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Do desert ants smell the scenery in stereo?

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Desert ants, *Cataglyphis fortis*, navigate individually in the inhospitable saltpans of Tunisia using path integration for long-distance navigation, and visual and olfactory landmarks for fine-scale orientation in the vicinity of the nest entrance. Here, we show in a field experiment that the ants are able to locate the nest entrance within a two-dimensional olfactory array. Ants were trained to forage in an open channel and to memorize the nest entrance relative to four odours that were applied at the corners of an invisible quadratic array. In a test situation, the ants pinpointed the fictive nest only when the odours were present at their learned positions. Our results suggest that the ants had learned the olfactory scenery around their nest. Furthermore, unilaterally antennectomized ants could not pinpoint the nest within a two-dimensional array. Hence, this kind of orientation depends on the simultaneous input of both antennae, that is, on a stereo sense of smell. Until now, insects and mammals, including humans, have only been known to use bilateral sensory input to follow a concentration gradient of an odour. Our evidence suggests that desert ants require a stereo sense of smell to make use of the olfactory scenery around their nest for homing.

Animals use olfactory information for navigational means over a range of spatial scales. Seabirds pinpoint food-rich habitats over hundreds of kilometres based on plankton-derived olfactory cues (Nevitt et al. 1995), while pigeons, *Columba livia*, seem to use the olfactory landscape for homing (Wallraff 2004). Behavioural studies on insect olfaction have mainly focused on odour-evoked upwind flights to reach food or a potential mating partner (Murlis et al. 1992). In this study we investigated how the desert ant *Cataglyphis fortis* uses complex olfactory surroundings for homing. Because of its sophisticated visual navigational capabilities, *C. fortis* has become a model organism for studying orientation (Wehner 2003). In search of food, individual ants depart on tortuous routes that can take them more than 100 m from the nest. After finding a food item, an ant returns directly to the inconspicuous nest entrance. This precise navigational feat is accomplished with help from two synergistic systems, namely path integration for long-distance navigation (Wehner 2003) and landmark orientation for navigating in the vicinity of the nest (Wehner & Raeber 1979). To accomplish the latter, foragers leaving the nest are known to take a snapshot of their visual surroundings. Returning from the foraging trip, they pinpoint the nest by matching the actual image with the stored snapshot (Akesson & Wehner 2002). Recently, we found that the habitat also provides the ants with potential olfactory landmarks and that *Cataglyphis* is able to make use of such information for homing by associating environmentally derived odours with the nest entrance (Steck et al. 2009). In the present study, we asked whether the ants not only learn to associate the nest with a single odour but even remember the topography of odour sources in the vicinity of the nest. We designed an experimental paradigm according to which the ants had to memorize the position of the nest entrance within an array of four spatially separated odour sources. We tested whether the presence of all odours and their correct positions within the array were mandatory for precise homing and, finally, whether accurate orientation within the two-dimensional array required input from both antennae or only one. To sum up our questions: do the ants learn the olfactory surroundings of the nest and do they ‘view’ this olfactory scenery using a stereo sense of smell?

METHODS

In a field experiment in a Tunisian saltpan, we trained ants to forage within an open channel. The visually inconspicuous nest entrance was a 1 cm hole in the channel wall, situated within a complex olfactory array. Four odours were placed on the channel ground at the corners of a fictive square (side length: 7 cm). Methyl salicylate (M) and decanal (D) were placed at the channel wall adjacent to the nest entrance, with M upwind and D

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downwind of the nest; nonanal (N) and indole (I) were placed opposite the nest entrance, with N upwind and I downwind of the nest (Fig. 1). We used 0.4 µl of each compound diluted in 20 µl hexane. These compounds are neither innately attractive nor repellent to naïve ants, can be learned equally well, and can be distinguished (Steck et al. 2009). As the ants do not antennate the ground while walking (see Supplementary Material), odours are perceived as volatiles via olfaction rather than via taste. Odours were reapplied every 20 min. A feeder, providing the ants with biscuit crumbs, was placed 8 m downwind from the nest entrance (Fig. 1).

