

Desert ants do not rely on sky compass information for the perception of inclined path segments

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SUMMARY

On their foraging excursions, desert ants (*Cataglyphis fortis*) navigate by means of path integration, exhibiting high precision even in undulating terrain. To avoid errors in their home vector the ants must measure the slopes of ascents and descents in their itinerary. This information is necessary to convert the distances actually walked on the slopes into the ground distance, which is the crucial parameter for vector navigation. However, it is as yet unknown how *Cataglyphis* perceives the slopes of inclines. We asked whether the ants gauge slopes by means of the changes in the sky's polarization pattern or the sun's position imposed by the unusual head posture when walking on inclines. During training on a ramp, we manipulated the sky compass cues available for the ants. If both sky compass cues, polarization pattern and sun position, were excluded during ramp training, the ants behaved in subsequent tests as if they had never encountered a slope. However, the presence of either of the two compass cues was sufficient for the ants to memorize the sloped path segment. In a third experiment, the supposed polarization cue was excluded by covering the training and test channels with a linear polarization filter. In these tests the ants clearly showed that they had perceived and memorized an ascent also without a change in the polarization pattern. Hence, changes in polarization perception can be ruled out as the crucial cue for gauging slope, and we conclude that slopes are monitored by a, still unknown, proprioceptive mechanism.

Key words: *Cataglyphis*, ants, path integration, vector navigation, 3-D orientation, Hymenoptera.

INTRODUCTION

Social insects like ants, bees and wasps must solve the problem of finding their way back to their nest after extended foraging excursions that may cover some hundred-thousand body lengths (von Frisch, 1965; Wehner and Wehner, 1990). A solution that suggests itself is 'route following', i.e. to mark the outbound trail, e.g. by pheromones, and then to trace these marks back to the starting points (for reviews, see Wehner and Wehner, 1986; Collett and Collett, 2002; Wehner and Srinivasan, 2003; Collett et al., 2006; Ronacher, 2008; Cheng et al., 2009). However, many insects use a different strategy, which is termed 'path integration' or 'vector navigation'. In the path integration process, the animals continuously combine information about their movement directions and travelling distances into a 'home vector' pointing to the starting point of the excursion, usually the nest (Müller and Wehner, 1988). A characteristic sign of this type of navigation is the observation that an animal returns to its nest on a fairly straight course that differs completely from its often meandering outbound path [see fig. 4 in Wehner and Wehner (Wehner and Wehner, 1990)].

Desert ants of the genus *Cataglyphis* are champions in path integration (Wehner and Wehner, 1986; Wehner and Wehner, 1990; Wehner, 1994b; Wehner, 2003). To compute their home vector, these animals assemble directional and distance information by compass and odometer modules, respectively. Ants can use different compass cues; however, the dominant one is the sky's polarization (POL) pattern (Wehner and Müller, 2006) [reviews on the polarization compass (Wehner, 1994a; Wehner, 1997; Homberg, 2004; Wehner and Labhart, 2006; Heinze and Homberg, 2007)]. The odometer was for a long time an enigma, until in 2006 Wittlinger and colleagues convincingly demonstrated

a stride integrator in *Cataglyphis fortis* (Wittlinger et al., 2006; Wittlinger et al., 2007a). Remarkably, *C. fortis* were found to perform precise path integration even when walking over hills (Wohlgemuth et al., 2001; Wohlgemuth et al., 2002; Grah et al., 2005). To solve this problem, the ants must be able to measure the slopes of ascents and descents with sufficient precision and to use this information to convert their actual walking distance into the ground distance, i.e. the projection of the slopes on the horizontal (Wohlgemuth et al., 2001; Wohlgemuth et al., 2002; Grah et al., 2005). However, it is still not clear how the ants do measure the slopes of inclined path segments. Experiments, in which hair plate sensors at different joints were immobilized or shaved, so far did not reveal conclusive clues to the mechanism(s) of slope perception (Wittlinger et al., 2007b) (see also Seidl and Wehner, 2008). However, recent investigations on the kinematics of locomotion on inclines found that although the ants showed some adjustment of their posture while walking on inclines, the compensation was far from being complete (T. Weihmann and R. Blickhan, personal communication). In particular, the angle between the head axis (measured from the mandibles to the neck) and the horizontal plane changed drastically. In flat terrain, the angle between the head axis and the ground was ~30 deg. whereas it shifted to ~165 deg. for a 60 deg. ascent (now the mandibles were pointing slightly upwards). By contrast, during a 60 deg. descent, the head axis was kept in an almost vertical position (the mandibles were pointing to the ground) (T. Weihmann and R. Blickhan, personal communication). These massive changes in head posture probably change the perception of the POL pattern of the sky, the sun position and the horizon. These observations were an incentive to ask whether the ants might derive essential

information about the slope of an ascent or descent from the corresponding changes in the perceived visual information, in particular of the sky's POL pattern.

