RESEARCH ARTICLE

The polarization compass dominates over idiothetic cues in path integration of desert ants

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SUMMARY

Desert ants, *Cataglyphis*, use the sky's pattern of polarized light as a compass reference for navigation. However, they do not fully exploit the complexity of this pattern, rather – as proposed previously – they assess their walking direction by means of an approximate solution based on a simplified internal template. Approximate rules are error-prone. We therefore asked whether the ants use additional cues to improve the accuracy of directional decisions, and focused on 'idiothetic' cues, i.e. cues based on information from proprioceptors. We trained ants in a channel system that was covered with a polarization filter, providing only a single e-vector direction as a directional 'celestial' cue. Then we observed their homebound runs on a test field, allowing full view of the sky. In crucial experiments, the ants were exposed to a cue conflict, in which sky compass and idiothetic information disagreed, by training them in a straight channel that provided a change in e-vector direction. The results indicated that the polarization information completely dominates over idiothetic cues. Two path segments with different e-vector orientations are combined linearly to a summed home vector. Our data provide additional evidence that *Cataglyphis* uses a simplified internal template to derive directional information from the sky's polarization pattern.

Key words: *Cataglyphis*, polarization compass, e-vector, sky compass, navigation, path integration, idiothetic.

INTRODUCTION

Desert ants (*Cataglyphis fortis*) perform large-scale foraging excursions from which they return to their inconspicuous nest entrances on the shortest path possible, by means of path integration (Müller and Wehner, 1988; Wehner, 2003; Wehner and Srinivasan, 2003). During an excursion, they continuously update a vector that points to their starting location, the nest. To ascertain this 'home vector', an ant must measure the directions of path segments as well as the distances walked in these directions, i.e. ants need a compass system and an odometer. The nature of the odometer, the second ingredient to path integration, remained enigmatic for a long time (Schäfer and Wehner, 1993; Ronacher and Wehner, 1995; Ronacher et al., 2000), until recently it was shown that the ants use a stride integrator to measure walking distances (Wittlinger et al., 2006; Wittlinger et al., 2007).

As concerns the compass, Wehner and coworkers have shown in a series of ingenious experiments that *Cataglyphis*, like honey bees, relies primarily on the polarization (POL) pattern of the sky as a compass reference (Duelli and Wehner, 1973; Wehner and Müller, 1985; Wehner and Müller, 2006; Fent, 1986; Wehner, 1989; Wehner, 1994; Wehner, 1997; Wehner, 2003; Wehner & Labhart, 2006). Like in other insects, this POL pattern is perceived by means of specialized ommatidia located in the dorsal rim area of their compound eyes [first discovered in *Cataglyphis* (Herrling, 1976) and then described in various other insect species as well (for reviews, see Labhart and Meyer, 1999; Wehner and Labhart, 2006)]. Apart from the POL information, ants can also use the sun's position, the spectral gradient across the celestial hemisphere (Wehner, 1997; Wehner and Müller, 2006) and even the wind direction as additional or substitute compass cues – in the ants'

habitats there is continuous wind usually blowing from a rather constant direction (Wehner and Duelli, 1971; Wolf and Wehner, 2000; Müller and Wehner, 2007).

The polarization pattern of the sky is complex, and the exact position of e-vector directions depends on the sun's elevation. How can insects like ants or bees use this changing pattern for their navigation? The solution proposed by Rossel and Wehner (Rossel and Wehner, 1982; Wehner and Rossel, 1985; Rossel and Wehner, 1986) is that these insects have an internal, largely simplified template representation of the celestial POL pattern [for ants, see Fent and others (Fent, 1986; Wehner, 1989; Wehner, 1994; Wehner, 1997) that allows them to determine the symmetry axis of the celestial POL pattern, and thus the solar meridian (see Fig. 1B).

Although many features of the ant's compass system have been elucidated, some important questions have yet to be answered. For example, in his 1997 review, Wehner states that 'we do not even know yet whether skylight patterns are used by these hymenopteran species simply to read a reference direction – e.g. the azimuthal position of the solar meridian – from the sky (Hypothesis I), or whether they are used to determine any particular point of the compass (Hypothesis II)'. According to Hypothesis I, 'a walking ant might well rely primarily on proprioceptive information derived from cuticular mechanoreceptors of its locomotor apparatus' with the danger of rapidly accumulating errors. '*Cataglyphis* might refer to skylight information simply for calibrating and, every now and then, recalibrating its internal compass scale' [pp. 177–178 in Wehner (Wehner, 1997)].

What kind of proprioceptors may be involved? Some mammals are able to return to a starting point by path integration even if all external cues are excluded, and the vestibular system seems to be

essential for this homing performance. The semicircular canals and the statolith organs provide information on rotatory and linear accelerations, which can be integrated to monitor the animal's own movements – hence the term 'idiothetic' (Mittelstaedt & Mittelstaedt, 1973) [additional graviceptors are presented elsewhere (Mittelstaedt and Mittelstaedt, 1996; Mittelstaedt, 1997)]. Successful homing based on idiothetic cues has been demonstrated both in freely moving mammals [e.g. golden hamsters, gerbils and humans (e.g. Etienne, 1980; Seguinot et al., 1993; Mittelstaedt & Glasauer, 1991)], as well as during passive displacements (Ivanenko et al., 1997; Nico et al., 2002) (for a review, see Wallace et al., 2008). Remarkably, in humans, the information about self-motion during active walking was found to be dominant over visual (optic flow) cues (Kearns et al., 2002). In contrast to vertebrates, insects do not possess statoliths or semicircular canals. Instead, they use fields of mechanoreceptors (hair plates) located on various joints between body segments as gravity and probably acceleration receptors (Markl, 1962; Wittlinger et al., 2007). There exists a plethora of other mechanoreceptors in insects, e.g. chordotonal organs (for a review, see Field and Matheson, 1998), but their potential contribution to path integration is not well understood. However, there is ample evidence that arthropods may use idiothetic cues to stabilize an intended course or memorize previous movements (Mittelstaedt & Mittelstaedt, 1973; Seyfarth et al., 1982).

Our experiments aimed at adding a piece of evidence to the above hypotheses proposed by Wehner. We designed an experimental paradigm in which ants were exposed to a conflict between different navigational cues. In particular, ants had to cope with opposing information from the POL compass and the proprioceptors (idiothetic cues).

MATERIALS AND METHODS

The experiments were performed on desert ants *Cataglyphis fortis* (Forel 1902) at two locations within their natural habitat: (1) a salt-pan area near the village Maharès in 2008 (34°52′N, 10°53′E), and (2) a salt pan near Menzel Chaker in 2010 (34°57′N, 10°24′E).

