

Supplemental Data: Ants use the panoramic skyline as a visual cue during navigation.

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Supplemental Experimental Procedures

Background

There have been a variety of approaches to the difficult task of analysing how insects extract and use visual landmark information for navigation in complex natural environments. Here we present examples of four different techniques:

Displacement studies. One approach is to displace an insect away from its nest or familiar route, to a variety of locations and observe the attempted return. With bees this technique has been used to map which parts of the world are familiar to a forager [e.g., S1]. Additionally, with well-chosen hive locations and release points it is possible to use such a study to determine what visual landmark cues are important for the bee. For example, Southwick and Buchmann [S2] found that bees were successful at returning to hives that were close enough to nearby hills such that a useful skyline profile could be perceived from distance. This type of displacement study is particularly informative when studying ant behaviour because their earthbound routes are easier to record and therefore it is easier to deduce which objects are visible from along the route. A series of studies where ants successfully recovered after being displaced laterally from their habitual route [e.g., in our study species, S3] indicate that ants use large features of the environment for guidance, rather than small landmarks local to their route [reviewed in S4].

Masking. One way to investigate which visual features are used for navigation is to mask portions of the insect's field of view. Using this technique van Iersel and van den Assem were able to show that the digger wasp *Bembix rostrata* searches longer for its nest entrance when the view of distant landmarks was obscured [S5]. Similar masking studies with ants have shown how in particular circumstances accurate homing is dependent on specific large landmarks [S6,S7]. With *M. bagoti* we have used masking studies to investigate which elevations within a panoramic view are necessary and sufficient for visually guided homing [S8]. We were able to show that *M. bagoti* do not rely simply on the most prominent landmarks in the higher elevations of the panorama. Rather, it seems they rely on information from a range of locations in the panorama.

Substituting a similar location. If one has a specific hypothesis about which environmental features may be used as landmarks, this can be tested by releasing animals at a novel location which also contains the feature in question. Classic and modern work on honeybees has used this approach to demonstrate that large-scale landmarks are used for determining direction. In seminal experiments by von Frisch and Lindauer [S9], honeybees were trained during an afternoon to follow some conspicuous large-scale landmark such as the shore of a lake or a line of trees. Overnight, the hive was displaced to a location at which such a feature (e.g., line of trees) ran in a different compass direction. The majority of bees followed the landmark feature, which conflicted with the learned compass direction. Modern work has replicated this pattern [S10,S11]. Towne and Moscrip's experiments [S11] are intriguing because they had a 'control' condition where honeybees trained in one landscape and displaced to a distant novel location nevertheless showed strong orientation. Panoramic photos at the pre- and post-displacement locales showed a resemblance in skyline, leading the authors to suggest the use of skyline information for directional determination. Earlier studies with *Cataglyphis* ants [S12] had led to a similar conclusion, i.e., two locations with similar skyline profiles can be confused during navigation.

Artificial panoramas. Experiments using the methods outlined above have provided circumstantial evidence about the role the skyline profile might play in insect navigation. However, an explicit test of the skyline hypothesis only comes when we take animals from a familiar natural location and test their orientation in a controlled visually impoverished environment where only the skyline profile matches the familiar place. This type of arena, with an artificial skyline, has been used to demonstrate innate visually driven orientation in species inhabiting an intertidal habitat, where the skyline reliably discriminates sea from land [Fiddler crabs, S13; Sandhoppers, S14].

Given the strength of this technique, we adopted a similar approach. We investigated whether ants that had learnt a route in a natural environment would replicate their orientation

response in an arena where the skyline is a crude match to the familiar skyline and all other features (e.g., colours, distances) have no resemblance to the natural environment.

Detailed methods

The ants. Experiments were undertaken with two nests of *Melophorus bagoti* Lubbock within their natural semi-arid habitat at a field site 10 km south of Alice Springs, NT, Australia. The study nests and their surroundings are shown in Figure 1 and Figure S1. *M. bagoti* are solitary foragers, primarily collecting insects that have perished in the heat and they are well adapted to desert conditions, often foraging when substrate temperatures have surpassed 70°C [S15]. Even these ants, however, avoid the heat of the mid-day sun and their active foraging periods during this experiment were 1000-1200 and 1430-1600. These ants use a variety of navigational strategies, including path integration, and the use of landmarks for route guidance and search for a goal [S16].

