# Desert ants use olfactory scenes for navigation

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## ARTICLE INFO

Desert ants, *Cataglyphis fortis*, forage for dead arthropods in the Tunisian salt pans. Both the unpredictable food distribution and the high surface temperatures might account for the fact that the ants do not use any pheromone trails. However, *Cataglyphis* has been shown to still use olfactory cues for navigation. For instance, the ants locate sparsely distributed food or pinpoint their inconspicuous nest entrance by following odour plumes. In this study we found that, as well as using odours to pinpoint a target, the ants might use environmental odours as olfactory landmarks when following habitual routes. When analysing odours collected at 100 positions in the desert, we found spatially distinct gradients of a range of different environmental odorants. Furthermore we confirm that individual foragers followed forager-specific routes when leaving the nest. Therefore these ants could potentially learn such olfactory landscape features along their stable routes. We, hence, asked whether ants could learn and use olfactory cues for route guidance. We trained ants to visit a stable feeder and presented them with a sequence of four different odours along the way. Homing ants that had already passed the odour alley on their way back were displaced to a remote test field and released at the starting point of an identical alley. Control ants that experienced the alley only during the test situation focused their search on the release point. Ants that had experienced the odours during training, however, biased their nest search towards the odour alley and performed straight walking segments along the alley. Hence, we found that ants learnt the olfactory cues along their homeward route and used these cues in the absence of other navigational information. Hence, desert ants seem to be able to use odour information to follow routes.
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Desert ants are expert navigators and their study has led to the unravelling of many mechanisms of navigation (for reviews see e.g. Wehner, 2003, 2009). While foraging, these ants have to travel through a hostile desert environment in which an unpredictable food distribution and high surface temperatures force the ants to forage individually rather than orienting along pheromone trails. The initial navigational mechanism for a forager is path integration (Muller & Wehner, 1988; Ronacher, 2008; Wehner & Srinivasan, 2003). Through the use of a skylight compass (Wehner & Muller, 2006) and some kind of odometer (Wittlinger, Wehner, & Wolf, 2006) ants can keep track of the direction and distance in which they have travelled such that they are continuously informed about their position relative to the nest. In addition to this strategy ants can use learnt information from visual cues to pinpoint a place of interest or to follow a habitual route (Wehner, Cheng, & Cruse, 2014; Zeil, 2012). Learning of visual cues for route guidance

allows ants to develop idiosyncratic routes and robustly travel back and forth between the nest and plentiful feeding sites (Collett, 2010; Collett, Dillmann, Giger, & Wehner, 1992; Graham, Fauria, & Collett, 2003; Kohler & Wehner, 2005; Mangan & Webb, 2012; Sommer, von Beeren, & Wehner, 2008; Wehner, Michel, & Antonsen, 1996; Wystrach, Schwarz, Schultheiss, Beugnon, & Cheng, 2011). Furthermore, it has been shown that ants can take advantage of other sensory cues when available (Buehlmann, Hansson, & Knaden, 2012a; Kleineidam, Ruchty, Casero-Montes, & Roces, 2007; Seidl & Wehner, 2006). One of these modalities is olfaction (Steck, 2012; Wolf, Wittlinger, & Bolek, 2012). For instance, *Cataglyphis fortis* ants are capable of using an odour to accurately localize the inconspicuous nest entrance after experiencing it during a training phase (Steck, Hansson, & Knaden, 2009). Moreover, they are even able to learn the nest position relative to a complex array of multiple odorants around the entrance (Steck, Knaden, & Hansson, 2010). These findings led to the question whether olfaction also plays a role in a more natural setting when the ants are foraging within the salt pan. *Cataglyphis fortis* ants use olfaction to detect and locate dead arthropods (Buehlmann, Graham, Hansson, & Knaden, 2014) and to augment other

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navigational systems to pinpoint their nest by following a nest-produced odour plume (Buehlmann, Hansson, & Knaden, 2012b).

Here we investigated whether the desert environment provides odour features that might be useful for route navigation. Furthermore, we asked whether *C. fortis* ants can use olfactory information as a route-defining cue.

## METHODS

### Chemical Environment

To study the ants' chemical environment we collected odour samples in their natural foraging area in the Tunisian salt pan. The salt pan near the village of Menzel Chaker (34°96′N, 10°41′E) is mainly devoid of vegetation and is a rather homogeneous habitat, although the flat ground has some structure, i.e. the salt crust can be interrupted by clefts, sandy areas or small pieces of wood or halophytic plants. A 100 m × 100 m grid was established using strings fixed with nails (mesh width, 10 m) and 100 odour samples were taken at the intersection points by using poly-dimethylsiloxane (PDMS) tubes. Pieces of PDMS analytical tube (length: 4 mm; inner (outer) diameter: 1.5 (2.3) mm; RCT Reichelt Chemietechnik GmbH &Co, Heidelberg, Germany) were cleaned overnight in the laboratory with 4:1 acetonitrile/methanol, pre-conditioned at 230 °C for 4 h under nitrogen flow in a tube conditioner (Gerstel) and stored in clean vials before use. In the field, PDMS tubes were placed at the sample sites by inserting them slightly into the ground. Five PDMS tubes were used per sample site. Cleaned glass petri dishes were used to cover the sampling sites in order to facilitate the relocation of the analytical tubes. To decrease the direct sun radiation, horizontal aluminium plates (250 × 250 × 0.5 mm) were installed 10 cm above each sampling site. Odours were collected for 84 h. Analytical tubes placed into cleaned glass petri dishes on top of the desert ground served as blanks. After collection the analytical tubes were stored in the freezer (−80 °C) for around 3 months until measurement.

