. 3A, lower panel).

The stride length and stride frequency data were also plotted against walking speed (Fig. 6) to provide a basis for comparison with literature data (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”, “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 data of 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 differ
ence from 0, and for differences against each other). Statistical methods are described in detail in 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, and to be reached through a narrow channel. Experimental ants were captured at the feeder, had their leg lengths 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. 3 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 posthoc 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, 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), respectively. No statistically significant difference was discernible between these groups (P = 0.502).

The half widths (“full width at half-maximum height”) of the search density distributions, as shown in Fig. 4, reflect the uncertainty of the ants’ estimates of their homing distance (Wolf and Wehner, 2005; Merkle et al., submitted). Half width values exhibit a conspicuous relationship to the imposed changes in leg length. In the data from “Test 1” (see Fig. 3A), half widths 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 - think of 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”), respectively, 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 stride frequency (Zollikofer, 1994a, 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 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. 5. It is evident that manipulation of leg length changed the relationship between stride length and stride frequency, as was to 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 relation
ships were used to normalise the stride length data of the experimental animals to a stride frequency of 23 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. 3A, 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 to be expected from the altered stride lengths as recorded in the high speed video analysis.

**Characteristics of walking behaviour in ants with manipulated leg lengths**

While the plot of relative stride length versus stride frequency in Fig. 5 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. 6 presents plots of stride frequency (Fig. 6A) and stride length (Fig. 3B) against walking speed. These diagrams lend themselves to direct comparison with data from other insects and indeed other walking animals (see Discussion).

The rise in stride frequency (Fig. 6A) and the rise in stride length (Fig. 6B) 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 e.g. Full and Tu, 1990, 1991), indicative of the absence of flight phases in the step cycle, or galloping.

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. 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 towards 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%), due to 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 due to 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% of the legs mass, and in a distal 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 honey bee navigation by 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”, while 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 already in 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” hypothesis has been confirmed in principle recently by studying the homing behaviour of desert ants with imposed changes in leg length (Wittlinger et al., in press). However, detailed analysis of walking behaviour in ants with manipulated leg lengths was not performed in this 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. Our present results are in unequivocal agreement with that 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 of walking behaviour remained with 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. 5). 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 independent of the experimental manipulations (Fig. 5; details see Methods). The predictions derived from the observed changes in stride
length are in good agreement with the experimental data of the homing experiments (Fig. 3A, 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. 3A, 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, due to 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 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, that 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 been ruled out by Ronacher and Wehner (1995) already. 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, namely, through grey paint on the channel walls and even coating of the channel floor with fine-grained sand and. 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 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, in particular, since the ants on stilts often walked indeed more slowly than did normal ants, their maximum speed being reduced to about 82%. Nevertheless, these animals overestimated homing distance by about 50%.

The width of search density distribution reflects navigation uncertainty

One may interpret the widths of the search density distributions (Fig. 3) as signs of the animals’ navigation uncertainty (Wolf and Wehner, 2005; Merkle et al., submitted), 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). In the present experiments stride length was manipulated, though, 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. 4, 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 in-bound travel, and thus also relative - and absolute - errors of distance estimation). This result is in clear agreement with the above interpretation regarding navigation uncer-
tainty. The data further suggest that the half width of the uncertainty distribution per step cycle is in the range of 25%.

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

The plots of stride frequency and stride length versus walking speed (in normal ants) follow the general observations made in other animals, and in insects in particular (e.g. Full & Tu, 1990). As noted above, there were no indications of galloping in the present experimental conditions, while *Cataglyphis* may gallop, in principle (Zollikofer, 1988, 1994b). The agreement of our data with previous results corroborates (Zollikofer, 1988, 1994a, 1994b) and extends (Full and Tu, 1990, 1991) these extends the rules governing walking behaviour 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. 6A) 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 & 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 & 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. 6A 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, among other things increasing the
risk of stumbling and getting caught on uneven structures. The ants on stilts carry some 56 % excess mass on their legs. Both groups have poorer ground contact due to the missing tarsal pads.

By a similar line of argument, a linear relationship would be expected between stride length and walking speed (Fig. 6B). 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, due to 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 due to the manipulations, resulting in different speeds).

Again, the graphs in Fig. 6B conform to expectation almost perfectly. And as noted above, this observation indicates that walking behaviour remains remarkably unchanged by the manipulations.
References


Fig. 1. Experimental situation. Schematic diagram of channel layout; training channel, top; test channel, bottom. 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.

