

RESEARCH ARTICLE

Combining sky and earth: desert ants (*Melophorus bagoti*) show weighted integration of celestial and terrestrial cues

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ABSTRACT

Insects typically use celestial sources of directional information for path integration, and terrestrial panoramic information for view-based navigation. Here we set celestial and terrestrial sources of directional information in conflict for homing desert ants (*Melophorus bagoti*). In the first experiment, ants learned to navigate out of a round experimental arena with a distinctive artificial panorama. On crucial tests, we rotated the arena to create a conflict between the artificial panorama and celestial information. In a second experiment, ants at a feeder in their natural visually-cluttered habitat were displaced prior to their homing journey so that the dictates of path integration (feeder to nest direction) based on a celestial compass conflicted with the dictates of view-based navigation (release point to nest direction) based on the natural terrestrial panorama. In both experiments, ants generally headed in a direction intermediate to the dictates of celestial and terrestrial information. In the second experiment, the ants put more weight on the terrestrial cues when they provided better directional information. We conclude that desert ants weight and integrate the dictates of celestial and terrestrial information in determining their initial heading, even when the two directional cues are highly discrepant.

KEY WORDS: Desert ant, Direction, Information, Integration, Navigation, Orientation

INTRODUCTION

Using multiple sources of information can improve decision making (Cheng et al., 2007; Körding, 2007). Because each source of information comes with its associated error, averaging multiple sources of information usually reduces the error, thus improving the decision. In some conditions, the optimal weighting of various sources of information can be specified, under what has been called a Bayesian analysis (Cheng et al., 2007; Deneve and Pouget, 2004; Körding, 2007). Roughly speaking, the smaller the variability or unreliability associated with a source, the more it should be weighted.

Many examples come from human perception and perceptual-motor actions (Cheng et al., 2007). Thus, cues from different modalities may be integrated. For example, Alais and Burr (Alais and Burr, 2004) showed that when both an auditory source and a visual source specify the direction to an object, the dictates of the two sources are averaged, and given approximately optimal

weighting. Visual and haptic sources of information are also integrated (Ernst and Banks, 2002; Gepstein and Banks, 2003), the cues again given approximately optimal weighting. Multiple sources of dynamic perceptual information may also be integrated. For example, when a visible beacon and a pattern of optical expansion provided in virtual reality each indicates a direction to walk in, humans use both sources (Warren et al., 2001), although it is not clear whether the integration was optimal in this case.

Examples of cue integration in other species are harder to come by, but some recent evidence is provided by studies of insect navigation. Reid and colleagues (Reid et al., 2011) presented homing night-active bull ants (*Myrmecia pyriformis*) with a conflict between celestial and terrestrial cues. This was achieved by using a polarising filter that rotated the pattern of polarisation by 45 deg, thus creating a discrepancy between this cue and the unmanipulated terrestrial panorama. The ants struck a compromise direction that reflected an average of the dictates of the two kinds of cue. In another myrmecine ant (the diurnal *Myrmecia croslandi*), Narendra and colleagues (Narendra et al., 2013) displaced them to examine the range of their familiar territory. The authors found that near the edge of their familiar terrain, *M. croslandi* struck a compromise between the dictates of celestial and terrestrial cues. Collett (Collett, 2012) tested North African desert ants (*Cataglyphis fortis*) on their outbound journey to a feeder via a two-legged path. Tests on displaced ants produced results suggesting a compromise between the dictates of path integration and those of the surrounding panorama, representing cues that had been set in conflict by the displacement. Collett (Collett, 2012) discussed the possibility that the ants were integrating cues in a Bayesian fashion.