For all experiments, ants were trained to visit a feeder filled with biscuit crumbs in a channel system covered with a polarization filter transparency (POL filter). In 2008, ants were individually marked and had to visit the feeder at least five times before being tested. In 2010, ants were not individually marked because the training channel was so crowded; therefore, we could not record the exact number of visits at the feeder before testing. However, as the ants continuously shuttled between the feeder and the nest at high speed, most of the tested individuals likely had visited the feeder several times. We can also not exclude the possibility that some individuals saw a different e-vector orientation on days before the actual training. However, earlier tests showed that ants use the actual outbound path to determine their home vector direction (e.g. Wehner et al., 2002). In addition, the results of 2010 were very similar to those of 2008 when the ants were individually marked and their experimental experience was recorded (data not shown). Individual ants visiting the feeder were caught and transported – without sight of the sky or surroundings – to a distant test field (distance to the nest >150 m). The test field was a flat area devoid of any vegetation or other landmarks, with a grid painted on the desert floor (grid width 1 m, 15×15 m), where the ant was released with a morsel of biscuit and where her homing direction could be recorded.

The width and height of the side walls of the training channel were 7 cm (for details see Grah et al., 2005). Fine grey sand was glued to the channel floor in order to increase grip while walking. The inner side walls were painted a matt grey 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 and minimal optic flow cues. A plastic enclosure surrounded the nest entrance and guided foraging ants into the training channel. In the first, short part of the foraging excursions, from the nest to the entrance of the channel covered with the POL filter, the ants had free view of the sky, along a distance of ~45 cm. An ant walking in the training channel had no direct sight of the sun because of a 50 cm high barrier erected next to the channel. Two different training directions, at different nests, were used in the morning and in the afternoon to exclude the direct view of the sun: in the morning, the ants had to walk from the nest in the southwest direction (225 deg; Fig. 1A); in the afternoon, the feeder was located in the southeast direction (135 deg) – the respective homing directions to the nest thus were 45 and 315 deg. At approximately noon, the shadowing of the training channel was not possible and therefore training and testing was suspended (see Fig. 2).

In order to manipulate the POL information, we covered the channels with a POL transparency (HN38 Polarisationsfolie linear, 0.3 mm; Fa. ITOS GmbH, Mainz, Germany), which produced linearly polarized light also in the UV range of the spectrum. The spectral properties of the POL filter are shown in Heß et al. (Heß et al., 2009), their fig. 3. This transparency has a reasonable transmission and a high POL filter efficiency in the UV range (300–400 nm), which is the relevant part of the spectrum *Cataglyphis* uses for its compass (Duelli and Wehner, 1973; Labhart, 2000). Thus, on their way from the nest to the feeder the ants experienced a single, specific direction of the e-vector of light, as the filter transparency excluded all e-vectors except one. In this respect, the present experimental setup differed from most earlier experiments in which ants were trained under full view of the sky, and were then exposed to a restricted view of the sky or reduced POL patterns on their homebound path, by means of a trolley, which was moved along with the homing ant (e.g. Fent and Wehner, 1985; Fent, 1986; Müller, 1989) (see also Wehner and Müller, 2006). Three orientations of the e-vector relative to the channel orientation were used: orthogonal (90 deg), parallel (0 deg) and oblique (135 deg). Different cohorts of ants were used for each POL-filter orientation and each individual was tested only once.

Three types of experiments were performed (see Fig. 1). As a control, ants were trained in a 6.6 m linear channel with uniform e-vector (orthogonal, parallel and oblique; Fig. 1C, note that the orientation of the schemes in Fig. 1C–E does not correspond to the experimental situation; all channels were oriented on the field as shown in Fig. 1A, depending on the time of day). In the first cue conflict experiment, ants were trained in a channel with a 90 deg turn (after 4 m; the second leg was 3.3 m); this channel was covered with a constant (orthogonal) e-vector pattern (see Fig. 1D and Results). In a second cue conflict experiment, ants were trained in a linear channel covered with different combinations of two e-vectors; e.g. 3.3 m orthogonal followed by 3.3 m parallel (Fig. 1E). In this series of experiments we used the following combinations of e-vector orientations: 90 and 0 deg, 0 and 90 deg, 90 and 135 deg (all equal length of the two segments), and 90, 0 and 90 deg (the two 90 deg segments combined had the same length as the 0 deg segment). In preliminary experiments we noticed that the ants tended to turn back at the sharp transition from a 0 deg to a 90 deg e-vector. To reduce confusion of the ants, we covered the border between the two e-vector orientations with a 15 cm piece of orange Perspex, so that at the transition the ants had to walk a 15 cm distance without POL-compass information (see Ronacher et al., 2006); this 15 cm
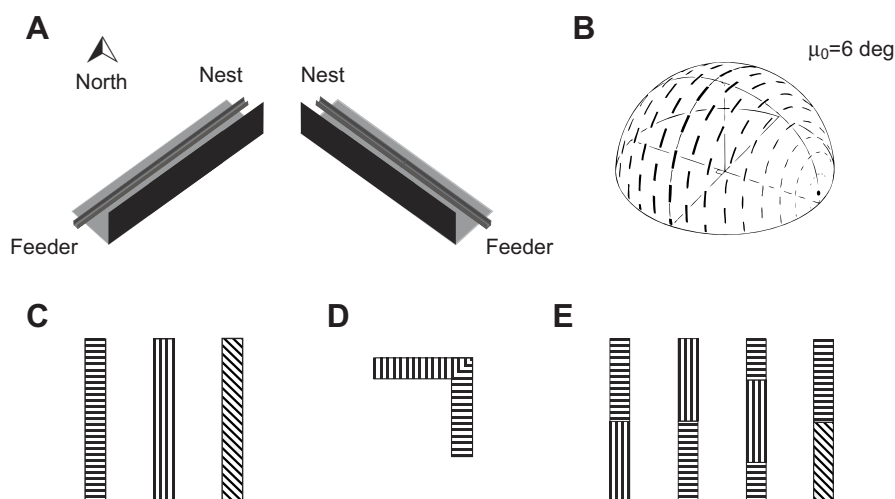
Fig. 1. (A) Experimental setup showing the orientation of the channel used in the morning (left) and the afternoon (right). In the morning, the ants had to walk from the nest in the southwest direction (225 deg); in the afternoon, the feeder was located in the southeast direction (135 deg); the respective homing directions to the nest thus were 45 and 315 deg. (B) Sky polarization pattern at sunrise; the sun elevation (μ_0) was 6 deg [from Wehner (Wehner, 1982), with permission]. (C) Straight channels with three different orientations of the e-vector [channel length with polarization (POL) cover= 6.6 m; channels not drawn to scale). Note that the orientation of the channels in C–E does not correspond to the experimental situation; on the field all channels were oriented as shown in A, depending on the time of day. (D) First cue conflict paradigm: channel with 90 deg bend, covered with uniform POL-filter orientation (e-vector orthogonal to the channel direction). (E) Second cue conflict paradigm: change of e-vector orientation in a straight channel (each POL pattern covered a distance of 3.3 m).

distance is not included in the length details given above. In the first cue conflict experiment (Fig. 1D), however, the bend was not covered by orange Perspex, thus the ants performed their turning movement under sight of the (orthogonal) e-vector.