To see what information this odour array provided the ants, we used a photoionization detector (Aurora Scientific Inc., Ontario, Canada, Model 200A) to visualize the distribution of a tracer volatile in the channel with wind blowing in a nest-to-feeder direction. The photoionization detector probes air and exposes the vapour sample to high-intensity ultraviolet light that ionizes the nonair chemical substances. Ions are then collected on positive and negative electrodes, creating a current proportional to the contaminant concentration. Under field conditions (wind blowing in the nest-to-feeder direction), we applied a tracer gas at a position in the channel corresponding to the position of one of the odours of the array and probed the air in the channel at each cross of a fictive 1 cm grid at a height corresponding to that of the antennae of walking ants. The concentration directly above the applied odour was set as 100% and the rest of the data were normalized. Owing to the symmetric application of the odour array, we could then extrapolate the distribution for all four odours. This technique does not provide any information about the absolute concentration of odours but describes how the relative concentrations change over several centimetres in the channel (Fig. 2a). The visualization of the volatiles revealed place-specific blends. The composition and the concentrations of the volatiles changed over a distance as short as the span of an ant's antennae (Fig. 2b). Thus, when homing ants approached their nest, they came across a 'landscape' of position-specific blends of odours. Note that the nest entrance does not converge with the highest odour concentration.

Would the ants be able to learn the position of the nest entrance relative to the surrounding odour sources and make use of this olfactory scenery to locate the nest entrance? To find out, we captured, at the nest entrance, homing ants that had accomplished at least 15 training runs and released them individually to a remote test channel 1 m downwind of an olfactory array (Fig. 1) that was either identical to the training array or had the positions of the odour sources exchanged. We expected the latter test to show whether the ants had memorized the positions of the four odour sources or had simply learned that they first had to pass the blend of the downwind odours D and I and then search directly downwind of M and N. To see whether ants need both antennae to accomplish the task, we trained and tested unilaterally antennectomized ants either with the olfactory array or with a point odour source (a mixture of all four odours placed directly at the nest entrance). To assess the impact of unilateral antennectomy on search accuracy, we trained and tested another group of ants with intact antennae with the point odour source. To see which odours of the training array were necessary and sufficient for generating a focused nest search, the test array was reduced to two odours, located either adjacent to the nest entrance or opposite it. For further details see Figs 4 and 5 in the Results. Finally, we tested whether the concentration of odours affected the ants' search accuracy (see Appendix for Methods and Results). The nest searches of 30 homing ants in each of the different test arrays were analysed and each ant was tested only once. Channel