Our study continues this line in the Australian desert ant *Melophorus bagoti* Lubbock 1883, also known as the red honey ant (Cheng et al., 2009; Cheng et al., 2014). Research over the past decade has shown that this ant possesses a suite of navigational capacities (Wystrach et al., 2013) like other well-studied insects such as *Cataglyphis* ants (Wehner, 2003; Wehner, 2009) or honeybees (Cheng, 2000; Srinivasan, 2011). *M. bagoti* integrates a path (Narendra, 2007a; Narendra, 2007b; Wehner et al., 2006), keeping track of the distance and direction travelled from its starting point (Collett and Collett, 2000; Wehner and Srinivasan, 2003). It uses panoramic terrestrial landmarks for navigation (Graham and Cheng, 2009; Wystrach et al., 2011a; Wystrach et al., 2012), often using the many trees, bushes and tussocks found in its central Australian semi-arid habitat to travel stereotypical routes (Kohler and Wehner, 2005; Sommer et al., 2008; Wehner et al., 2006; Wystrach et al., 2011b). It sometimes also uses the well-studied celestial compass (Wehner, 1994; Wehner, 1997; Wystrach et al., 2014) to navigate routes outside of the context of global path integration for finding the direct path to the nest, including finding the path to an exit (Legge et al., 2010), heading in the last direction travelled (Schwarz et al., 2011; Wystrach et al., 2013) or compensating for displacement by wind (Wystrach and Schwarz, 2013). As a last resort for finding places, it

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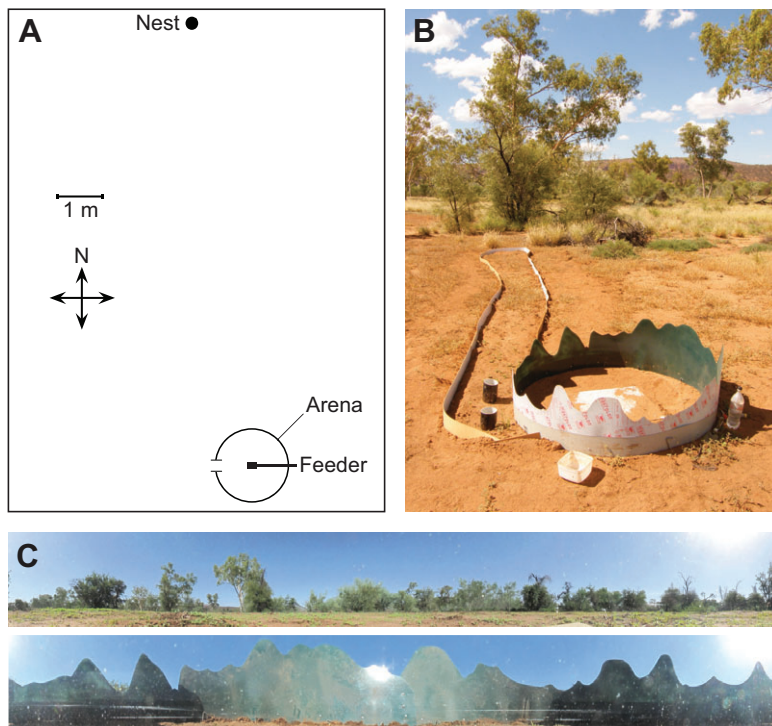


Fig. 1. Experimental setup using an arena to assess the importance of terrestrial cues in the Australian desert ant *Melophorus bagoti*. (A) Diagram depicting the position of the ants' nest relative to the training arena used in experiment 1. (B) Training arena used in experiment 1. The channel outside of the arena extends from the nest to the arena wall to prevent ants from foraging naturally. (C) Panoramic images (360 deg) taken from the centre of the arena (bottom image). The top image is a 360 deg panoramic image taken at the same location without the arena present. Note that the arena completely obscures terrestrial cues from the natural skyline, but is open at the top so that natural celestial cues remain available.

engages in systematic search (Schultheiss and Cheng, 2011; Schultheiss and Cheng, 2013; Schultheiss et al., 2013). Like published studies on humans (Alais and Burr, 2004; Warren et al., 2001), the current study investigated the integration of directional sources of information – both terrestrial and celestial – in these ants.

In one experiment, we set up an artificial terrestrial panorama in the form of a round arena with a 'skyline' of varying height (Fig. 1). Ants were trained to enter the arena and find a feeder at its centre. They had to enter and exit in a different direction (at 90 deg discrepancy) from the beeline direction connecting feeder and home. The entrance and the exit were in the same direction from the feeder. On crucial tests, the arena was rotated, putting terrestrial panorama and celestial cues in conflict. To be clear about what these cues are, the arena was uniformly coloured. The terrestrial cues consisted of the pattern of skyline formed by the arena wall. Celestial cues are cues with a celestial origin, sometimes reflected off terrestrial objects. They consist of the pattern of polarised light, which is known to be used by insects in path integration (Wehner, 1994; Wehner, 1997), the position of the sun (Wehner and Müller, 2006) and spectral cues (Wehner 1994; Wehner, 1997). Shadows in the arena are determined by the direction of the sun. The side of the arena opposite the direction of the sun has higher light intensity, and also reflects relatively more UV light.