We performed three types of experiments. In a first paradigm, we trained ants either in a flat horizontal channel to a feeder on ground level or *via* a steep ramp to an elevated feeder (see Fig. 1). The sight of sky compass cues could be prevented on the ramp. Then we tested whether or not the ants accepted a descent slope on their homebound path. These tests were guided by the observation that ants trained in a flat horizontal channel consistently refused to walk on an inclined test ramp (Grah et al., 2007; Grah and Ronacher, 2008), while after ramp training ants readily accepted the test ramp. Hence, by the acceptance or rejection of a test ramp we can infer whether or not ants incorporated the ascent part of their foraging excursion into their route memory. In a second paradigm, we trained ants to walk over an artificial hill with a steep ascent and descent to reach a feeder on ground level. While crossing the hill, various types of visual information were withheld: either direct sight of the sun, perception of the POL pattern or both. Again acceptance or rejection of test ramps was recorded. For a third training paradigm, we used ramp or flat training (as in the first experiment) but now the ants experienced only a single direction of polarized light during their foraging excursions; the horizontal as well as the ramp parts of the channel were covered by a POL transparency that filtered out all but one e-vector direction. Hence, the ants could not notice any difference between the POL pattern in the horizontal and the ascending parts of the channel. The expectation was that this manipulation should influence the acceptance of slopes if under normal conditions slope perception depends mainly on the changed perception of POL patterns caused by different head inclinations.

MATERIALS AND METHODS

Experimental setup

Experiments were performed from early July until early September in 2006 to 2008 on desert ants (*Cataglyphis fortis* Forel 1902) in their natural habitat, a saltpan area at 34.52 deg.N, 10.53 deg.E, near Maharrès, Tunisia. The ants belonged to 12 different nests. Except for one control experiment (see Results), each animal was tested only once.

Training and testing took place in aluminum channels (width and height of side walls: 7 cm) (see Grah et al., 2005). The channels were positioned in an East–West direction for experiments 1 and 2 and in a North–South direction for experiment 3. A plastic enclosure surrounded the nest entrance and guided foraging ants into the training channel. Fine gray sand was glued to the channel floor in order to increase grip while walking. The inner side walls were painted a matt gray to prevent possibly irritating reflections from metallic surfaces. The upper ends of the walls were covered with smooth adhesive tape in order to impede escape attempts. The channels provided no visual landmarks or optic flow cues. Ants were trained to visit a feeder filled with biscuit crumbs. In experiments 1 and 2, they were tested on their way home from the feeder towards the nest. In experiment 3, they were tested on their outbound way. Ants were individually marked with acrylic paint (Motip Dupli GmbH, Haßmersheim, Germany) with a three dot color code on their thorax and gaster in order to count visits to the feeder and to exclude repeated testing.

Experiment 1

Different groups of animals underwent three different training procedures: (1) the 'open ramp training' was performed in a 5 m horizontal channel that led into a steep 1.5 m ramp (70 deg.

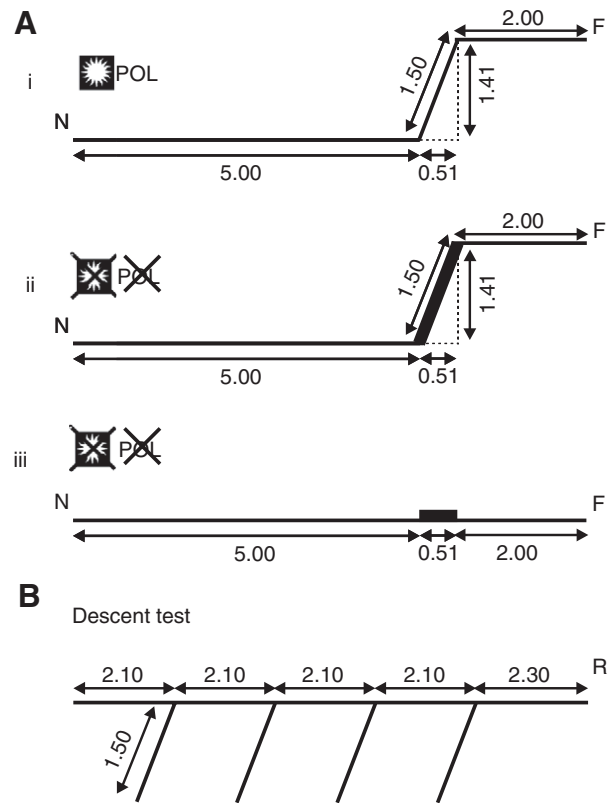


Fig. 1. Schematic side view of the setup used in experiment 1. Three different training channels: (Ai) open ramp training with open view of the sky; (Aii) closed ramp training with ramp covered with orange Perspex and cardboard shield, no sky compass cues available on the ramp; (Aiii) flat training with open view of the sky, except for a short segment that was covered with orange Perspex and cardboard shield (see pictograms). (B) Schematic drawing of the test channel system with four options for a descent. N, nest; F, feeder; R, release point; POL, polarization. Dimensions in meters. The angle of the ascent was 70 deg.