Data evaluation and statistics

From the recorded homebound paths, the homing direction relative to the sun azimuth was determined as the vector connecting the release point to the ant's current location, at distances of 2, 3 and 4 m from the release point; homing directions were then assembled in a circular diagram and subjected to circular statistics. The length of the mean vector and the circular standard deviation describe the concentration of data around a specific angle. To test whether a population's mean angle corresponded to a theoretical value, we applied the one-sample test (Zar, 1999). However, viewing a single e-vector direction in the zenith yields ambiguous directional information. For example, having been trained with an e-vector parallel to the channel, on the test field the ant can choose to walk either 90 deg to the left or to the right of the sun azimuth, as these are the positions of a 'vertical' e-vector, at least when the sun is at the horizon (see Fig. 1B) [the terminology relates to the e-vector orientation relative to the meridian when seen from the inside of the celestial hemisphere (cf. Wehner, 1982; Wehner, 1997)]. Ideally, this leads to a bimodal distribution of homing directions in a circular plot. Hence, the mean vector becomes very small in spite of a strong clustering of the walking directions. In the case of a clear bimodal separation of data, we applied the one-sample test separately to each half of the bimodal distribution (see Results). As an alternative, we also transformed the bimodal distribution to a unimodal distribution before applying significance tests (e.g. Batschelet, 1981; Zar, 1999). To compare two distributions we applied the Mardia–Watson–Wheeler test, after transformation to a unimodal distribution.

In some cue conflict experiments, a more complex, quadrimodal distribution of homing directions was expected, for which no simple

formulas exist in circular statistics. We applied two types of tests. First, we compared the counts in a 30 deg sector around the expected directions with the counts outside this range, and tested whether more ants than the expected 0.33 proportion headed in the predicted directions. The procedure was repeated for a 45 deg sector around the expected directions. As a second test of whether the actual homing directions of ants would correspond to these expectations, we applied a Monte-Carlo simulation in combination with the Kolmogorov–Smirnov test; the details of this procedure will be described in the Results section.

In addition, the length of an ant's home vector was determined as the distance between the release point and the point where the ant switched from a straight path to search loops (Wehner and Srinivasan, 1981). To compare these data with the actual distances between nest and feeder, we estimated the confidence intervals of the medians, according to the procedure given by Sachs [p. 336 in (Sachs, 1999)]. All statistical tests were two-sided.

RESULTS
Training with a single e-vector orientation

In a first set of experiments, an ant was exposed to a single e-vector direction during her foraging excursion in a straight channel (see Fig. 1C). Performing her homebound run on the test field, now with full view of the sky's polarization pattern and the sun, the ant should orient in a particular direction relative to the sun azimuth, depending on where on the sky it expects to see that specific POL direction (see Wehner, 1997). For example, when trained with the e-vector orthogonal to the walking direction, the expected homing directions are along the solar meridian, either towards the sun azimuth or in the opposite, antisolar direction, as horizontal e-vectors are restricted to the solar–antisolar meridian (see Fig. 1B). Obviously, during the day the sky-bound homing directions will change relative to the earth coordinates of the test field. In Fig. 2A, the actual homing directions of the ants, recorded at different times of the day, are shown in earth coordinates (0 deg corresponding to north). In this
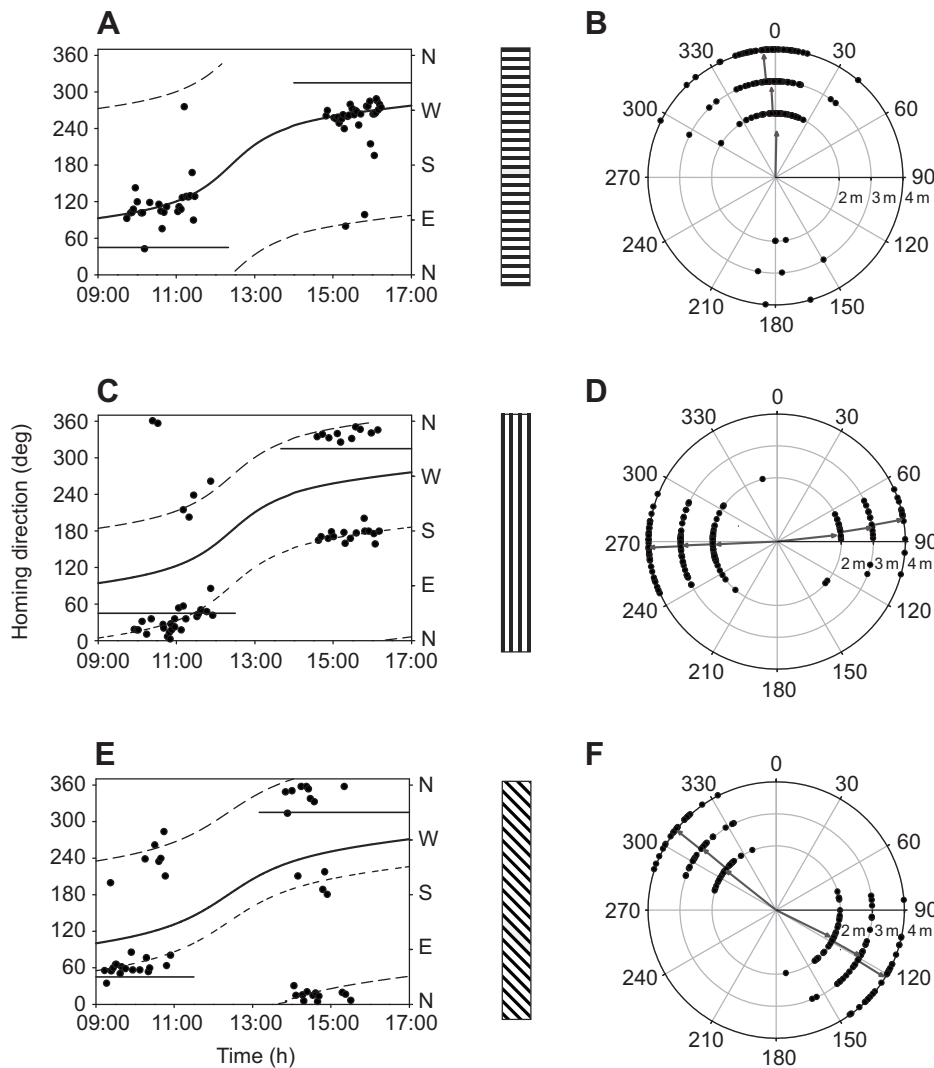
Fig. 2. Homing directions of ants recorded on the test field, after training with a single e-vector direction (as in Fig. 1C). Left diagrams: direction of the homebound path at different times of the day, taken at 3 m distance from the release point. Curves represent the sun azimuth (solid lines) and the change in expected homing directions during the day (dashed lines); 0 deg corresponds to north (the four points of the compass are indicated on the right ordinate); horizontal lines at 45 and 315 deg indicate the nest position. The sun azimuth curves differ slightly between the diagrams because of the different test days. Each point represents the course of one individual. Right diagrams: homing directions relative to the sun azimuth (at 0 deg), measured 2, 3 and 4 m from the release point. Black arrows: mean vectors. (A,B) Orthogonal e-vector orientation (90 deg). Lengths of mean vectors were >0.96 (evaluated for the solar half). At 2 and 3 m, the direction was not significantly different from 0 deg; at 4 m, the difference was significant (0.01<P<0.05) (N=56, 54 and 52 at 2, 3 and 4 m, respectively). (C,D) Parallel e-vector orientation (0 deg). Mean vectors were evaluated separately for the left and right halves (all r>0.94). Mean vectors were not significantly different from the 270 deg expectation at all distances (N=43, 42 and 37); although the mean vector was not significantly different from 90 deg at 2 m, at 3 and 4 m the difference was significant (0.01<P<0.05 and P<0.01, respectively) (N=14, 14 and 12). (E,F) Oblique e-vector orientation (135 deg). Mean vectors were evaluated separately for the left and right halves. The mean vector was not significantly different from 315 deg at 3 m, but this difference was significant at 2 m (0.01<P<0.05) and at 4 m (P<0.01; all N=21). Mean vectors were significantly different from the expected 135 deg (all P<0.01; N=30, 28 and 25).