Fig. 2. Manipulation of leg length. The right hind leg of a *Cataglyphis fortis* worker is shown from anterior (adapted from in Wehner (1983); Fig. 1 therein), and the manipulations performed in the present study are indicated. “stumps II”, “stumps I”, “normal”, and “stilts” denote the different experimental situations, as they are termed throughout the text. Details see text.
Fig. 3. Homing distances of experimental ants. The top panels shows search density plots (abscissae, homing distance; ordinates, cumulated relative search densities between the first and sixth turning points; see Methods), the bottoms panels 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). The ants in (A) had been 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). The ants in (B) 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; details see text. Colour code: red, stilts; blue, normal; yellow, stumps I; green, stumps II.
**Fig. 4.** Widths of search density distributions change with altered leg length. The half-widths (curve width at half-maximum height) of the search density distributions in Fig. 3 were determined. They are shown for the different experimental situations, “Test 1”, top, “Test 2”, bottom. Colour code as in previous Fig. 3: red, stilts; blue, normal; yellow, stumps I; green, stumps II.

**Fig. 5.** 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 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 graphs: Stilts,
stride length (mm) = 0.124 * stride frequency (Hz) + 2.14 (mm); normal, stride length (mm) = 0.084 * stride frequency (Hz) + 1.89 (mm); stumps I, stride length (mm) = 0.061 * stride frequency (Hz) + 1.788 (mm); stumps II, stride length (mm) = 0.057 * stride frequency (Hz) + 1.42 (mm). Colour code as in previous figures: red, stilts; blue, normal; yellow, stumps I; green, stumps II.

**Fig. 6.** The relationship between stride frequency and walking speed is shown in (A), and that between (relative) stride length and walking speed in (B). The different experimental situation are colour-coded as in the previous figures: red, stilts; blue, normal; yellow, stumps I; green, stumps II. Linear regression lines are indicated. The
terms describing the regression lines for the normal ants are stride frequency (Hz) = 0.054 * walking speed (mm/s) + 7.72 (Hz) (R^2=0.87) in (A) and stride length (mm) = 0.018 * walking speed (mm/s) + 6.80 (mm) (R^2=0.81) and in (B).
3 Hair plate mechanoreceptors associated with body segments are not necessary for 3-dimensional path integration in desert ants, *Cataglyphis fortis*

Matthias Wittlinger, Department of Neurobiology, University of Ulm, Germany
Harald Wolf, Department of Neurobiology, University of Ulm, Germany
Rüdiger Wehner, Institute of Zoology, University of Zürich, Switzerland

Abstract

In formicine ants, the hair fields associated with the neck and the petiole (alitrunk-petiole and petiole-gaster joints) have long been established to function in graviception. Here we examine a possible role of these hair receptors in three-dimensional (3D) path integration of the (formicine) desert ant, *Cataglyphis fortis*. *Cataglyphis* judge the ground distance when travelling over hills, allowing correct homing even in (unpredictably) uneven terrain. We eliminated the function of these hair sensors in graviception either by shaving the hairs, or by immobilising the joints monitored by the hair plates. With that major component of their sense of graviception eliminated, one would expect the ants to disregard, or at least misgauge, the ascents and descents performed across hills during outbound journey. The ants should thus consider the (much longer) actual walking trajectory, instead of the base distance, when calculating their homing distance. Surprisingly, neither shaving nor immobilisation of the hair sensillae affected correct path integration, across both, uneven terrain (3D) and level surface. If anything, the ants underestimated homing distance, which may reflect a general, safety-oriented navigation strategy. Animals that had performed the outbound journey with their gaster fixed in a horizontal position underestimated their homing so dramatically that this latter explanation cannot hold.
Introduction

Desert ants, *Cataglyphis fortis*, perform dead reckoning, or path integration (Mittelstaedt, 1983; Müller and Wehner, 1988), as a major means of orientation when foraging in their typically flat and featureless desert habitat. Path integration relies on two input components. The first is the direction of travel, measured by a celestial compass. This compass is sensitive to the polarisation pattern of sky light, to the sun’s azimuth, and to spectral gradients of sky light (Wehner, 1992). The second component for path integration is distance measurement, provided by an odometer module. The ant odometer is basically a step integrator, that also considers stride length and stride frequency, though (Wittlinger, in press).