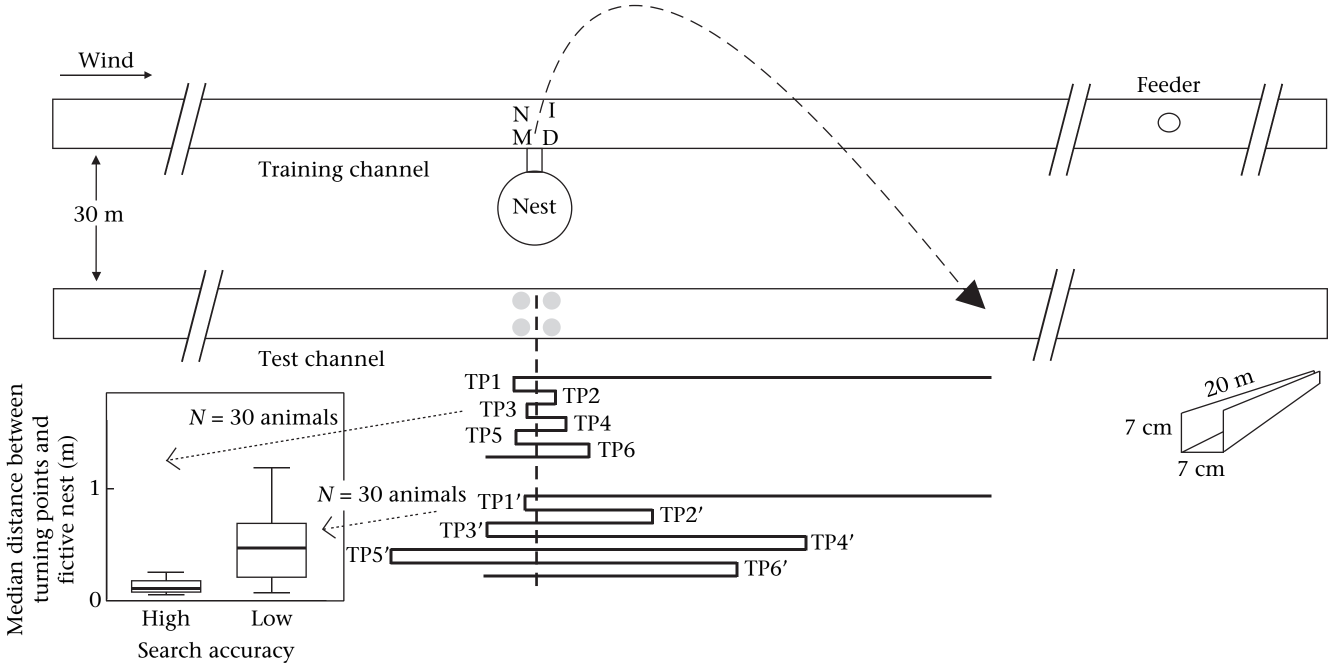

Figure 1. Experimental paradigm. The training channel consisted of 10 identical 2 m modules. The nest was connected to the training channel via a tube. The training array consisted of 0.4 µl of decanal (D), methyl salicylate (M), indole (I) and nonanal (N), each diluted 1:50 in hexane. A feeder was placed 8 m downwind from the nest entrance. The dashed arrow indicates transfer of individually marked homing ants (that had accomplished at least 15 foraging runs) from the nest entrance to a remote test channel. Odours were placed in the test array (grey circles) in the test channel 1 m upwind of the point of release. The various test arrays used are shown in Figs 4 and 5. Inset figure depicts the channel dimensions. Two schematic search runs (grey lines) are shown, one of a narrow and one of a broad nest search. For clarity, the runs are projected outside of the channel. Six turning points (TP1–TP6) after the ant had passed the test array for the first time were analysed for their median distance to the fictive nest (dashed line). Short median distances result in high search accuracy and long median distances in low search accuracy. The example box-and-whisker plots show the median, interquartile range and 10th and 90th percentiles.