The key results came from testing ants that had returned home from a foraging trip, but were caught just before they entered their nest. These ants have run off the vector according to path integration, and are thus called zero-vector ants. In the setting of an arena, we have evidence that *M. bagoti* can and sometimes do use celestial cues for orientation in the absence of path integration information (Legge et al., 2010). We also have evidence that they use the terrestrial skyline panorama formed by an arena (Graham and Cheng, 2009). Thus, we suspected that given a conflict between celestial and terrestrial panorama cues, ants without path integration information might integrate the two sources of information. We also tested ants taken directly from the feeder, which still have their vector to home based on path integration (full-vector ants).

In a second experiment, we displaced full-vector homing ants in their natural habitat before their journey home to create conflicts between celestial and terrestrial cues. Narendra (Narendra, 2007b) had effected such a manipulation, and found suggestive evidence that the ants used both terrestrial and celestial cues for orientation. We now provide a thorough and systematic assessment, using different release points that degraded the familiar view to different extents. Full-vector ants were displaced to three different release points (Fig. 2). The furthest release point from the feeder was at a location with little resemblance in the terrestrial panorama to the scenery at the feeder. This provided a control condition in which the ants were expected to use solely celestial information for homing, an expectation that was borne out by the results about to be reported. The other two release points, which are closer to the feeder, can then be compared with the control condition to estimate the extent to which terrestrial and celestial sources of information were used for homing. At these release points, the path integration system based on celestial compass cues points in the feeder–nest direction, whereas terrestrial cues based on the panoramic scenery points in the release-point–nest direction. Zero-vector ants were also tested to ensure that the terrestrial cues at the two closer release points could be and would be used. Past research has shown that when trained in natural conditions, the navigation of zero-vector *M. bagoti* ants is dominated by terrestrial cues (Graham and Cheng, 2009; Wystrach et al., 2012).

RESULTS

Use of an artificial skyline to investigate the importance of terrestrial cues

Zero-vector ants

In the first experiment, zero-vector ants had to navigate their way out of a circular arena based on the irregular skyline of the arena wall, with the exit at ~90 deg discrepancy with the beeline direction home. When the test arena was in the same orientation as the training arena, zero-vector ants were significantly oriented ($r=0.74$, $N=43$, $P<0.05$; Fig. 3A). Interestingly, ants did not choose a