graph, the shifts of the solar and antisolar direction are represented by solid and dashed curves, respectively. The 'correct' homing directions, towards the nest, are indicated as horizontal lines at 45 and 315 deg (different training directions were used in the morning and in the afternoon, to exclude the direct view of the sun; Fig. 1A). Evidently, after this training the ants were not able to walk in the respective nest directions. Most ants headed in the solar direction, whereas only three animals chose the antisolar sector (Fig. 2B). To quantify the accuracy of orientation, we plotted the homing directions relative to the sun's direction, in a circular diagram (Fig. 2B; sun azimuth at 0 deg). Focusing on the data in the upper half of the diagram, the length of the mean vector (**r**=0.96) and the moderate circular standard deviation (±15.8 deg) indicate a strong concentration of homing directions. The mean vector of this distribution (μ=357.4 deg) was not significantly different from the expected value 0 deg (one-sample test, 95% confidence interval for μ=353.2–1.7 deg, N=54).

The training with an e-vector direction parallel to the channel's axis mimics – from the ant's perspective – a foraging excursion in a direction at right angles to the sun's position; this is the expectation according to the ant's simplified internal template (Wehner, 1997) (see also Fig. 1B). For different times of the day, the two expected directions are shown in Fig. 2C as dashed curves, whereas the solar

direction is plotted as solid curve. The right diagram shows again the orientation in coordinates relative to the sun's position (Fig. 2D). Evaluating the right and left half of the diagram separately, mean (±circular s.d.) vectors were 81.6±12.2 and 267.7±12.8 deg (**r**=0.978 and 0.975, N=14 and 42, respectively). The mean angle of 267.7 deg did not deviate significantly from the expected 270 deg (P>0.05), whereas the mean angle of 81.6 deg deviated significantly, although weakly, from the 90 deg expectation (0.01<P<0.05). After training with the 135 deg orientation of the POL filter (Fig. 2E,F), the homing directions again clustered near the expected values of 135 and 315 deg. Evaluating the two halves of the diagram separately, the mean vector of 309.8±12.4 deg (N=21) did not deviate from the expectation (P>0.05), whereas the opposite vector (118.9±9.0 deg, N=28) deviated significantly from 135 deg (P<0.01). Possible causes for the deviations from the expected values shown in Fig. 2D,F will be discussed later.

When insects experience a single e-vector direction in the zenith as a compass cue, one expects a bimodal distribution of heading directions (Wehner and Strasser, 1985; Wehner, 1994) (see also Fig. 2D,F). This bimodality was absent in the experiment with orthogonal e-vector orientation – ants showed a strong bias to the solar azimuth (Fig. 2B) – whereas the other two training conditions exhibited a bimodal distribution of homing directions (Fig. 2D,F).
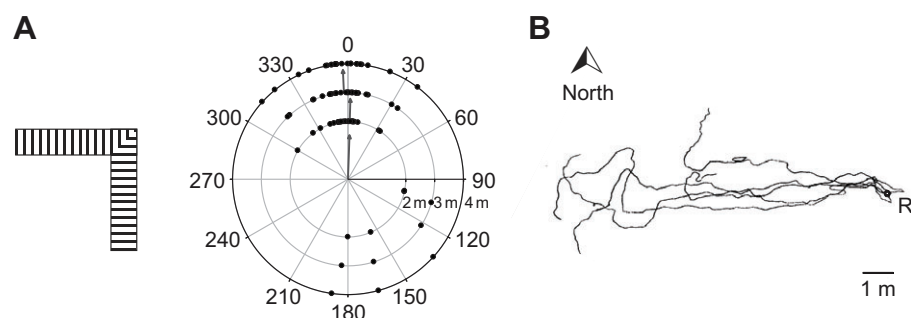
A

B

Fig. 3. (A) Homing directions of ants on the test field, after training in a channel with 90 deg bend, covered by a POL filter with orthogonal e-vector direction (N=30, **r**=0.925); the distribution of homing directions was not different from that shown in Fig. 2B (Mardia–Watson–Wheeler test: W=3.297, P=0.192). (B) Sample tracks of five individuals. R, release point.

A closer look at Fig. 2C,E, however, reveals a similar bias in these data. In the parallel e-vector training, the ants strongly preferred one of the optional homing directions during the morning (stippled curves in Fig. 2C): 27 animals headed to the 270 deg sector, and only four to the 90 deg sector; this bias was almost gone in the afternoon (15 *versus* 10). In the oblique e-vector training (Fig. 2E), the bias (17 *versus* 7) was directed to the 315 deg sector in the morning, and reverted during the afternoon (4 *versus* 21). Possible causes for these deviations from bimodality will be discussed later.

Cue conflict experiments

In the next set of experiments we put the sky compass cue in competition with the idiothetic cues derived from the animals' own movements. The inset in Fig. 3A depicts the situation of the channel with a rectangular bend, in which, however, the continuous 90 deg POL filter mimicked a linear course. This experiment could be performed only in the late afternoon (16:00–17:15 h) because both legs of the channel had to be shaded against direct view of the sun. Fig. 3B shows some sample tracks of individual ants, demonstrating the straight path segments until they switched to search loops (see Wehner and Srinivasan, 1981). The outcome of this experiment is very clear: the ants ignored the actual bend of the channel, and they behaved exactly like animals trained in the straight channel with 90 deg POL-filter direction (compare Fig. 3A and Fig. 2B). The two distributions in Fig. 2B and Fig. 3A did not differ significantly (Mardia–Watson–Wheeler test after transformation to unimodality, W=3.297, P=0.192).

A 'reciprocal' experiment was performed in a straight channel, but now simulating a virtual bend by a change in the POL-filter direction. In this series of experiments we used four combinations of e-vector orientations (see Fig. 1E): 90 and 0 deg, 0 and 90 deg, 90 and 135 deg (all equal length of the two segments), and 90, 0 and 90 deg (the two 90 deg segments combined had the same length as the 0 deg segment).