Path integration in desert ants is surprisingly accurate and successful, due to a number of compensatory and backup mechanisms that deal with the inevitable navigation errors inherent in all integration mechanisms (Müller and Wehner, 1988). This accuracy is not too surprising for the compass module which relies on exact external directional cues, namely, sun azimuth and sky light pattern. The odometer module, by contrast, has to rely primarily on idiothetic cues, namely, proprioceptive signals that measure leg movement or monitor the output of a locomotor central pattern generator (Wehner, 1992). It might thus be expected to be more error-prone than the directional component of the path integrator. All the more surprising are unexpected features of the odometer module that have been reported in recent years. Most notably, the odometer is able to determine travel distance not just in the typical flat desert pans but also in hilly terrain with slope inclinations of up to 60° (Wohlgemuth et al., 2001, 2002; Grah et al., 2005). Ants walking over such hilly terrain determine the ground, or base line, distance irrespective of the actual shape of the surmounted hills. This allows accurate return to the nest on a different route than that taken during outbound travel, independent of the substrate structure on these routes. Not only is this feature of the ant odometer surprising but so is its accuracy that is comparable to that achieved on level ground.

It is as yet completely unclear how base line distance is determined by the ants when walking on hilly terrain. It is clear, however, that the ants must be able to measure the slope of their walking substrate quite exactly to achieve this feat. In the present account we examine one particular hypothesis of how desert ants might be able to determine the inclination of the ground they travel on. According to Markl (1962), ants determine the relative positions of their body parts, such as head, thorax (or in ants, alitrunk) and abdomen (or in ants, gaster), by means of hair fields associated with the joints between
these body segments. And since the pull of gravity on these segments is dependent on body position and inclination, the above hair fields might indeed serve as graviceptors involved in adjusting the odometer module to substrate inclination.

Materials and Methods

Animals and experimental site

The field site for our experiments was located near Maharês, Tunisia (34°30’N, 19°29’E). Large and viable nests of *Cataglyphis fortis* Forel 1902 (Wehner, 1983) were selected. The experimental season lasted from the mid of June to the start of September in the years 2004 and 2005.

Preparation

To manipulate graviception, in a first set of experiments the hair sensillae in the neck region, and/or those associated with the petiole were eliminated by shaving with a razor splinter. There are two groups of hair plates associated with the petiole, one between alitrunk and petiole, and a second between petiole and gaster. (Fig. 1). In a second set of experiments the joints between head and alitrunk, and those between alitrunk and gaster were immobilised with a bees’ wax-resin mixture (1:2, v:v). In detail, the head was fixed to the alitrunk in a normal attitude, while the gaster was fixed to the alitrunk either in an extended position (i.e. gaster, petiole, and alitrunk forming a roughly straight line, 180°) or in an erect position (the angle between alitrunk and gaster was <90°) (Fig. 2).

Experimental set-up

The basic experimental set-up was adapted after Wohlgemuth and co-workers (2001, 2002). Ants were trained to forage at a feeder they could reach only via a series of artificial hills (Fig. 3, hill training), affording a walking distance of 10 m. That walking distance corresponded to a distance on ground level between nest entrance and feeder of 6m. As a control, a group of ants was trained to walk a 10 m distance in a flat channel (Fig. 3, flat training). For testing, the experimental ants were put into a horizontal channel, 21 m long and oriented in parallel to the outbound (hill and flat training) channel (Fig. 3, test). Both, the artificial hill channel set-up for training (ten symmetric triangular “hills”, consisting of 50 cm ascent and 50 cm descent each, with slopes of 54°) and the flat channel set-up for control training and testing consisted of alloy U-profiles.
Width and height of the channels were 7 cm (see Fig. 3, top left), such that the ants experienced a strip-like view of the sky of approximately 53° when walking in the centre of the channel profile (“centring response”, Heusser and Wehner, 2002) to read the sky-light pattern. To provide traction for walking the channel floor was coated with grey quartz sand, and to reduce optic movement cues and avoid distracting reflections the walls were painted with matt grey varnish.