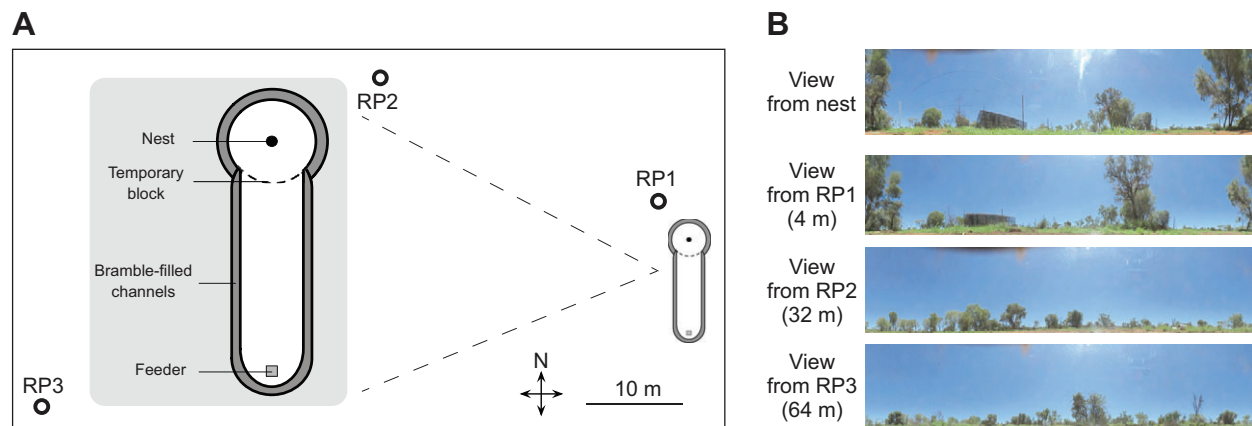


Fig. 2. Setup of the conflict experiment using the natural panorama (experiment 2). (A) Diagram of the setup used in experiment 2, showing the relative locations of the nest, feeder and release points (RP). Inset shows the setup around the nest and feeder. Channels were filled with brambles to discourage foragers from leaving the main training path. (B) Panoramic images (360 deg) taken at the nest entrance, and at the centre of each of the three release points used in experiment 2. Note that the images are ordered from top to bottom according to their Euclidian distance from the nest entrance. Euclidian distance from the nest entrance is indicated in parentheses for each image.

direction that would bring them directly to the arena exit (270 deg). Instead, they showed a systematic bias towards a point of the arena wall centred at 298.0 deg. Full-vector ants also headed initially in a similar direction (mean, 308.8 deg; supplementary material Fig. S1).

When the arena was rotated by 180 deg, celestial and arena cues indicated diametrically opposite directions of 298.0 deg and 118.0 deg, respectively. In this condition, ants were not significantly oriented ($r=0.14$, $N=45$, $P>0.05$; Fig. 3C), and the distribution appeared to be random with no sign of bimodality (Rayleigh test for

bimodality: $r=0.08$, $N=45$, $P>0.05$). When the arena was rotated by 90 deg, ants were significantly oriented (Fig. 3B; $r=0.71$, $N=48$, $P<0.001$). We compared the distribution in this condition against the distribution found on the control test. Their mean directions differed significantly (Watson–Williams test, $F_{1,89}=5.80$, $P=0.018$), indicating a significant influence of the rotated arena. When the arena was rotated by 270 deg, ants were once again significantly oriented (Fig. 3D; $r=0.55$, $N=45$, $P<0.001$). Comparing the headings in this condition against the headings found on the control test, their