Analytical tubes were analysed using a GERSTEL thermodesorption unit (TDU) coupled to a GERSTEL cooled injection system (CIS 4) on an Agilent GC (gas chromatography) 7890 A connected to a mass selective detector (MSD) 5975 C. The MS (mass spectrometry) operated in electron ionization mode (70 eV) with scans from 33 to 450 atomic mass units. Chemical compounds were separated on a 30 m × 250 μm HP-5 MS ultra inert column with a 0.25 μm film coating (Agilent Technologies; 19091S-433UI). Helium was used as carrier gas (constant flow 1 ml/min). The TDU temperature was increased from 30 °C to 210 °C at a rate of 30 °C/min and was held at 210 °C for 10 min. The thermodesorbed compounds were trapped in the cooled injection system (CIS 4) at −50 °C. The GC run started by heating the injection system from −50 °C to 220 °C at a rate of 12 °C/min and kept the end temperature for 5 min. The GC oven was set to 40 °C for 2 min and, a temperature ramp of 10 °C/min followed. The end temperature of 260 °C was held for 5 min. In each measurement two pieces of analytical tubes were used. Data were processed with MSD ChemStation Data Analysis Application (Agilent Technologies) and DataTrans.

Compounds were identified by comparison of mass spectra and KI values with those available in the NIST 2.0 mass spectra database (http://www.nist.gov/srd/nist1a.cfm). We do not intend to provide a complete description of the chromatograms but rather wanted to test, for a few selected substances, whether spatial gradients of environmental odours exist in the desert environment. Because of the huge number of peaks in the chromatograms (874 peaks with different retention times) we selected those peaks that reached a fit with NIST that was over 800 and additionally were found in at least six of the 100 sample sites. The relative quantity of a compound was calculated from the detected peak area normalized against the internal tube peak present throughout the measurements and the maximal value was set to 1. After selecting eight compounds by these rules we plotted the occurrence of these selected compounds for the 100 sample sites.

### Individual Foraging Tracks

Foraging paths of individual *C. fortis* foragers from one colony were tracked with GPS. Ants were individually colour coded and several foraging paths were tracked by following the ants with a GPS measurement device (GARMIN eTrex 20 or 30). GPS co-ordinates were taken every second. Owing to high satellite coverage the GPS units indicated an expected error for absolute position of less than 2 m. However, by following a predefined route and comparing the GPS output we found that even small movements of less than 20 cm were correctly tracked. GPS data were transferred to a PC using EasyGPS (http://www.easygps.com) and paths were analysed in Matlab (Mathworks, Natick, MA, U.S.A.). To analyse whether individual ants take consistent paths through the salt pan, the overall heading direction for the path segments from 6 to 50 m from the nest was determined. Having 10 ants with only one path each allowed us to calculate the mean pairwise difference between these paths (between-ant comparisons). Then for each ant from another group of 10 ants we took the difference in trajectory direction of two paths (within-ant comparisons). We could then ascertain the proportion of ants whose heading direction difference was smaller than the mean pairwise difference in trajectory direction between ants. We tested the significance of this proportion with a sign test.

### Olfactory Route Cues

To test whether ants are able to use olfactory information as part of their general route navigation we trained *C. fortis* foragers for at least 1 day to visit a feeding site containing biscuit crumbs that was located 10 m from their nest. The ants rapidly learnt to shuttle back and forth between the nest and the feeder. During training, ants experienced the following odours along the beeline of the route: (1) methyl salicylate, (2) decanal, (3) indole and (4) nonanal. As described in previous studies, these odours neither innately attract nor repel naïve ants, are learnt equally well and can be distinguished by the ants when applied in the current concentrations (Steck et al., 2009). Odours were presented in the sequence 11223344, with intervals of 1 m between each odour and 1.5 m gaps between the end of the odour array and nest and feeder. Odours were diluted in hexane (1:50) and 30 μl was pipetted onto a filter paper that was put in a 2 ml glass vial placed into the ground such that the opening was slightly lower than the ground level. The odours were renewed every 30 min which should guarantee that odour sources were never depleted (Steck et al., 2010). As the wind direction was mostly relatively stable, we were able to align the odour alley perpendicular to wind direction.

For tests, experienced ants returning from the feeder were captured just before they entered the nest and the search paths of these so-called zero-vector ants was recorded after releasing the ants at a distant test field either in the presence of the same (+/+ ants) or the reversed odour (+/+∗ ants) sequence. A 25 m × 25 m test field (mesh width, 1 m) was painted on the desert ground about 100 m from the training ground and the paths were recorded for 5 min on gridded paper. Wind direction was measured by using a small wind vane placed on the test field. As a control we recorded the search paths of ants that had been trained to the same feeder distance but had not experienced any odours along the route. These ants were later tested either without (−/− ants) or with (−/+ ants)