In all experiments, each ant was marked by an individual three- or four-digit colour code, so that each animal of an experimental group could be run individually through the particular series of treatments and tests. The animals were caught and operated under a dissection microscope (Wild M8, magnification 50x, Heerbrugg, Switzerland) at the feeding site, and then placed into a small container with an assortment of food. As soon as a given ant had picked up a food item it was placed into the test channel to perform its homebound run. With a food item in their mandibles, the animals were highly motivated to return home (Wehner, 1982) and performed determined homebound runs. This experimental group had performed the outbound travel in a normal condition (“0”), and the homebound travel in a manipulated condition (“1”), and it is thus termed “0-1” throughout the text. After this first test the ants were released into their nest. A further (control) test was performed in these manipulated ants after they had re-emerged from the nest, and performed another journey to the feeder (outbound, manipulated – homebound, manipulated: “1-1” ants). In animals with immobilised body segments (head-alitrunk or alitrunk-petiole-gaster joints incapacitated) additional tests were carried out. Namely, one test was made after removal of the bee’s wax-resin glue at the feeding site, thus making the previously immobilised body parts again free to move (outbound, manipulated – homebound, normal, 1-0), and one final control test was made after these 1-0 animals had re-emerged from the nest, being in a normal condition again (outbound normal – homebound normal, 0-0a; as opposed to 0-0 as the group of ants tested prior to any manipulation, except colour marking). Of course, the shaving of hair plates could not be reverted in this way, the final test for these animals being 1-1.

Once transferred into the (flat) test channel, the ants performed their homebound runs. We recorded the point at which the ants switched from their steady, straight return path to their typical nest-searching behaviour. This point is marked by a conspicuous U-turn, followed by a run pacing back and forth around the initial turning point. The median distances of the six initial U-turns, relative to the release point, and the search densities were evaluated (see Figs 4, 5, 6). The test channel was divided into 10 cm bins for dis-
tance recording. Search density distributions (Figs 4, 5, 6, upper panels) were evaluated 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. Search density distributions were normalised to their peak values.

**Statistical tests**

For descriptive statistics box and whisker plots (box plots) are shown (see Figs 4, 5, 6, lower panels). They display median values (bar in the box) and interquartile ranges (IQR) as measures of statistical dispersion, with first an third quartile (the box margins) and the 5th and 95th percentiles (whisker margins). For all tests we performed non-parametric statistics. Friedman Repeated Measures ANOVA on Ranks compared effects of a series of different experimental test situations on a single group. If a difference was found, Tukey’s test was performed as *post hoc*-test for multiple comparisons between test situations (Sachs, 1992). All statistical analyses were made with SigmaStat Version 2.03, SPSS Inc., Chicago, USA.

**Results**

*Shaving of hair fields*

We trained ants to walk from the nest entrance to a feeder over an array of artificial hills (Fig. 3, hill training). The actual walking distance for the animals was 10 m, corresponding to a distance of 6 m on ground level. Ants that had reached the feeder in this artificial hill set-up were caught and subjected to experimental manipulation, and their homing distances were subsequently measured in a flat channel (see Materials and Methods). In accord with previous studies (Wohlgemuth et al., 2002), control animals without any manipulation exhibited homing distances close to the actual ground distance of the training set-up (0-0 in Figs 3 A, B, C). In experimental animals the hair plates between head, alitrunk, and gaster were manipulated, by shaving the hair sensillae in a first set of experiments. Unexpectedly, none of the shaving operations changed the animals’ homing distances, and particularly they did not extend them towards the 10 m actual walking distance experienced during the outbound journeys (Fig. 4). If anything, the ants slightly underestimated, rather than overestimated, the distance to the
nest. This held true for all combinations of hair plate manipulation, shaving of the hair sensillae in the neck region, of the hair plates associated with petiole and gaster, and shaving of all these sets of hair plates (summary in Tab.1). The slight underestimation of homing distance just noted was statistically significant only for the animals that had all these hair sensillae incapacitated and were tested after they had accomplished their first manipulated homing run (1-1 animals, P<0.05, Tukey test) (Fig. 4 C).

**Shaving of hair fields – flat channel training controls**

In these control experiments, the ants were trained from the nest entrance to the feeder over a 10 m distance in a flat, linear channel, instead of the artificial hill array used otherwise. The ants were tested in the usual 21 m long flat channel. Interestingly, the slight underestimation of homing distance after the shaving operations also occurred with the flat channel training array (Fig. 5 A, B). Actually, animals that re-emerged from the nest after having been shaved in the neck region previously (1-1) significantly (P<0.5, Tukey test) undershot in their homebound runs, compared to the control recordings in these same animals (0-0). Animals with their hair sensillae shaved in the petiole-gaster region showed a significant underestimation of their homing distance, compared to the control situation (0-0), in both tests that followed the manipulation ((0-1) and (1-1), both P<0.5, Tukey test). These slight underestimations of travel distance correspond to those observed with training in the artificial hill array.