Because a single e-vector orientation leads, in principle, to a bimodal distribution of homing directions (as in Fig. 2D,F), the expected homing directions in this type of experiment become more complex. For the combination of 90 and 0 deg, in principle a quadrimodal distribution of homing directions is expected (see scheme on top of Fig. 4) because an ant trained with the orthogonal (90 deg) e-vector first has two options, the solar or antisolar direction. When the POL pattern then is changed to a parallel (0 deg) e-vector, she may choose to either run to the left or to the right of the sun position. As the two channel segments with 90 and 0 deg orientation of the e-vector had the same length (3.3 m), the combination leads to expected homing directions at 45, 135, 225 and 315 deg, respectively, provided that the ants combine the two segments in a linear way (see inset in Fig. 4). A potential drawback of this experimental paradigm is that it may be difficult to distinguish such a quadrimodal distribution from a uniform distribution of

homing directions. We expected that the strong preference of the solar direction observed in the experiment shown in Fig. 2A,B could reduce the ambiguity described in the scheme of Fig. 4, and would lead to an actual preference of fewer than four directions. This was indeed the case (Fig. 4). In particular, there were virtually no individuals heading in the 135 deg direction, and the 225 deg quadrant was also underrepresented (Fig. 4B,D,F). As circular statistics books (Batschelet, 1981; Zar, 1999) offered no ideal solution for a statistical treatment of this type of data, we applied a Monte-Carlo simulation approach, combined with a Kolmogorov–Smirnov test (Sachs, 1999). First, we calculated the expected distribution of homing directions using two Gaussian distributions with the 'ideal' mean values at 0 and 90 deg (or 0 and 270 deg) and a standard deviation of 19 deg, which is well in the range of standard deviations observed in Fig. 2 (range of circular s.d. between 10.5 and 22.8 deg). Fig. 5 compares the observed homing directions at 3 m distance from the release point (grey columns) with the expected distributions. We then used the Kolmogorov–Smirnov test to check for significant differences between the expectation and the actual data; we applied an expectation with four peaks, weighted by the number of data in the respective quadrants. Fig. 5A demonstrates a reasonable agreement between the observed and expected distributions in the combination of orthogonal–parallel e-vectors (the data at 4 m distance yielded very similar statistics; in the following, the 4 m probabilities are given in parentheses). The Kolmogorov–Smirnov test indicates that the differences were not significant [D=0.1264, P=0.31, N=58 (P=0.72, N=49)]. For the combination of parallel–orthogonal e-vectors (Fig. 5B), the difference between the expectation and the data was significant [D=0.213, P=0.025, N=48 (P=0.004, N=39)]. However, there was good agreement if the expected peak at +45 deg was shifted to 35 deg [now D=0.131, P=0.386 (P=0.32, N=39)]. Fig. 5C shows the results of the e-vector combination orthogonal–parallel–orthogonal. These data corresponded well to the expectation [D=0.1165, P=0.40, N=59 (P=0.22, N=46)]. In summary, the linear combination of the two directions (0 and 90 deg, or 0 and 270 deg) described the observed homing directions remarkably well (Fig. 5).

As an additional, less sophisticated statistical test, we checked whether a higher proportion of walking directions fell into 30 deg sectors centred on the four expected homing directions (45, 135, 225 and 315 deg), i.e. 'hits', or in the surrounding sectors, i.e. 'misses'. The results indicate a high proportion of hits in three experiments. For the parallel–orthogonal e-vector combination (Fig. 4D) the proportions were 25 hits and 23 misses – compared with the uniform expectation of 16 hits *versus* 32 misses (χ^2=7.59, P<0.01). For the orthogonal–parallel–orthogonal combination (Fig. 4F), there were 31 hits and 28 misses (χ^2=9.79, P<0.01). For the orthogonal–oblique e-vector combination (Fig. 6B), there were 24 hits and 18 misses (χ^2=10.71, P≈0.001). For the
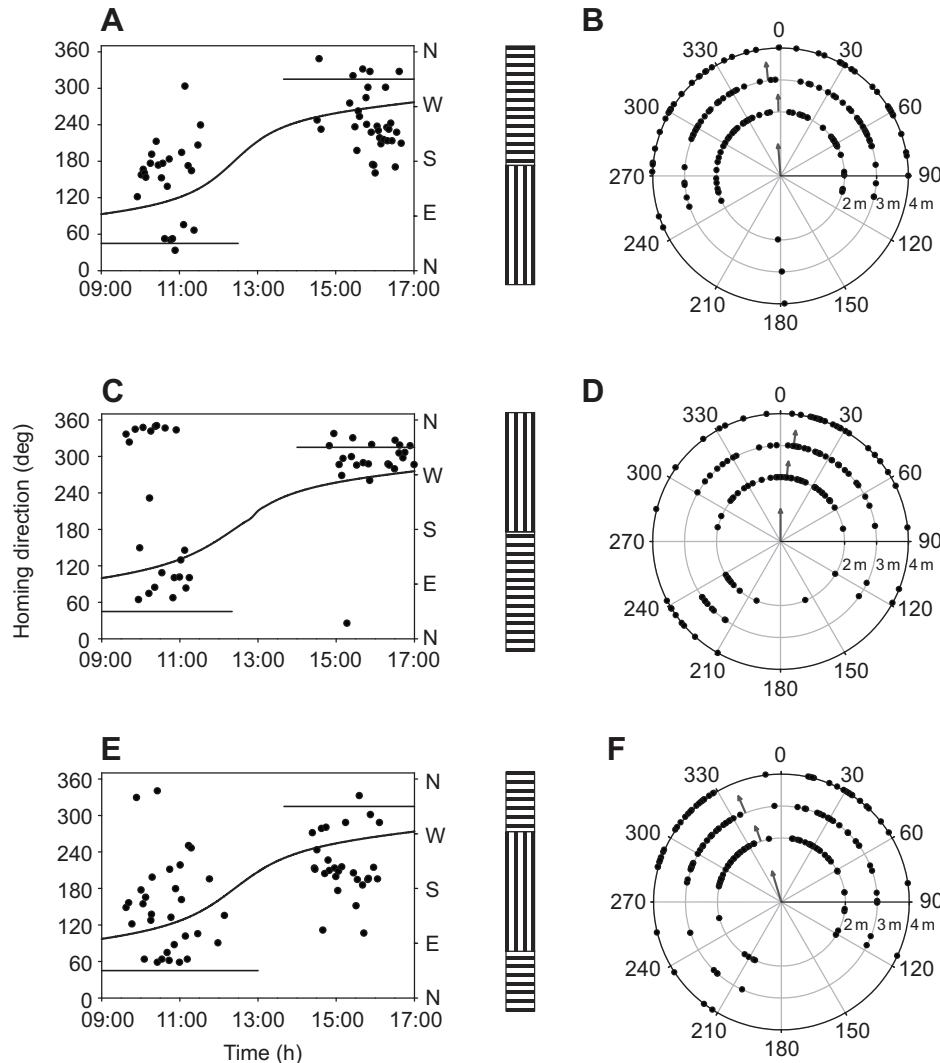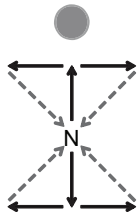
Fig. 4. Cue conflict experiments in a straight channel simulating an 'optical bend' by combining 0 and 90 deg e-vector directions. Top: scheme of expected homing directions. N, nest (the grey disc marks the sun azimuth). (A,C,E) Distribution of homing directions during the day; as in Fig. 2, the solid curve represents the sun azimuth; expected homing directions are not indicated in these diagrams. Horizontal lines at 45 and 315 deg indicate the respective nest positions. (B,D,F) Homing directions plotted relative to the sun azimuth. The respective vector lengths are small compared with those shown in Fig. 2 because of the broad distribution of homing directions. For the correspondence with the expected homing directions, see Fig. 5. In the sketches of the channels, the upper part represents the e-vector direction seen first by the ants on their outbound foraging trips (cf. Fig. 1A). For further explanations, see Results.