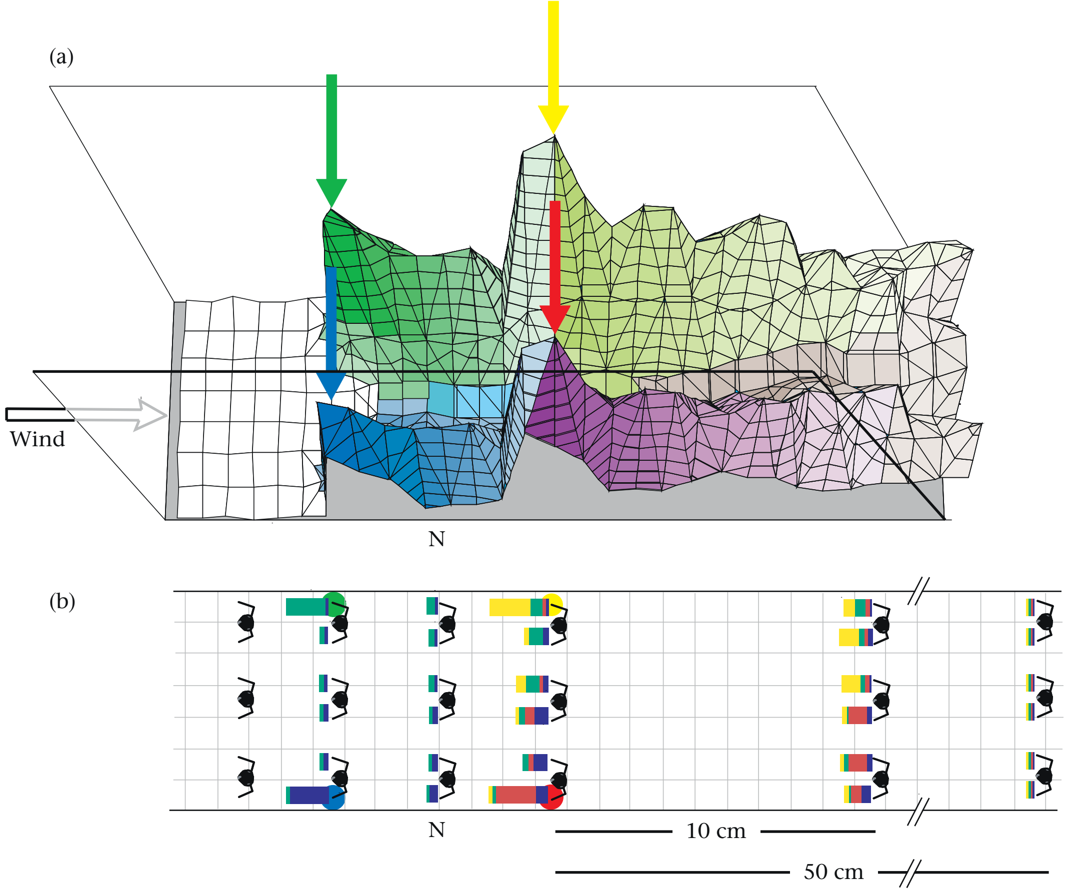

Figure 2. Odour distribution within the training channel. (a) Visualization of the odour distribution by the photoionization detector. Concentration of a tracer volatile was measured on each cross of a fictive 1 cm grid on the channel ground. Concentration measured directly above the odour source was set as 100% and the rest of the data were normalized. Data were extrapolated for each odour source of the array. Green: nonanal; blue: methyl salicylate; red: decanal; yellow: indole; N: position of nest entrance. Coloured arrows point to the position of the odour sources on the channel ground. (b) Relative concentrations of the four volatiles as detected by each antenna of ants at different positions within the channel. The size of the bars over the antennae depicts normalized odour concentration, from 100% (directly at odour source) to <5% (e.g. 50 cm downwind from odour source). Odours are colour coded as in (a). Circles depict the positions of odour sources.

modules with the test array were never used again either as no-odour modules or as other test array modules.

Experiments with visual landmarks have demonstrated that the search broadens when homing ants face a new landmark panorama and can no longer match the stored and the actual snapshots (Wehner & Raeber 1979). Hence, the breadth of the search reflects the ants' accuracy while pinpointing the nest. Therefore, we used as a measure of search accuracy the median distance between the first six turning points and the nest location in the array relative to the odours (called 'fictive nest', Figs 1, 3). We used a measuring tape placed alongside the test channel to track and record the positions of the ants' turning points. If the training and the test arrays appeared identical to the ants, a narrow search would have been likely, whereas any detected differences between training and test arrays, that is, any changes in the olfactory scenery, would have caused the ants' search pattern to become less precise and they would have broadened their search (Fig. 1).

RESULTS

Ants that were tested with the same array that was used in the training situation displayed a narrow search (Fig. 3) with short distances between turning points and the fictive nest (Fig. 4). When the odour pairs adjacent and opposite the nest were exchanged, the ants broadened the search (Fig. 3) and search accuracy significantly decreased (Fig. 4); thus memorization of the blend sequences appears unlikely to have been the only characteristic guiding the ants home. Rather, the ants had also memorized an odour's location, that is, whether it was left or right during homing.

Compared to intact ants, unilaterally antennectomized ants displayed a significantly broader nest search (Fig. 3) and travelled five times longer between turning points and the fictive nest (Fig. 4). When ants were trained to associate the nest entrance with a point source landmark, antennectomized animals headed for the nest as accurately as the intact control ants (Fig. 4). Thus, unilateral antennectomy did not affect the ants' ability to orient by olfactory cues in general, but impeded the orientation within an olfactory array.

When the test array was reduced to two odours, either adjacent or opposite the nest entrance, the ants still searched for the fictive nest in the correct position but were less focused (Fig. 5). The downwind odours I and D alone were not accepted as a nest-defining cue and search accuracy was low (Fig. 5). When tested with only the two upwind odours M and N, ants showed the same search accuracy as they showed when tested with the full array control (Fig. 5). Therefore, the actual odour composition at the nest entrance seemed to be necessary and sufficient for pinpointing the entrance, while the presence of those odours perceived on the way to the nest was not crucial. However, when the downwind odours were present, they had to be at the correct position (Fig. 5). Thus,