**Immobilising body parts**

There are two major differences between the shaving of hair plates and the immobilisation of body segments. First, the hair plates associated with immobilised body parts will constantly signal the particular fixed body posture, while signalling is absent or corresponds to a “null” position after shaving. Second, the immobilisation of body parts - here head and petiole-gaster - is reversible. This expanded the test runs to be performed for any given ant by another two: outbound journey, manipulated – homebound journey, normal (1-0), and outbound journey, normal – homebound journey, normal (0-0)a (in addition to the usual 0-1 and 1-1 tests). That is, the animals were tested right after removal of the bee’s wax-resin glue at the feeding site (1-0) and later, after they had once again re-emerged from the nest and travelled to the feeder (0-0a). Unfortunately, ants with their head fixed to the alitrunk could not be tested right after this manipulation, even when care was taken to adjust the head in a fairly normal post-
ture. They were confused, had problems to clean their antennae with the comb on their front legs, and thus refused to accept a food crumb. Carrying food in their mandibles, however, is evidence for the ants’ normal motivation to carry their booty home (Wehner, 1982), and was thus a prerequisite for recording homing distances (see Materials and Methods). Nevertheless, the operated animals appeared one day later from the nest with their heads still immobilised, now apparently without problems, and travelled to the feeder. The 1-1 test series could now be performed, though without the normally preceding 0-1 test run. No differences were observed in the homing distances between these tests and the subsequent 1-0 and 0-0a tests (Fig. 6 C). In all these tests the ants appeared to slightly underestimate ground distance, instead of overestimating it due to the much longer outbound walking distance of 10m across the artificial hills array (see above).

Animals that had their gaster fixed in an extended position (180°) were tested immediately after the gaster had been glued to the alitrunk at the feeder. These ants, too, slightly underestimated their homeward travel distance on level ground. In the subsequent tests (1-1; 1-0; 0-0a), they searched even closer to the release point than right after the manipulation (0-1) (differences significant in (1-0) and (0-0a), compared to (0-1), P<0.05, Tukey test). The search density distributions in Fig.6 A (upper panel) demonstrate that this undershot was quite distinct, the distribution of test 0-1, by comparison, showing a consistent shift toward larger distances.

The most striking results were observed in ants that had their gaster fixed in an erect position (<90°). Ants that had their gaster immobilised during outbound as well as homebound runs (1-1) significantly underestimated homing distances, both after training in the horizontal channel and in the artificial hill array (P<0.05, Tukey test) (see Figs 6 C and 5 C). Very distinct displacements of the search density distributions towards the shorter homing distances were observed in these experiments (Figs 6 C and 5 C, upper panels). This held true to some extent even after removal of the immobilising glue before the animals started their homebound journey (1-0) (see Fig. 6 C). This pronounced underestimation in the (1-1) and (1-0) tests of the artificial hill training and in the (1-1) tests of the flat channel training were conspicuous indeed, particularly when considering the rather consistent results of all the other experiments.

The above results are summarised in Tab. 1.
Discussion

Path integration on slopes, and graviception

One might expect that, after elimination of the hair plates between adjacent body segments that are presumed to function as their sensory system for graviception (Markl, 1962), ants foraging in the artificial hill channel array have no perception of the surmounted up- and downward slopes any more. They should thus perform homebound journeys in the flat test channel that correspond to the actual walking distance of 10 m of their outbound journey in the artificial hill array (in the critical 1-1 tests). At the very least, one would expect the animals to extend their search for the nest entrance well beyond the base line distance of 6 m. Contrasting with this expectation, the experimental animals consistently performed homebound journeys that corresponded to the ground level distance of their outbound journeys. In several instances the ants actually searched for the nest entrance at even shorter distances from the release point. This was true for both experimental interferences, shaving of the hair fields and immobilisation of the body parts monitored by these position sensors (summary in Tab. 1). These results demonstrate that operated animals were still able to perform (almost) correct path integration during their up- and downhill outbound journeys.