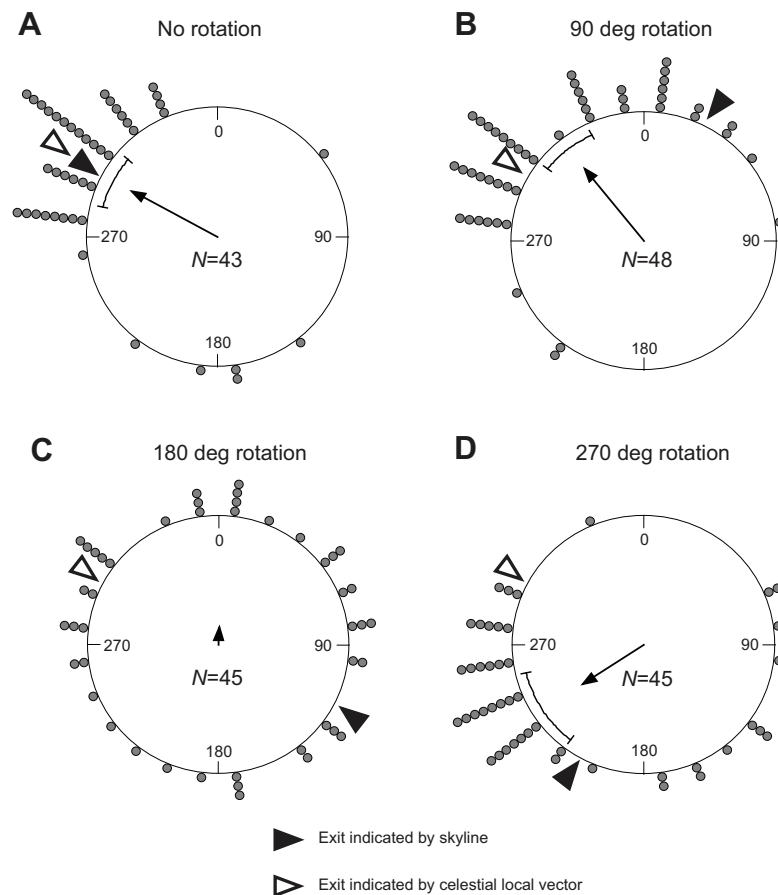


Fig. 3. Ants' directional bearings in the conflict experiment using the arena (experiment 1). Each grey circle indicates the directional bearing of one ant after crossing a marked goniometer line 30 cm from a central release location. Open triangular markers indicate the exit direction according to a learned route (celestial local vector). Closed triangular markers indicate the exit direction according to the view-based navigational system of the ants (terrestrial panorama). These were obtained by taking the closed triangle in the no-rotation condition and rotating it by the amount of rotation in each of the rotated conditions. Thus, these theoretical directions were determined by the performance of the control group rather than specified *a priori*. The true feeder–nest direction is 0 deg whereas the true feeder–exit direction is 270 deg. (A–D) Headers above each circular plot indicate the degree the testing arena was rotated relative to the orientation of the training arena. When ants were significantly oriented, the 95% confidence interval of the mean is displayed as a curved marker on the inside of the circular plot.

mean directions also differed significantly (Watson–Williams test, $F_{1,86}=26.11$, $P<0.001$). These comparisons show that both these groups were not using solely celestial cues for orientation, and that terrestrial cues had an effect on their navigation.

To test whether the unrotated cues – the celestial cues – also influenced the initial orientation of the ants in the 90 deg and 270 deg conditions, we then compared the distribution in each of these conditions against the headings in the control condition rotated by the same amount, thus 90 deg against control distribution rotated by 90 deg, and 270 deg against control distribution rotated by 270 deg. Both comparisons indicated significant differences in mean direction from the respective rotated controls (Watson–Williams test, 90 deg condition: $F_{1,89}=45.73$, $P<0.001$, 270 deg condition: $F_{1,89}=5.91$, $P=0.017$). Thus, in both cases, the celestial cues had an effect on initial orientation. The ants used both terrestrial and celestial sources of information to determine the direction of travel.

Full-vector ants

Although the exit of the arena was also at 270 deg for full-vector ants, they did not learn to fully adjust to that direction, with mean directions in all conditions 30–42 deg off (supplementary material Fig. S1). Ants in all of the conditions were significantly oriented (Rayleigh tests, $Z>31$, $P<0.0001$). None of the rotated conditions differed significantly from the control condition (Watson–Williams tests on pairwise comparisons, $F<1.74$, $P>0.19$; see supplementary material Table S1 for mean heading directions and scatter).

Numerically, however, the 90 deg and 270 deg conditions had mean vectors displaced slightly from the controls in the direction of rotation of the arena (supplementary material Table S1).

Conflict between celestial and terrestrial cues within a natural panorama (experiment 2)

In the second experiment, ants were attracted to a feeder in their natural habitat with an unobstructed view. Both full-vector and zero-vector ants were displaced to three different release point (RP) locations for testing, with one location (RP3) a long way away (64 m), where the ants were not expected to be able to use terrestrial cues. Examination of ants tested at RP3 showed that full-vector ants were significantly oriented ($r=0.88$, $N=72$, $P<0.0001$; Fig. 4F) but zero-vector ants were not ($r=0.05$, $N=68$, $P>0.05$; Fig. 4C). This shows that at this location the ants were only using celestial information to orient. It also shows that zero-vector ants tested in these conditions did not use the celestial cues to orient in the absence of directional dictates from path integration. We used the distribution of the full-vector RP3 group as a reference direction for the use of celestial cues.