inclination) followed by an elevated horizontal 2 m channel (the total ground distance from the nest to the feeder was thus 7.51 m) (see Fig. 1Ai). (2) A 'closed ramp training' system was identical to the first system except for the fact that the ramp was now covered by orange Perspex (Ronacher et al., 2006) in order to eliminate cues of the polarization compass, which are perceived *via* the UV-receptors of the dorsal rim area (Duelli and Wehner, 1973; Labhart, 2000). Direct view of the sun was also prevented by attached cardboard (see Fig. 1Aii). (3) A third training paradigm ('flat training') consisted of a horizontal channel of 7.5 m length with a 0.5 m piece being covered by orange Perspex and shielded from direct view of the sun (see Fig. 1Aiii). For testing, individual ants were transferred from the feeder into an open test channel, which was laid out in parallel to the training channel. The test channel offered four 'decision points' at which the ant could either continue to walk horizontally or descend on a ramp (Fig. 1B). All ramps used in tests were 1.5 m long and had a slope of 70 deg. [for details of the apparatus, see Grah et al. (Grah et al., 2007)]. In the test channel system, the ants had free view of the sky (with the restrictions imposed by the channel's side walls). As also observed by Grah et al. (Grah et al., 2007), most ants used the first two ramps to attempt a descent (67% on ramp No. 1, 25% on ramp No. 2).

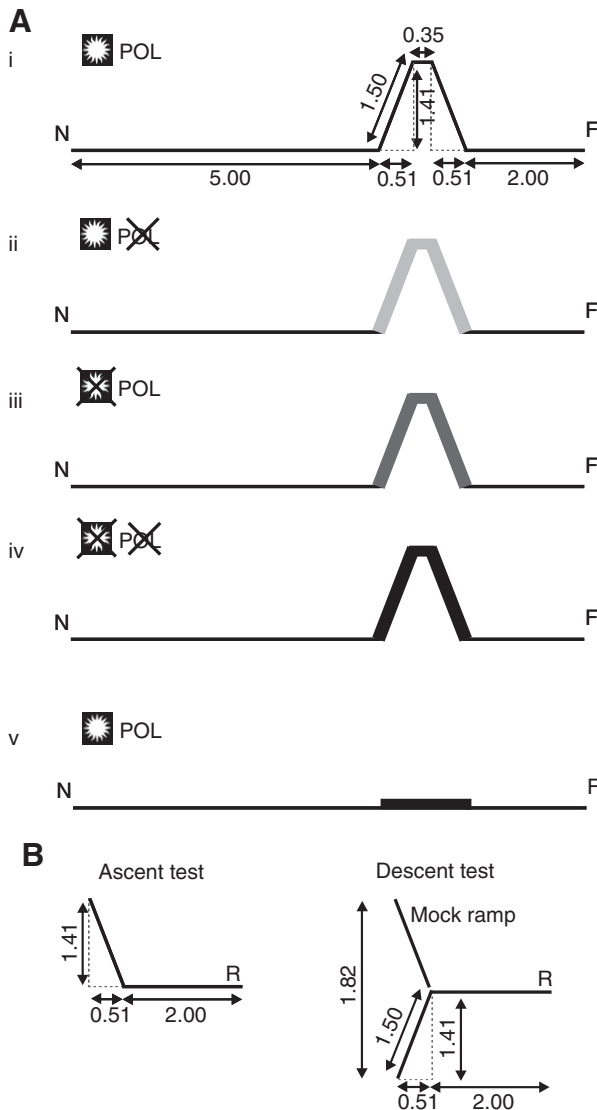


Fig. 2. (A) Schematic drawing of the training channels used for experiment 2 ('hill' training). (B) Side view of the test channels. (i) Unimpeded view of the sky (except for the restrictions imposed by the channel's side walls); (ii) blocking of the sky's POL pattern (via orange Perspex) with view of the sun still possible; (iii) access to the POL pattern but blocking of the sun by means of a cardboard shield; (iv) complete blocking of sky compass information (via orange Perspex and exclusion of direct view of the sun); and (v) as a control, a fifth group of ants underwent a flat training with open view of the sky, except for a 1.37 m segment covered with orange Perspex. N, nest; F, feeder; R, release point; POL, polarization. Dimensions in meters. The angle of the ascent was 70 deg.