This result is surprising when considering the facts that *Cataglyphis* ants usually keep their gaster in an upward (erect) position (see Fig. 2 A), and that, due to their long legs and elevated centre of mass, the gaster would suggest itself as a major means of detecting terrain slope. More significantly, Markl (1962) has implied the hair sensillae in the neck and petiole regions as important sensors for graviception.

In the above experiments, the hair plates associated with the legs and antennae remain as possible sensors of the slopes of ascent and descent during outbound travel, of course. Unfortunately, the hair fields of coxa and trochanter are not accessible for operation or immobilisation, at least not without incapacitating the animals seriously and thus preventing further homing experiments. Operating the antennae has similarly severe effects. However, Markl (1962) suggests that the neck joint and the alitrunk-petiole-gaster joints are of particular importance to graviception and slope detection. At the very least, the relative contributions to graviception of these hair plates and those associated with the legs are in the same order of magnitude and cannot completely replace each others’ function. Thus, the hair plates remaining on the legs do not appear to be responsible for the mostly unaffected path integration in uneven terrain. Wohlgemuth and
co-workers (2002) further showed that additional load applied on the alitrunk did not induce a misjudgement of base line distance, and thus presumably of the slopes of the hills, in a very similar experimental set-up. The authors conclude that the artificial load could only have affected the legs, and thus the hair sensillae associated with the joints between leg segments, and could not have impaired other body parts such as head, petiole and gaster. Hence, the detection of slopes must have remained intact with altered leg load and slightly changed leg coordination.

In summary, it appears unlikely that terrain slope is measured by desert ants through sensory systems employed in graviception, namely, hair fields associated with neck and petiole, but also those associated with the legs. At least these organs are dispensable for 3D path integration and their loss can be compensated. It remains to be examined whether the ants use different sensory modalities altogether, such as vision and the detection of sky light polarisation, to assess the slope of their walking substrate.

Possible reasons for underestimation of homing distance

Apparent underestimation of homing distance was observed in several of our experiments (see e.g. Figs 4, 6; summary in Tab. 1). Modest undershoot may in fact represent an element of homing strategy, rather than a genuine mistake in distance estimation. Starting to search for the nest after a homing distance that is shorter than the actual estimate by the average margin of error will lead the animal into the familiar side of the nest surrounds, rather than into unfamiliar terrain past the nest (see also Sommer and Wehner, 2004; Wolf and Wehner 2005). Such undershoot in the range of about 5-10% of homing distance is thus frequently observed in homing experiments, not just with desert ants (Sommer and Wehner, 2004). This effect may become more pronounced in cases where the animals experience disturbances or are even disabled. In such cases it may be advantageous to opt for more safety at the expense of homing speed. According to this line of argument, a more severely impaired ants should exhibit shorter homing distances, at least within the short-distance ranges examined here (6-10m, compared to more than 100m in some recorded foraging runs; for these distances this argument may not be valid any more).

Gluing the gaster to the alitrunk in an (extremely) erect position (<90°) had the most noticeable effects on homing distance, both in the up- and downhill channel training and in the flat channel training (Figs 5 C; 6 C). The undershoot was so pronounced in these experiments that the above general explanation for shortened homing distances appears
very unlikely here. At the same time, this manipulation had the largest impact on body posture, as the position of an ant’s centre of mass is influenced noticeably by fixing the heavy gaster onto the alitrunk (see Fig 2 D) (Zollikofer, 1988). In the artificial hill training, these animals underestimated their travel distance considerably, by an average 75.6 %, when tested after they emerged with their fixed gaster at the feeding site (1-1). This held true when the manipulation was reversed at the feeder and the animals were tested again, although the undershoot was somewhat smaller (50 %) (1-0) (Fig. 6 C). Intriguingly, the ants also misgauged their travel distance in the flat channel (control) experiment, though only when the gaster was fixed during both outbound and homebound runs (1-1). They did not undershoot when the manipulation was reversed before the homebound run was performed (1-0) (Fig. 5 C).

Outbound journey with the gaster fixed in an extreme erect position thus seems to be the major determinant in misgauging homing distance in these experiments. Only speculations are possible at present regarding possible reasons for these observations.