orthogonal–parallel e-vector combination (Fig. 4B), there was no significant accumulation in the 30 deg sector (22 hits *versus* 36 misses); however, in a 45 deg sector around the expected directions the counts were 36 hits *versus* 22 misses, approaching significance (χ^2=3.38, $P\approx0.066$). Taken together, this alternative test supports the notion that the ants headed more often than expected by chance in the directions predicted for these cue conflict experiments.

The combination of an orthogonal and an oblique e-vector orientation (90 and 135 deg) was chosen because it represents a (virtual) 45 deg bend in one direction *versus* a 135 deg bend in the other direction (see scheme in Fig. 6). Müller reported that it was much more difficult to induce ants to make a sharp turn leading backwards when trained in channels with view of the sky (Müller, 1989). Hence, we expected that this combination of two e-vector orientations would further reduce the ambiguities introduced by a

single e-vector orientation. Indeed, this experiment yielded a strong concentration of homing directions around 337.5 deg, that is, the expected value for a linear combination of 360 and 315 deg (mean vector at 3 m=336.7±35 deg, R=0.832, N=41; Fig. 6B). Fig. 6C shows an almost perfect correspondence between expected and observed homing directions in this experiment [Kolmogorov–Smirnov test: D=0.114, P=0.69, N=41 (P=0.07, N=36)].

Length of home vectors

From the paths recorded on the test field we extracted the distance between the release point and the first distinct turning of the ant, which indicates the switch from the rather straight path to the characteristic search behaviour, and thus the length of the home vector (see Wehner and Srinivasan, 1981). Fig. 7 presents a
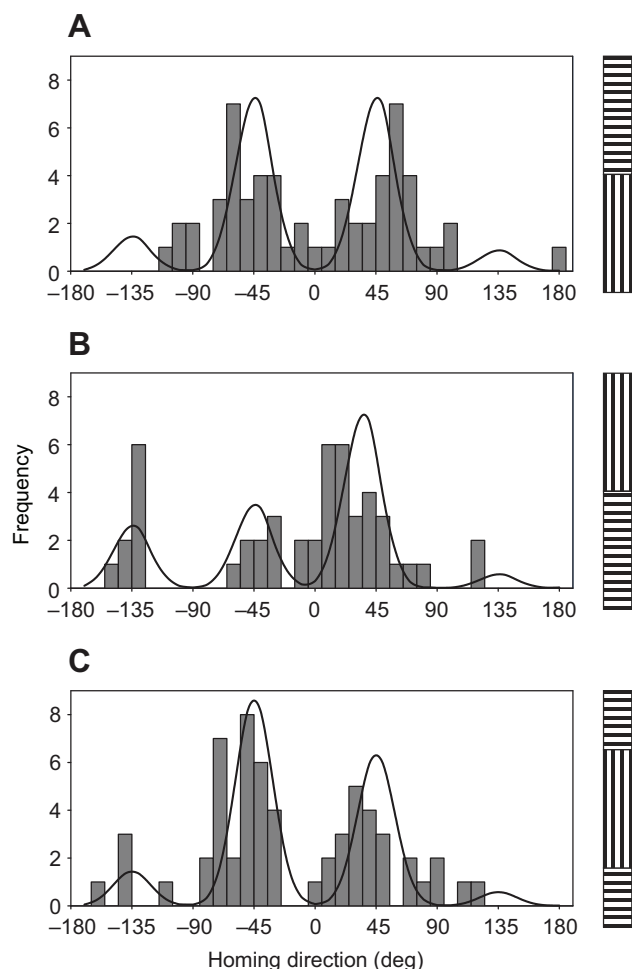
Fig. 5. Statistical evaluation of the data shown in Fig. 4. Homing directions are shown as frequency histograms (grey bars). Curves represent the expected homing directions; the height of the expectation curves was adjusted by the number of actual occurrences in the respective quadrants. For further explanations, see Results.

compilation of the homing distances observed in the different experimental paradigms. Shown are medians, quartile ranges (grey bars) and 10th and 90th percentiles (whiskers). The actual distances an ant had covered in the training channel under the POL filter are indicated by bold black bars, whereas triangles represent the distances for a virtual (or actual) bend between nest and feeder in the cue conflict experiments. For path lengths longer than 8 m, the switch to search loops could not be recorded exactly; therefore, these data were fixed at 8 m. Although the spread of observed distances is large, some trends are visible (Fig. 7). After training with a single e-vector direction, the ants tended to underestimate the true distance (the difference to the expected 6.6 m was significant at $P<0.01$ for parallel and oblique e-vector orientations, but not significant for orthogonal e-vector orientations). A shortening of home vectors, however, is not uncommon if the training and test situations differ (Müller, 1989; Grah et al., 2005). In the cue conflict experiments with combinations of orthogonal and parallel e-vectors (cf. Fig. 4), the observed distances were always significantly smaller than the actual walking distances ($P<0.01$), whereas they did not differ significantly from the virtual nest-feeder distance indicated by the triangles (4.67 m). In the combination of orthogonal and oblique e-

vectors (cf. Fig. 6), the actual and expected virtual homing distances are rather similar, and the ants showed an underestimation for both (observed lengths of home vectors compared with the walking distance, $P<0.01$; compared with the expected distance marked by the triangle, $P<0.05$). Remarkably, in the first cue conflict experiment with the 90 deg bend of the channel (see Fig. 3), the median distance is close to the walking distance in the channel ($P>0.05$); however, it differs significantly from the real distance between nest and feeder ($P<0.01$; note that in this experiment, for technical reasons, the two legs of the channel were 4 and 3.3 m, and thus the diagonal was 5.19 m). This last result corroborates the conclusion of Fig. 3 that the ants interpreted this experimental paradigm as a straight path.