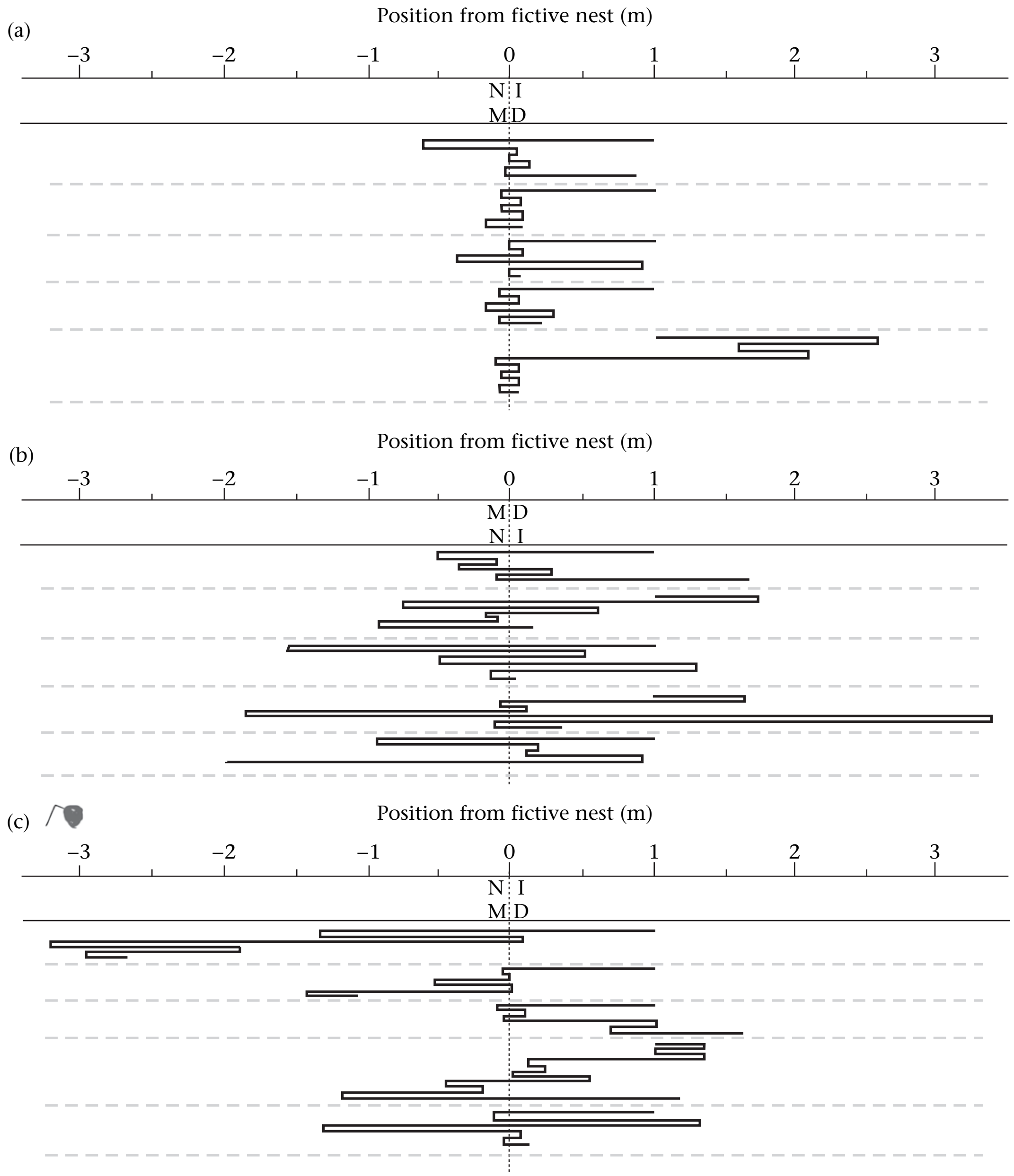

Figure 3. Example runs of intact ants tested with (a) the training array and (b) an exchanged array (with the positions of the odour sources exchanged) and (c) of unilaterally antennectomized ants tested with the training array. Compare with Fig. 4. Position of odour array, i.e. fictive nest: 0 m; point of release: 1 m. The first six turning points after the ant passed the nest for the first time were taken for analysis. For clarity, the runs are projected outside the channel on two dimensions. Horizontal dashed lines separate example runs.

ants had not simply learned mixtures of odours but, instead, had memorized the exact positions of the odour sources and the nest entrance relative to them.

Furthermore, the total amount of volatiles, that is, the amount of olfactory input, did not alter the ants' homing performance (Appendix Fig. A1).