References


Fig. 1. Manipulated hair plates. (A) Schematic drawing of a *Cataglyphis* worker ant, lateral view. The positions of the manipulated hair plates are indicated (red rectangles); (a) neck joint, (b) alitrunk-petiole joint, (c) petiole-gaster joint. Drawing adapted from Wehner (1983). (B) Scanning electron micrograph (SEM) of the petiole-gaster joint; ventral view. Hair plates are in the centre of the image, not manipulated. (C) SEM of the petiole-gaster joint, ventral view, the hair plates had been shaved with a razor splinter (one of the experimental animals that had contributed to the data in Fig. 4 B).
Fig. 2. Immobilisation of body parts. (A) Body posture in walking ants, *Cataglyphis fortis*. (B) Animal with its head fixed to the alitrunk. (C) Gaster (metasoma) fixed to the alitrunk in an prolate position of approximately 180°. (D) Gaster fixed onto the alitrunk in an erect (<90°) position. Drawing adapted from Wehner (1983). Bees’ wax-resin glue indicated in red.
Fig. 3. Experimental situation and channel layout. Channel cross section, top left; single channel segment for hill training, top right; hill training and flat training channels, middle; test channel, bottom. The search behaviour exhibited by *Cataglyphis fortis* foragers after having run off their home vector is illustrated schematically below the test channel, search trajectory. Not drawn to scale.
Fig. 4. Training across the artificial hill set-up (compare Fig. 1, middle), experimental data I. The top panels show search density plots (abscissae, homing distances; ordinates, cumulated relative search densities between the first and sixth turning points; see Materials and Methods). The bottom diagrams display box-and-whisker plots (medians of the initial six turning points), derived from the same data sets (n = 15-20 ants for each experimental situation). 0-0, 0-1 and 1-1 are double-digit codes for the particular test situation, the first digit represents the outbound run and the second digit, the homebound run; 0 denotes the normal situation, 1 the manipulated condition. Left column (A), animals with the hair plates in their neck region shaved. Middle column (B), animals with the hair plates in the petiole-gaster region shaved. Right column (C), animals with both, neck and petiole-gaster regions shaved.
Fig. 5. Training across the flat channel set-up, experimental data. Diagrams and details of presentation are the same as described in the legend of Fig. 4. Left column (A), animals with the hair plates in their neck region shaved. Middle column (B), animals with the hair plates in their petiole-gaster region shaved. Right column (C), animals with their gaster fixed in an erect (<90°) position.
Fig. 6. Training across the artificial hill set-up, experimental data II. Diagrams and details of presentation are the same as described in the legend of Fig. 4. Left column (A), animals with their gaster fixed in a prolate (ca. 180°) position. Middle column (B), animals with their head fixed in a normal position. Right column (C), animals with their gaster fixed in an erect (<90°) position.
Table 1. Statistical data of all experimental groups including all test situations.

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<th>IQR [m]</th>
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Mein ganz besonderer Dank gilt Herrn Prof. Dr. Harald Wolf für die Idee zu diesem Projekt, die intensive Betreuung und die ständige Hilfs- und Diskussionsbereitschaft während der gesamten Zeit. Er hat mir die Freiheit gelassen eigene Ideen zu entwickeln und diese weiterzuverfolgen und war dabei immer ein inspirierender Schatz an experimentellem Wissen.

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mich verzichten, wenn ich die Sommer in Griechenland oder Tunesien bei den Freilandexperimenten verbrachte.
Curriculum vitae

Persönliche Angaben
Name Matthias Wittlinger
Geburtsdatum 05. Dezember 1974
Geburtsort Kirchheim unter Teck
Nationalität Deutscher Staatsbürger
Familienstand verheiratet seit April 2002

Schulbildung
1981 - 1985 Grundschule Boll
1985 - 1995 Hohenstaufen Gymnasium Göppingen

Wehrdienst
1995 - 1996 Wehrdienst bei der Luftwaffe im Jagdbombergeschwader 34 Allgäu, Memmingerberg

Studium
1996 - 2003 Studium der Biologie an der Universität Ulm
Thema der Diplomarbeit „Untersuchungen an bewegungssensitiven Neuronen in der Lobula der Honigbiene (Apis mellifera)“
2003 - 2006 Arbeit an der Dissertation in der Abteilung Neurobiologie an der Universität Ulm
Eidesstattliche Erklärung

Hiermit erkläre ich, die vorliegende Dissertationsarbeit selbständig angefertigt und keine anderen als die in der Arbeit aufgeführten Hilfsmittel verwendet zu haben. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Ulm, den 1. Juni 2006