DISCUSSION

The experiments reported here aimed at revealing the respective influences of sky compass information and idiothetic cues on the navigation of *C. fortis*. In different experiments, the ants were exposed to a situation in which the idiothetic information about the actual movement direction disagreed with the information from the POL compass. All these experiments supported the same conclusion: the ants relied primarily on the sky compass and ignored conflicting idiothetic information. Remarkably, the exposure to a rather artificial situation – with only a single e-vector direction available in the training channel – did not impair the accuracy of homing paths on the test field (see the low circular standard deviations in Fig. 2). Even in some of the cue conflict experiments, the clustering of homing directions was very tight (Figs 3, 6). The results will now be discussed in detail.

Training with a single e-vector direction

Ants that saw only a single e-vector orientation during an extended path segment behaved quite normally on the test field – now with full view of the sky. The ants followed rather straight homebound paths (Fig. 3B) and mostly adhered to the expected directions that correspond to the position of these e-vectors in the sky and in their internal template (see Fig. 1E) (see Wehner, 1997). Hence, a single e-vector direction seen during training allows for a quite accurate navigation on the test field – this is evident from the large mean vectors ($r=0.92–0.98$) and the small circular standard deviations shown in Fig. 2 (s.d. range=10.7–22.8 deg). The variances are in the same range as reported in earlier investigations (e.g. Müller, 1989; Wehner, 1997; Wehner and Müller, 2006). However, a few significant deviations from the expected homing directions occurred.

The first was the strong bias towards the solar direction (visible in Fig. 2B and Fig. 3A), which is in contrast to the bimodal distribution expected for a single e-vector stimulus in the zenith (Wehner and Strasser, 1985; Wehner, 1994). However, on closer examination of different test times, less obvious biases were also visible in the other experiments (see Results, Fig. 2C,E and Fig. 4). When trying to explain an obviously general lack of bimodality in several of these experiments, we should be aware that a tiny additional influence may be sufficient to turn the balance between two equivalent homing directions. As mentioned in the Introduction, ants can use several additional or substitute compass cues: the sun's position, the spectral gradient across the celestial hemisphere (Wehner, 1997; Wehner and Müller, 2006), and even the wind direction, which is rather constant for longer time periods in the study sites (Wehner and Duelli, 1971; Müller and Wehner, 2007). The direct view of the sun was shielded in our training paradigm (Fig. 1A), hence spectral gradients on the sky and wind remain as major additional compass cues that could have caused these biases.
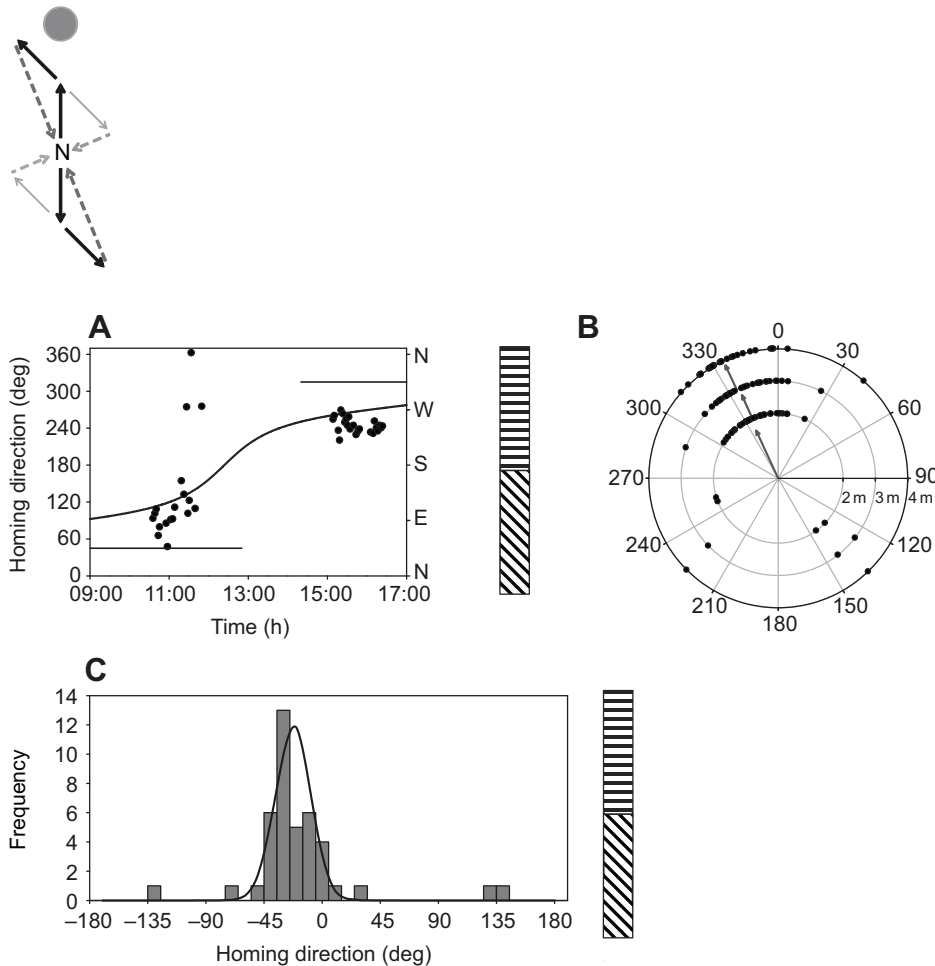
On her outbound run in the morning, an ant experienced a spectral gradient with more UV on her right side (see Fig. 1A). Thus, for her homebound run on the field she should prefer that of the two possible homing directions which yields more UV on her left side. This consideration perfectly explains the strong morning bias for 310 deg and the reversed afternoon bias for 120 deg found for the oblique e-vector training (Fig. 2E,F). The same argument is able to explain the strong morning bias to 270 deg in the parallel e-vector training (Fig. 2C). In the afternoon, however, a preference for 90 deg would have been predicted according to the spectral gradient hypothesis, which was not observed (10 ants heading to 90 deg *versus* 15 heading to 270 deg). For the cue conflict experiments shown in Fig. 4, we also observed a bias for certain directions, depending on the training type and test times. The spectral gradient hypothesis is in perfect agreement with the opposing biases found both in the morning and afternoon in the parallel–orthogonal e-vector training (Fig. 4C). The data shown in Fig. 4E are equivocal with respect to the hypothesis, as they do not exhibit such a strong bias. However, the preferred directions in the morning and afternoon sessions, shown in Fig. 4A, reveal a bias that is opposite to the predictions from the spectral gradient.

For the strong preference of the solar azimuth in the experiments with orthogonal e-vector orientation (Fig. 2B, Fig. 3) we can offer no conclusive explanation. The spectral gradient hypothesis is not able to explain these biases. Conceivably, an additional influence of wind – which blew mostly from a northern to a northeastern direction in the morning, while turning in the afternoon to the southeast or south – may have caused these preferences. However, as we could not continuously record the wind direction and wind speed during these experiments, this explanation must remain tentative.