DISCUSSION

In addition to their vision-guided orientation, *Cataglyphis* ants can learn to associate environmental odours with the nest entrance and use this knowledge to pinpoint the nest (Steck et al. 2009). The ability to pinpoint an odour source has been described for a wide range of insect species such as bark beetles, fruit flies, moths and mosquitoes (Murlis et al. 1992, and references therein). In these experiments, the animals simply had to follow a scent plume to find the odour. Furthermore, when honeybees, *Apis mellifera*, were trained to associate an odour with a food source, blowing that odour into the hive triggered these bees to carry out foraging flights in the direction of the food source (Reinhard et al. 2004). The olfactory navigation hypothesis suggests that birds learn an olfactory landscape by associating smells perceived at the home with the directions from which they are carried by winds (Papi et al. 1971; Wallraff 2004 and references therein; for seabirds see De Bose & Nevitt 2008 and references therein). Apparently, wind is the crosslink embedding atmospheric blends into a directional reference scale provided by the birds' compass. A plausible explanation for how pigeons can home after long-distance displacement is given by a simulated navigation based on the olfactory landscape (Wallraff 2004). Stable olfactory landscapes that could provide sufficient navigational information have been shown for mid-scale ranges of about 300 km in diameter (Wallraff & Andreae 2000) and

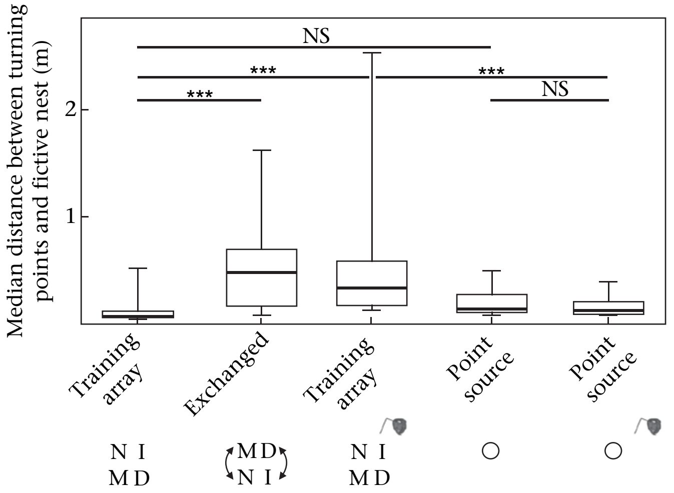

Figure 4. Median distances between turning points and the fictive nest of intact ants tested with the training array or an exchanged array (with the positions of the odour sources exchanged), unilaterally antennectomized ants trained and tested with the training array, intact ants trained and tested with a point odour source and unilaterally antennectomized ants trained and tested with a point odour source. Circle depicts point source landmark consisting of a mixture of all four odours. $N = 30$ ants per plot. Black line: median; box: interquartile range; whiskers: 10th and 90th percentiles. Statistical differences between the groups are shown for selected pairs (***$P < 0.001$, Kruskal–Wallis test with a Dunn's post hoc analysis).

for large-scale global ranges (Kettle et al. 1999). However, the difficulty of manipulating odours on such large scales complicates the in-depth investigation into how birds learn and use the olfactory landscape for navigational means. Our recent findings that *Cataglyphis* ants make use of environmental odours on a much smaller scale (Steck et al. 2009) enabled us to manipulate side-specific local blends and by doing so to decipher the navigational impact of the olfactory scenery.

Here we investigated whether ants were able to pinpoint the nest relative to surrounding odour sources. In short, did *Cataglyphis* ants memorize the topographic distribution of olfactory landmarks as if they were elements of an olfactory scenery? To answer this question, we trained ants in a situation where the nest entrance was situated within a two-dimensional array of four odours (Fig. 1). Therefore, the nest did not converge with the highest odour concentration (Fig. 2). Ants facing the same situation during the test focused their search on the fictive nest, that is, they learned the nest position relative to the surrounding odour sources (Figs 3, 4).

What are the underlying mechanisms of orientation within complex olfactory surroundings? The following two hypotheses are possible.

(1) The ants simply memorize the sequence of changing blends while moving upwind, that is, they focus their search at the position where they no longer smell the blend of the downwind odours but still smell the blend of the upwind odours.

(2) The ants memorize the olfactory topography, that is, which odours are positioned on the right or the left side during the ants' home-bound runs.

To discriminate between these hypotheses, we tested trained ants with a manipulated array (Figs 3, 4). When the down- and upwind odour pairs appeared in the training order (i.e. the ants had to pass I and D first) but the sides of the odours within the pairs were exchanged, the animals were less confident about the nest location (Fig. 4). Therefore, hypothesis 1, the ants learn the sequences of blends only, appears unlikely. The homing ants moreover memorized an odour to be on their left or on their right side. A further strong indication that the ants learned the spatial distribution of the odour sources rather than just the sequence of blends is the finding that unilateral antennectomy affected the ants' ability to orient in the complex array (Figs 3, 4). Learning the sequence of blends would have been possible with only one antenna. To sum up, we demonstrated that homing ants make use of olfactory topography in the vicinity of the nest entrance.