Experiment 2

In this experiment, ants had to cross a 1.41 m high 'hill' in order to arrive at the feeder (see Fig. 2 for the dimensions of this channel system; ground distance between nest and feeder was 8.37 m). Different groups of ants experienced four different training conditions on the hill segment of the channel, which were designed to disentangle different celestial compass cues: (i) unimpeded view of the sky (except for the restrictions imposed by the channel's side walls); (ii) blocking of the sky's POL pattern (via orange Perspex) with view of the sun still possible; (iii) access to the POL pattern but blocking of the sun by means of a cardboard shield; (iv) complete

blocking of sky compass information (via orange Perspex and exclusion of direct view of the sun); and (v) as a control, a fifth group of ants underwent a flat training with open view of the sky, except for a 1.37 m segment covered with orange Perspex (Fig. 2Ai-v). For this experiment, we modified the test paradigm. Ants of all training conditions were tested on their way home for their acceptance of ramps in two ways; either they were captured at the feeder and transferred to a flat 2 m channel segment that led into a 1.5 m ramp (70 deg. ascent) or transferred to an elevated horizontal channel that led into a steeply descending ramp (70 deg. descent). In order to provide a similar visual surround as on the homebound path during training, a mock ramp was adjusted over the onset of the descent (see Fig. 2B). Different groups of ants were tested for the acceptance of ascents or descents.

Experiment 3

In order to manipulate the POL pattern visible for the ants, we used a POL transparency (HN38 Polarisationsfolie linear, 0.3 mm; Fa. ITOS GmbH, Mainz, Germany), which produces linearly polarized light also in the UV range of the spectrum. The curve for crossed filters demonstrates that the efficiency of the filter is also high in the UV range (Fig. 3B). This is the relevant part of the spectrum that *Cataglyphis* uses for its compass (Duelli and Wehner, 1973; Labhart, 2000).

Two groups of ants underwent different training conditions: flat training and ramp training. For technical reasons, in these experiments, ants had to be tested on their outbound foraging excursion, and the channel's orientation was to the South. The arrival of individually marked ants at the feeder was noted and individuals had to be sighted at least five times at the feeder before they were tested in the (crucial) flat training paradigm. For the ramp training (see below) the criterion was relaxed to at least three sightings at the feeder; however, most animals had visited the feeder more often (mean number of visits \pm s.d., 7.7 ± 4.0).

In the flat training paradigm, ants first crossed a distance of 1.3 m with open view of the sky, thereby passing a 'valve' on the way that allowed to guide single individuals into the parallel test channel. Then they walked for 3.5 m to a feeder in a channel covered with a POL transparency (Fig. 3A). In the training and test channels, the orientation of the e-vector was always perpendicular to the channel's length axis thus mimicking a course in the direction of the solar/antisolar meridian. The channel's orientation was to the South in order to exclude the direct view of the sun during the afternoon by wooden plates (50 cm height). Training began at 14:30 h when the training channel was completely shaded. Testing began at 15:30 h when the parallel test channel and its ramp were shaded. The test channel was laid out in parallel, at 10 cm distance, and was connected to the training channel via a switch. The test channel consisted of a 2.5 m flat part followed by a 1.5 m ramp (70 deg. inclination). In a second version of this paradigm (used in 2008 testing), the ramp in the test channel began after a 2 m flat part (see Results for the rationale). The horizontal and ramp segments of the test channel were both covered with POL transparency in the same orientation as in the training channel. In the 2008 training, a mock ramp was erected over the training channel, at the position of the test ramp, in order to get the animals accustomed to the approach towards a ramp that could be interpreted as landmark. As a control, ants were also tested for the acceptance of a ramp on their homebound runs. To this aim ants were captured at the feeder and transferred to a parallel test channel located at 5 m distance. This test channel began with a 2 m horizontal channel at 1.41 m height, followed by a steep descent (1.5 m ramp, 70 deg. inclination). A 1 m