In Fig. 2D, a small but significant (P<0.05) deviation from the 90 deg expectation was observed, whereas in Fig. 2F, the deviation from the 135 deg expectation amounted to ~16 deg (P<0.01). It is unlikely that these deviations could have been caused by an additional influence of spectral gradients or wind. Wind probably can be ruled out, as the wind compass is neglected if put in competition with the POL compass (Müller and Wehner, 2007). Another explanation for these deviations may be that during training the ants first walked a short distance of ~45 cm between nest entrance and channel opening with full sight of the sky, and here experienced a different compass direction, compared with the POL pattern in the channel (see Materials and methods). This could have introduced a deviation from the solar meridian of up to 5 deg; remarkably, this effect was smaller in the morning and larger in the afternoon, which fits the data shown in Fig. 2C–F. Taking this potential effect into account, the deviations from the 90 deg value in Fig. 2D are not longer significant; however, this effect can not completely explain the larger deviation from 135 deg in Fig. 2F.

Additional evidence for a simplified internal template

It has to be emphasized that the expected directions shown in Fig. 2C–F correspond to the internal, simplified template that has been elaborated previously (Rossel and Wehner, 1982; Fent and
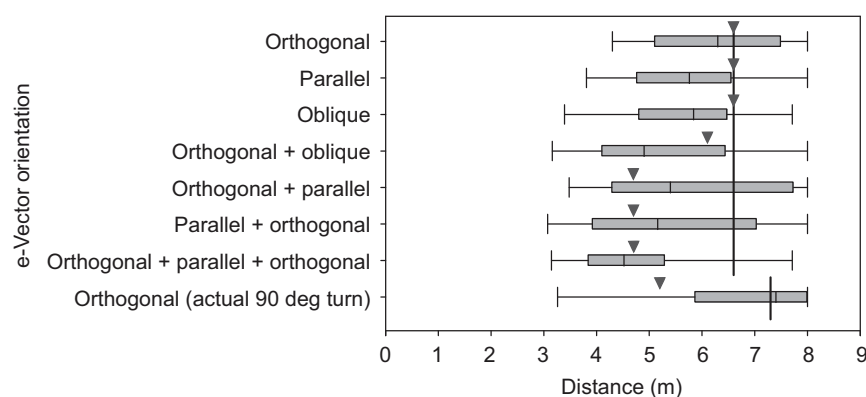
Fig. 7. Length of home vectors for the eight training paradigms, i.e. distance between release point and begin of search loops. Shown are medians, quartile ranges (grey bars), and 10th and 90th percentiles (whiskers). Bold vertical bars indicate the expectation according to the actual walking distances under the POL filter, i.e. distance between channel entrance and feeder. Arrowheads indicate expected vector lengths if the ants had perceived an actual (experiment of Fig. 3) or virtual bend of the channel (see Figs 4, 6). From top to bottom, the number of individuals tested is 59, 57, 51, 45, 60, 50, 61 and 30.

Wehner, 1985; Fent, 1986; Rossel and Wehner, 1986). However, it is only at sunrise and at sunset that this template fits well the actual distribution of e-vectors on the sky. At other times of the day, in particular around noon, there are deviations [for a detailed discussion, see Wehner (Wehner, 1982; Wehner, 1989; Wehner, 1994; Wehner, 1997)]. The 'vertical' e-vector (in our paradigm corresponding to the e-vector parallel to the channel; Fig. 2D), for example, is shifted towards 45–60 deg azimuthal distance to the sun at 11:00 h (Coulsen et al., 1960). Thus, if the ants had relied on the actual sky position of the vertical e-vector, at around noon their homing direction should have deviated by 30–45 deg from the 90/270 deg expectation. We checked the homing directions shown in Fig. 2C,D separately for times early in the morning (or late in the afternoon) and times between 11:00 and 15:00 h, and found no difference: the mean vectors at 2, 3 and 4 m differed by only –2.0, + 4.3 and –0.8 deg, respectively (Mardia–Watson–Wheeler test: $P>0.65$ in all cases, N=36 and 21, 35 and 21, and 31 and 18 animals, respectively). Thus, our results provide an independent piece of evidence for the model proposed by Wehner (for a review, see Wehner, 1994; Wehner, 1997), i.e. that the ants navigate by comparing the sky's polarization pattern with a simplified internal template for POL directions. In this template, the vertical e-vectors are fixed at 90 deg distance to the solar meridian, in contrast to the sky's polarization pattern, in which the azimuth positions of vertical (and other) e-vectors depend on the sun's elevation (Wehner, 1982; Wehner, 1994; Wehner, 1997).

Cue conflict experiments

Both types of cue conflict experiments showed that orientation in *C. fortis* is based on POL information as the completely dominant cue, rather than on idiothetic information. In the experiment shown in Fig. 3A – with a 90 deg bend in the channel – the ants behaved exactly like those trained in a straight channel. In the experiments shown in Figs 4 and 5, a marked influence of idiothetic cues would have led to a distinct deviation from the 45 and 315 deg expectations. In general, the data correspond quite well to the theoretical expectations (Figs 5, 6). The only exception (Fig. 5B) where a significant deviation existed between data and theoretical curves became non-significant if we shifted the 45 deg peak of the expectation by only 10 deg. This deviation is not larger than the deviations found in the experiments with a single e-vector orientation (see Fig. 2D,E). Hence, the ants seem to combine the path segments corresponding to two e-vector orientations (0 and 90 deg) in a linear fashion. This interpretation is corroborated by the results shown in Fig. 6, in which a strong concentration of homing directions and an almost perfect correspondence between expected and observed homing directions was found.

Deviations from the expected directions (see scheme in Fig. 4) could have several causes. One could be an influence of additional idiothetic cues – if an ant perceives the straightness of the channel *via* her proprioreceptors, this could lead to a compromise direction between the visual 90 deg bend and the straight path, resulting in a smaller virtual turning angle, e.g. 60 deg. A deviation could also result from a different weighting of the first and second channel segment. In addition, ants that are trained in a channel system (with sight of the sky) that forces them to a sharp turn later exhibit systematic navigational errors in their homing directions recorded on a test field (Müller and Wehner, 1988). As the combination of 0 and 90 deg e-vectors simulates a 90 deg turn of the channel, ants should exhibit this type of 'integration error' (Müller and Wehner, 1988). The expected error for a 90 deg turn and equal length of the two legs is ~12 deg (Fig. 5); for a 45 deg turn the error is ~5.6 deg (Fig. 6). The shift of the distributions shown in Fig. 5B,C towards 0 deg, compared with the expectations, may be due to this type of error. However, the data shown in Fig. 5A rather indicate a trend in the opposite direction. In Fig. 6C, no deviation from the expected homing directions is visible; however, in this paradigm the integration error would be rather small (<6 deg).

In summary, the results show that in path integration the polarization compass completely dominates over idiothetic cues. In addition, the good correspondence between expected and measured homing directions in the cue conflict experiments (Figs 4–6), does not only support the template hypothesis (Wehner, 1994; Wehner, 1997), but also suggests as the most parsimonious explanation that the two path segments with different e-vector directions are combined in a linear way.

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