Do the ants succeed in navigating in an olfactory landscape by using a stereo sense of smell? When the olfactory system of *Drosophila*, which usually receives bilateral input, is restricted to unilateral input, the animals can no longer pinpoint odour sources (Borst & Heisenberg 1982; Louis et al. 2007). Louis et al. (2007) argued that this is a result of a decreased signal-to-noise ratio rather than of a loss of stereo smell. In our study, the loss of orientation within the complex array of unilaterally antennectomized ants was not caused by a decreased sensory input, as the loss of one antenna did not compromise the ants' ability to locate a point odour source (Fig. 3). Thus the signal-to-noise-ratio hypothesis does not explain our results.

What morphological requirements could be needed to process bilateral sensory input simultaneously? Although in *Drosophila*

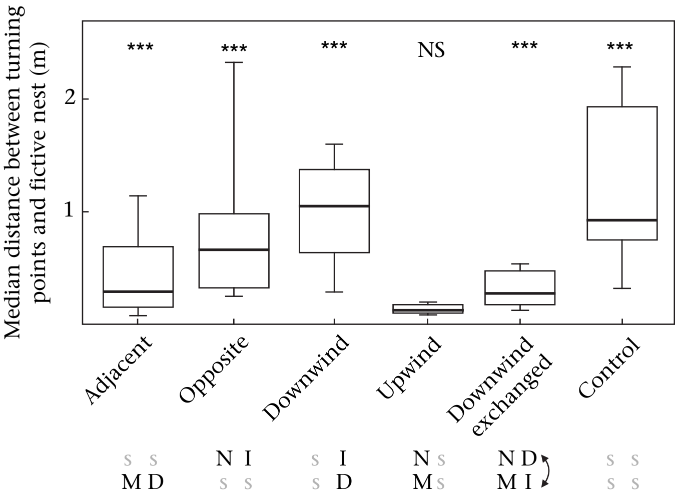

Figure 5. Median distances between turning points and the fictive nest of ants tested with two odours adjacent to the nest, opposite to the nest entrance, downwind of the nest, upwind of the nest, with the upwind odour pair at its training position but the downwind odours exchanged, and with the solvent (S) control. $N = 30$ ants per plot. Black line: median; box: interquartile range; whiskers: 10th and 90th percentiles. Statistical differences between the group of ants trained and tested with the olfactory array (see Fig. 4 left box) and the other groups are shown for selected pairs (***$P < 0.001$, Kruskal–Wallis test with a Dunn's post hoc analysis).

stimulation of one antenna results in ipsi- and contralateral activation in the antennal lobes (Stocker et al. 1990; Younossi-Hartenstein et al. 2002) fruit flies can discriminate an intensity gradient across the two antennae (Borst & Heisenberg 1982; Duistermars et al. 2009). In contrast to *Drosophila*, in hymenoptera odours activate the antennal lobes only ipsilaterally (Sandoz et al. 2002) which might even strengthen the animals' ability to process bidirectional input. Indeed, conditioning experiments with honeybees revealed antenna-specific odour learning, that is, the bees' olfactory system side-specifically processes the input from the left and the right antenna (Sandoz et al. 2002). In our experiments the visualization of the volatiles' distributions in the channel revealed considerable concentration differences over a distance as small as the span of an ant's antennae (Fig. 2b). Therefore it is very likely that the ants use the different input of both antennae, that is, a stereo sense of smell, to navigate in complex olfactory surroundings.

Stereo olfaction has been demonstrated for rats, *Rattus norvegicus*, and humans: rats can localize an odour source with a single sniff (Rajan et al. 2006) and humans are able to follow an odour trail (Porter et al. 2007). Both rats and humans perform less accurately when the bilateral olfactory input is reduced to a unilateral one. The use of a bilateral olfactory input has also been shown in insects. Honeybees (Martin 1965) and *Drosophila* (Borst & Heisenberg 1982; Duistermars et al. 2009) can localize an odour source most efficiently when they receive bilateral input from both antennae, and *Lasius fuliginosus* ants use bilateral olfactory input to follow a pheromone trail (Hangartner 1967). Our results that unilaterally antennectomized ants lose their ability to orient in an olfactory landscape, whereas they are still able to pinpoint a simple odour point source, can best be explained by the use of a stereo sense of smell.