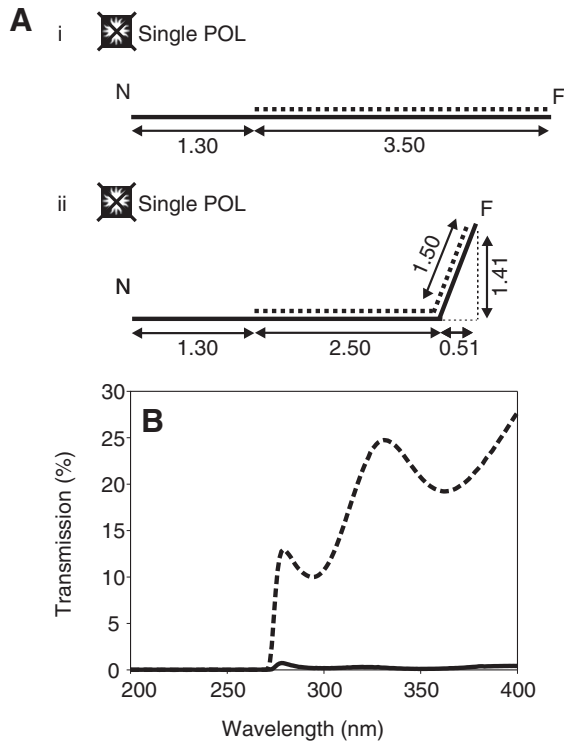


Fig. 3. (A) Training channels used in experiment 3: (i) flat training and (ii) ramp training. Broken line over the channel indicates cover with POL filter transparency. The test channel had the same dimensions as shown in ii and was laid in parallel to the training channel at a distance of 10 cm. Ants could be guided individually into the test channel via a switch. (B) Transmission of the POL filter in the UV range of the spectrum. Note the near zero curve for crossed filters, demonstrating high filter quality. N, nest; F, feeder; R, release point; POL, polarization. Dimensions in meters. The angle of the ascent was 70 deg.

part of the horizontal channel and the ramp were also covered with POL transparency. Ants were released at a distance of 1.25 m from the descent and could walk for 25 cm with open view of the sky before entering the POL part of the channel. In the ramp training paradigm, the ants again had to pass the switch to enter the POL channel [2.5 m flat and 1.5 m ramp (70 deg. inclination)]. The channel for the outbound tests was connected *via* a switch to the parallel training channel, run in 10 cm distance, and had the same dimensions as the training channel.

Statistical analysis

Frequencies of animals choosing to descend or ascend were analyzed using the χ^2 homogeneity test. The lengths of descents and ascents following the different training paradigms were compared for each experiment using the Kruskal–Wallis H-test. Differences between pairs of sample groups were localized using the Games–Howell *post-hoc* test for pairwise comparisons. All statistical analysis was carried out using SPSS 2003 software for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Experiment 1

A first group of ants was trained to the elevated feeder *via* a ramp that allowed free sight of the sky. Forty-four out of 46 of these ants showed a fast, unhesitating and complete (1.5 m) descent on one of the test ramps. By contrast, after flat training most ants turned around

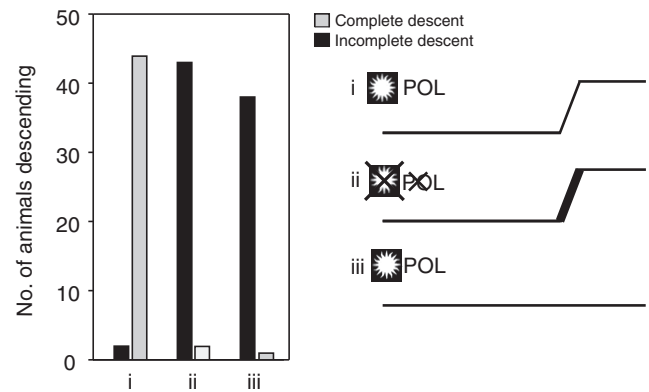


Fig. 4. Proportion of complete and incomplete descents (i.e. rejections of the ramp) after ramp training. Ants that had no sky compass cues while on the ramp (ii) behaved like ants after flat training (iii). The drawing on the right gives a simplified sketch of the training conditions (for details, see Fig. 1). Proportions of incomplete *versus* complete descents in ii and iii were not significantly different; however, both were different from i ($P \ll 0.001$). Pol, polarization.

on the test ramp after some 20–50 cm of descent, thereby confirming earlier results (Grah et al., 2007). How would the ants perform in the crucial experiment if during training the sky compass cues were absent on the ramp? As evident in Fig. 4, these animals behaved as if they had experienced a flat training, only 2 out of 45 showed a complete descent (compare ii with iii in Fig. 4). Note that in the test channel system the ants had normal view of the sky! The proportion of descents was not significantly different from the flat training.

Experiment 2

With the next series of experiments we tried to disentangle the respective influences of the two major sky compass cues: sun position and POL pattern (cf. Wehner and Müller, 2006). In this experiment, ants were trained to walk over a hill before arriving at the feeder situated at ground level (see Fig. 2 for details of the training paradigm). After this training ants accept both ascending as well as descending ramps on their homebound way (Grah et al., 2007; Grah and Ronacher, 2008). In addition to the two control trainings and the training under exclusion of POL and sun position information, animals had to traverse the hill with sight of the sun but without POL pattern or with POL pattern present but direct view of the sun excluded (see descriptions of training procedures in Fig. 2 legend).