We released zero-vector ants at RP1 (4 m from the nest) and RP2 (32 m from the nest) to test whether ants could use terrestrial cues to orient when information from path integration and its associated sky compass was not available. We found that at both release points, ants were significantly oriented (RP1: $r=0.79$, $N=108$, $P<0.001$; RP2: $r=0.47$, $N=75$, $P<0.001$) towards the true nest direction,

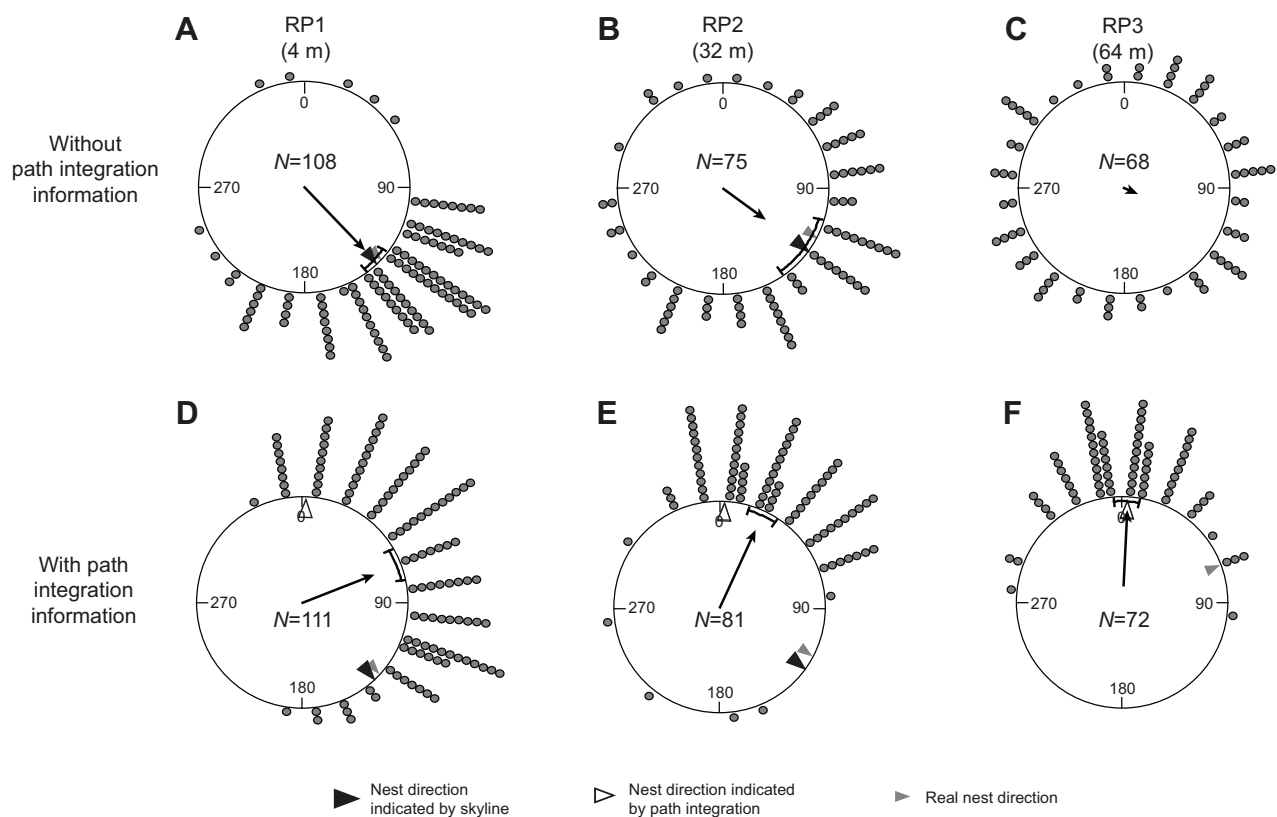


Fig. 4. Ants' directional bearings in the conflict experiment using the natural panorama (experiment 2). (A–F) Directional bearings of ants after crossing a marked goniometer line 30 cm from a central release location (grey circles). Closed black triangular markers indicate the nest direction according to the view-based navigation system, whereas the true nest direction is indicated by grey triangles. (D–F) Open triangular markers indicate the nest direction according to the path integration system (the true feeder–nest direction is 0 deg). Note that there are no open triangular markers in A–C because all ants tested here were zero-vector, and did not have access to path integration information. There are no closed triangular markers in F and C, because ants could not use view-based navigational cues to orient themselves at this release point (RP). When ants were significantly oriented, the 95% confidence interval of the mean is displayed as a curved marker on the inside of the circular plot.

indicating that ants could use terrestrial cues for orientation at these two locations (Fig. 4A,B). We used the distribution of these groups as reference directions for the use of terrestrial cues at each release point.