Training. A feeder was established 5 m from the entrance to Nest 1 and for at least 1½ days prior to any tests, ants were allowed *ad lib* access to cookie crumbs. With this arrangement we observed a steady stream of foragers arriving at and departing from the feeder. Ants from a second nest approximately 35 m away also foraged at the feeder. To record the natural homing direction of ants from both nests and to demonstrate that ants were familiar with the feeder location we recorded the departure direction of zero-vector ants from the feeder.

Ants were selected for tests if they were observed to arrive at the feeder from the Nest → Feeder direction and if they also headed back to the nest along a reasonably direct route. These ants were captured near the nest entrance using a plastic cup. By temporarily blocking the nest entrance we could gain some control over the position of capture relative to the nest entrance. Thus we ensured that there was no systematic bias in the direction of any residual portions of ants' path integrated home vectors. A goniometer was used to measure ants' headings. The goniometer was a plastic board marked with 15° sectors so that headings could be measured after 15 cm and 30 cm. For a test the goniometer was briefly placed directly on top of the feeder and the zero-vector ant released at the board's centre by inverting the plastic cup.

Testing. The retinal elevation of the panoramic skyline from the feeder location was measured at 15° azimuthal intervals using a purpose built transit. At a distant test field an arena 1 m in radius was created using black plastic sheeting pegged to metal spikes. The height of the black plastic wall was varied so that from the centre of the arena the retinal elevation of the artificial skyline matched that of the natural scene viewed from the feeder at 15° intervals. In other respects the artificial panorama bore no resemblance to the natural scene. The test site was approximately 150 m from the training location and was specifically chosen so that no natural landmarks were large or close enough to interfere with the artificial skyline. The site also allowed the arena to be rotated (by 150°), again with minimal interference to the skyline from nearby landmarks.

For tests, ants were captured and released as in the zero-vector controls and their headings were recorded after 15 cm with a goniometer marked in 15° sectors. Headings were recorded after a short distance because we wanted to measure heading before the ants' movement radically altered the perceived skyline profile. Interestingly, we observed that often, following release, ants performed a scanning manoeuvre consisting of pure rotation. We suggest that this behaviour may be used to assess the visual surroundings before choosing a departure direction. Following tests, ants were recaptured to ensure that individuals were not tested multiple times. The time of day of testing consisted of the earlier part of the morning and the later part of the afternoon. These times of testing meant that with a rotated test arena, the direction according to the artificial skyline and according to the sky compass were placed in strong conflict.

Recording panoramic images. We recorded panoramic images from the feeder and test locations using a GoPano panoramic mirror mounted on a Canon Powershot A720. These images were unwrapped using Photowarp software (EyeSee 360 Inc.).

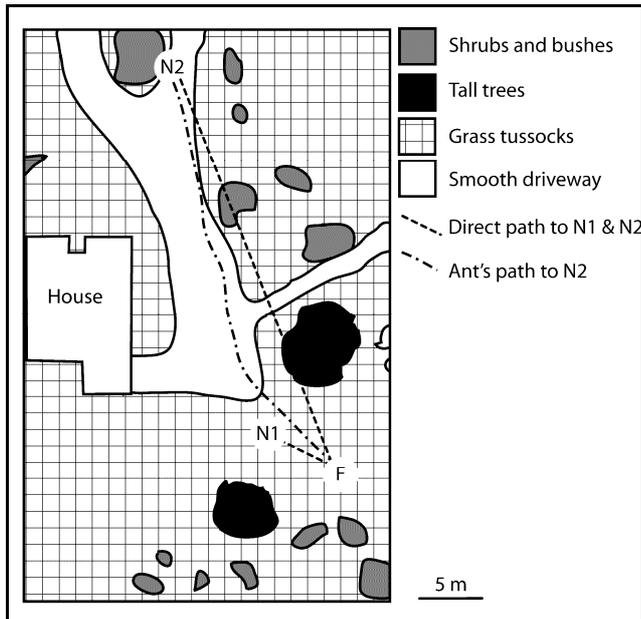


Figure S1.

Sketch of the field site and its surroundings. The area was characterized by grass tussocks and shrubs with two tall trees close to the training location. These trees formed the prominent beacons in the skyline profile. Ants from Nest 2 had to travel over 35 m to the feeder. Typically they did not take the direct route, instead they adopted a curved route which avoided grass tussocks and utilised the smooth driveway.

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