In separate tests the acceptance of ascending ramps or descending ramps was recorded; again the test ramps were not covered so that the ants had a normal view of the sky during testing. Shown in Fig. 5A are the proportions of complete (gray columns) *versus* incomplete ascents (black columns). As in the first experiment (Fig. 4), the complete absence of compass cues on the ramp led to a predominant rejection of ascents, only 5 of 25 animals climbed the whole test ramp up to 1.5 m (see iv in Fig. 5A). The proportion of ascents was not significantly different from the flat control ($P=0.18$; Fisher's exact test) whereas it differed significantly from the three other conditions ($P < 0.001$; χ^2 test). If only one of the two possible sky compass cues was present, the majority of ants accepted the ascent and performed complete ascents (the proportions do not differ from the 'open' control training nor between the two experimental conditions ($P > 0.2$; χ^2 test). The respective ascent heights are shown in Fig. 5B. For the three hill training paradigms,

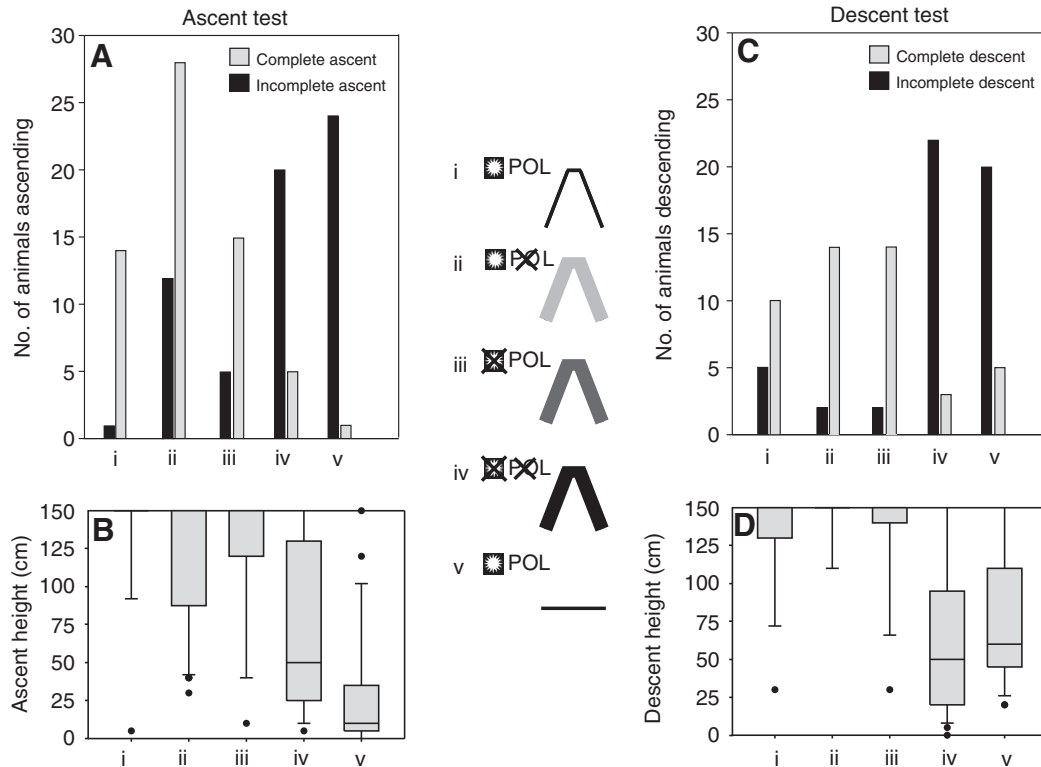


Fig. 5. Results of ascent and descent tests after hill training. (A) Proportions of complete versus incomplete ascents; (C) proportions of complete versus incomplete descents; (B,D) ascent and descent heights, respectively. Different training conditions are indicated in the sketches between A and C, for details of the training see Fig. 2. The proportions of complete versus incomplete ascents in A were not significantly different between training i,ii,iii ($P>0.2$; χ^2 test) and were not significantly different between training iv and v ($P=0.18$; Fisher's exact test). However, i-iii versus iv and v differed highly significantly ($P<0.001$). (B) A similar picture was found for the ascent heights using the Games-Howell *post-hoc* test: i-iii, $P>0.7$; iv compared with v, $P=0.03$; however, $P=0.14$ if the complete ascents were not considered. i-iii versus iv and v, $P=0.003$. (C) Proportions of complete and incomplete descents. i-iii, $P=0.38$; iv versus v, $P>0.7$; i-iii versus iv and v, $P<0.0001$. (D) Descent heights. i-iii, $P>0.7$; iv versus v, $P>0.8$; i-iii versus iv and v, $P<0.0001$. Closed circles, outliers.