At the beginning of the last century, Forel (1910) predicted that insects might also use bilateral antennal input to 'read' the topographical information conveyed by olfactory landmarks. This idea was later picked up by Von Frisch (1947). Our evidence suggests that homing *Cataglyphis* perceives the olfactory scenery around the nest in stereo and uses these olfactory landscapes for homing.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at doi:10.1016/j.anbehav.2010.01.011.

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APPENDIX

Does the amount of volatiles affect the ants' homing accuracy?

During training and testing, we reapplied the odours every 20 min on the channel ground. Owing to the high environmental temperature and the high volatility of the odours, we expected the odour landmarks to change considerably within this 20 min time window. We collected air samples from the landmarks during four consecutive 5 min periods after odour application and analysed the samples in a gas chromatograph. The amount of each of the four volatiles detected in the first sample during minutes 0–5 was defined as 100%. The concentrations of volatiles in the following three samples were normalized. During the 20 min between the reapplications of the odours, their concentrations dropped by at least 80% (Fig. A1a). However, when ants were trained with an array that was 20 min old and were tested with a newly applied one, they still displayed an accurate search pattern (Fig. A1b). In addition, ants that were trained with a continuously renewed odour array and tested with one 20 min old again displayed a focused search (Fig. A1b). Hence, it was not the intensity of the landmarks, that is, the total amount of volatiles, but rather the mixture of odours that was crucial for the ants' search accuracy.

When the odour array was reduced by two odour sources as well as when the ants were antennectomized, the accuracy of the ants' nest search decreased. This effect was not due to the reduced amount of volatiles or the reduced number of receptors. If it had been, one would have expected an effect of the changing odour concentrations, too.

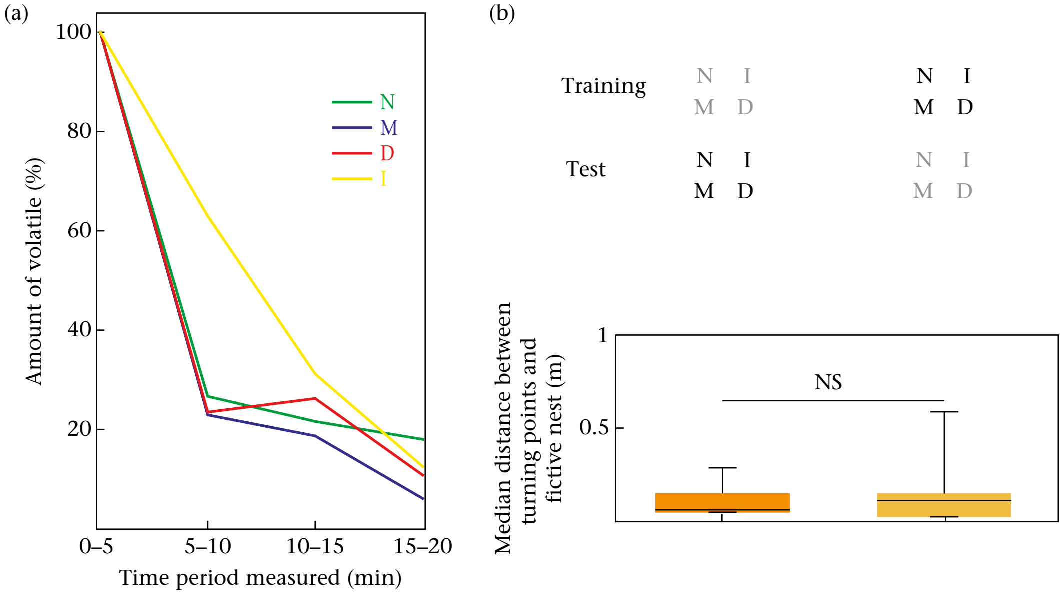

Figure A1. (a) Normalized amounts of volatiles measured for time periods between reapplications of odour array. (b) Effect of changing volatile concentration between training and test runs on search accuracy. Grey caption depicts old arrays, i.e. with lower amounts of volatiles; black caption depicts freshly reapplied arrays, i.e. with higher amounts of volatiles. $N = 15$ ants per plot. For further details, see Fig. 4.