with at least one sky compass cue present, the median ascent heights were uniformly 1.5 m ($P>0.7$) and differed significantly from the two other conditions ($P=0.003$; Games-Howell *post-hoc* test). The ascent heights shown after flat training and training on the hill without compass did differ significantly (compare v with iv in Fig. 5B; $P=0.03$). However, this difference disappeared if the animals with complete ascents ($N=5$ and 1) were excluded from the analysis. Then the median ascent height in Fig. 5iv shifts to ~ 35 cm and also the quartile ranges are reduced (compare v with iv, $P=0.14$; Games-Howell *post-hoc* test). Other groups of animals were tested for their acceptance of descents (Fig. 5C,D). These data confirm those of Fig. 5A,B. The proportions of complete descents were uniformly high if at least one sky compass cue was present during hill training (Fig. 3C) ($P=0.38$) and the descent heights did not differ significantly ($P>0.7$; median 1.5 m). By contrast, the absence of both compass cues (Fig. 5Civ) led to a clear rejection of descents – the ants now behaved as after flat training (compare iv with v: $P>0.7$ for proportion of descents and $P>0.8$ for descent heights). However, these two groups differed highly significantly from the other three training situations ($P<0.0001$) in both the proportion of complete descents and descent height.

To summarize these results, *Catalgypis* seems to ignore the ramp parts of their itinerary if deprived of all sky compass cues. If only one of the two main cues is present, sight of the sun or of the POL pattern, this is sufficient for the animals to remember the slopes and exhibit the appropriate behavior in tests. However, it would be premature to conclude from these results that the ants specifically

rely on the POL pattern (or sun position) for the perception of slopes. Rather, when updating their home vector, ants seem to generally ignore those segments of their path for which there is no simultaneous input of the sky compass. This omission of path segments in the absence of sky compass information has been shown previously in a linear channel arrangement (Sommer and Wehner, 2005) as well as in a two-dimensional training situation (Ronacher et al., 2006). Hence, the above results do not allow for an unequivocal conclusion.

Experiment 3

We therefore modified the first experiment into a training situation in which the animals still had input to their POL compass but would not experience a change of the POL pattern while climbing a ramp. This was achieved by covering the training and test channels with a polarization transparency (see Materials and methods). Preliminary tests had shown that the ants do not ignore the path segments under a single linear POL direction; they behave differently compared with a situation in which sky compass cues are completely absent. As the transparency produced a linear polarization of the light, the ants experienced a single POL direction both in the horizontal and the ascending parts of the training or test channels. As before, two training paradigms were compared, training in a flat horizontal channel and training over a ramp leading to an elevated feeder and, again, the acceptance of an ascent was used as criterion. Note that now the flat training is the crucial one, leading to the following prediction: if the perception of the slope would normally depend

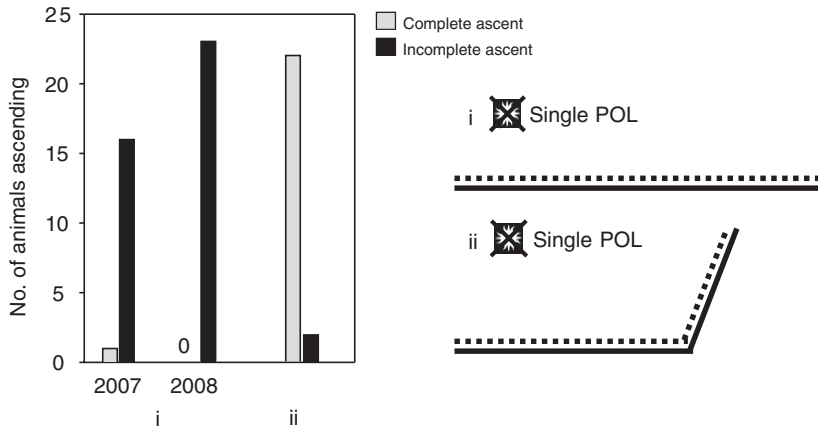


Fig. 6. Results of training under a linear POL (polarization) filter, i.e. with a single e-vector direction, and under the exclusion of the direct view of the sun. After flat training (i) the ants refused to climb on the ramp, contrary to the expectation of the POL hypothesis whereas they readily accepted the ramp after ramp training (ii); i versus ii, $P < 0.0001$.

on a shift of the POL pattern induced by a change in head inclination, ants that had experienced a flat training should now willingly ascend the test ramp covered with POL transparency. Due to the single uniform POL direction present in the test channel, the hypothetic cue for the detection of the slope, i.e. a change of the POL pattern, would now be absent on the test ramp.

Contrary to this prediction, in the flat training paradigm of 2007, only one individual out of 17 ants performed a complete ascent. Most animals performed their first U-turn within a few centimeters after the ramp onset (mean ascent height 24.4 ± 52.8 cm, median 0 cm) (see Fig. 6). In the flat training paradigm of 2008, the test ramp was positioned nearer to the nest to exclude a possible underestimation of the feeder distance, which could have caused the rejection of ascents. In addition, a mock ramp was erected over the flat training channel to make the visual experience of approaching a ramp as similar as possible between training and test situation. These modifications, however, did not change the results at all. Out of 23 animals tested, none climbed the ramp to its full height (Fig. 6), the two highest turning points were 1.15 and 1.25 m whereas 17 individuals turned around within the first 10 cm of the ramp (mean ascent height 22.4 ± 39.4 cm, median 0 cm). The next day, 13 of these 23 ants could be tested again on their homebound way, whether they would accept a descent (see Materials and methods). Only two showed a complete descent (1.5 m), one individual turned around at -40 cm, 10 others turned at the onset of the ramp or within the first 10 cm (mean descent height 27.7 ± 55.3 cm, median 5 cm). This result nicely confirms the result from the outbound foraging excursions, i.e. the rejection of ramps after flat training. The ramp training under constant POL condition resulted in a strongly contrasting picture, as expected on the basis of the observations of Grah and colleagues (Grah et al., 2007). Again ants were tested on their outbound excursions. After ramp training, 22 out of 24 individuals climbed the ramp to the top, without hesitation, only one individual turned at the foot of the ramp and another at 1.15 m. The proportion of animals that fully accepted the ramp (22/2) was highly significantly different from that after flat training (1/16 and 0/22; $P < 0.0001$; median ascent height 1.5 m, mean 142.3 ± 31.1 cm, compared with median 0 cm in the other two experiments).

DISCUSSION

Previous experiments in which desert ants were induced to perform foraging excursions over artificial hills (Wohlgegmuth et al., 2001; Wohlgegmuth et al., 2002) had shown that they can derive the ground distance while walking over undulating terrain. The ground distance and not the distance actually walked is incorporated into the home vector with remarkable precision (Grah et al., 2005). To calculate

the ground distance, ants must be able to perceive the slopes of uneven path segments and use this information to correct their odometric distance, which is based on a stride integrator (Wittlinger et al., 2006; Wittlinger et al., 2007a). So far, however, no convincing mechanism has been reported how *Cataglyphis* actually does measure slopes. As energy expenditure has been ruled out as a cue (Schäfer and Wehner, 1993; Wohlgegmuth et al., 2002; Lipp et al., 2005), the most likely hypothesis was that ants use hair fields at various joints to monitor changes in body posture or load distribution (Markl, 1962). However, although Wittlinger et al. (Wittlinger et al., 2007b) eliminated or immobilized a series of hair sensors at different joints, these hair fields appeared not to contribute to, or at least were dispensable for, slope perception. An alternative hypothesis was based on the observation that the head posture of ants changes considerably while ants are walking on an ascent or descent (see Introduction) (Seidl and Wehner, 2008). It therefore seemed conceivable that slopes could be perceived by the changing POL pattern distribution induced by different head postures. The experiments reported in the present study aimed at testing this hypothesis.

The first experiment in which during ramp training all sky compass information was withheld demonstrated that ants behave as if they had never encountered a slope (see Fig. 4ii). At first glance this seems to indicate a strong contribution of sky compass cues to the perception of an inclined path segment. In the second experiment, the two celestial compass cues, POL pattern and sun position, were separated (Fig. 5). These experiments further demonstrated that either of these sky compass cues was sufficient for a correct interpretation of the 3-D architecture of the itinerary and for the integration of slopes into the route memory (cf. Grah et al., 2007; Grah and Ronacher, 2008). However, it would be rash to conclude from these results that the ants specifically rely on the POL pattern (or sun position) as the major cue for the perception of slopes. Previous experiments in the horizontal plane showed that ants seem to generally ignore those segments of their path for the calculation of the home vector for which there is no simultaneous input of the sky compass (Sommer and Wehner, 2005; Ronacher et al., 2006).

This ambiguity was the reason for designing the third experiment, in which (uniform) POL information was still present but there was no change in the POL pattern between the flat and the ramp parts of the channel that could be used to derive slope information. Assuming that the shift of the POL pattern experienced on a slope would be the major clue for the perception of slopes, we postulated that even after flat training an ant should readily ascend the ramp in the test channel covered with POL transparency, as no change in the POL pattern occurred between the flat and the ramp segments

of the channel. This expectation was clearly disproved. After the (crucial) flat training the ants consistently refused to climb on the ramp (Fig. 6), showing that they perceived a slope also in the absence of a usable change in the POL pattern cue. This result is further corroborated by a laboratory experiment conducted by Wohlge-muth et al. (Wohlge-muth et al., 2002). Our original hypothesis therefore can be rejected. Obviously, the change in POL pattern accompanying an excursion on sloped path segments is not the major cue on which the perception of slopes is based. Hence, we must further conclude that *Cataglyphis* relies on some proprioceptive information, even though different manipulations so far could not reveal the crucial ones (Wittlinger et al., 2007b; Seidl and Wehner, 2008).

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