We conducted tests on full-vector ants at RP1 and RP2 to test the ants' orientation when celestial and terrestrial cues are placed in conflict. Celestial cues would point in the feeder–nest direction, whereas terrestrial cues would point in the release-point–nest direction. Ants were significantly oriented at both release points (Fig. 4D,E; RP1: $r=0.66$, $N=111$, $P<0.001$; RP2: $r=0.81$, $N=81$, $P<0.001$). At RP1, the distribution of headings differed significantly from both the full-vector RP3 group (representing the use of celestial information) and the zero-vector RP1 group (representing the use of terrestrial information) (Watson–Williams test; versus full-vector RP3: $F_{1,181}=112.45$, $P<0.001$; versus zero-vector RP1: $F_{1,217}=134.68$, $P<0.001$). Likewise at RP2, the distribution of headings differed significantly from both the full-vector RP3 group (representing the use of celestial information) and the zero-vector RP1 group (representing the use of terrestrial information) (Watson–Williams test; versus full-vector RP3: $F_{1,151}=13.81$, $P<0.001$; versus zero-vector RP2: $F_{1,154}=119.72$, $P<0.001$). Thus, at both release points, ants with a conflict between celestial and terrestrial information headed in an intermediate direction that differed significantly from the dictates of either celestial cues or terrestrial cues.

Finally, we estimated the weight placed on terrestrial versus celestial cues at RP1 and RP2 by using the mean directions of those groups, and calculating the proportion of angular distance they were from the reference directions representing the use of celestial and terrestrial cues, respectively. The full-vector ants at RP1 (Fig. 4D: 4 m distance from the nest) placed double the weight on terrestrial cues as did the full-vector ants at RP2 (Fig. 4E: 32 m from the nest; 0.36 versus 0.18). Thus, at the nearer release point, more weight was placed on terrestrial cues. Indeed, results with zero-vector ants show that the ants were more accurate at using the terrestrial cues at RP1 (Fig. 4A) than RP2 (Fig. 4B) (circular Wilcoxon test on scatter of zero-vector ants at RP2 versus those at RP1: $Z=4.43$, $N=183$, $P<0.001$).

DISCUSSION

In two experiments, we provided homing desert ants with conflicts between the dictates of terrestrial and celestial cues for direction. In the first experiment, this was done by rotating the arena that constituted the terrestrial cues for zero-vector ants. In the second experiment, this was done by displacing the ants from the feeder (full-vector ants) before letting them return home. In both experiments, we found evidence that the mean heading of the ants differed significantly from the dictates of both the celestial and the terrestrial cues, and was found to be in an intermediate direction. In experiment 1, however, an intermediate mean heading was only found with rotations of 90 deg (the 90 deg and 270 deg conditions). It was not found when the terrestrial cues (the arena) were rotated 180 deg; in that case, the ants showed a random distribution of headings.

Consistent with Collett (Collett, 2012), we interpret the results to mean that the ants were using both kinds of cues in these test situations. Their headings were based on a weighted average of the dictates of the two kinds of cues. The findings extend the integration of multiple cues for navigation to another ant species tested in different conditions. Integration of multiple cues has now been shown in *M. pyriformis* (Reid et al., 2011), *M. croslandi* (Narendra et al., 2013), *C. fortis* (Collett, 2012) and *M. bagoti* (current results), across a range of cue manipulations.

The 90 deg and 270 deg conditions in experiment 1 differed in that in the former condition, the ants placed more reliance (weight) on the unrotated (celestial) cues while in the latter condition, they placed more reliance on the rotated (terrestrial) cues. It is possible that this difference in weighting occurred because of the pattern of light reflected off the arena wall by the sun. Perhaps by rotating the arena by 90 deg, the visual appearance of the arena changed more than when the arena was rotated by 270 deg. If this was the case, the informative value of the visual panorama, and hence the weight assigned to it, was altered asymmetrically by the arena rotation. Future experiments will be required to determine whether this is indeed the case.

The second experiment also shows that the weight assigned to terrestrial versus celestial cues can vary, but in this case, a clear *a priori* explanation can be provided. At the release point closer to the feeder, with presumably a panorama that is more familiar and more similar to that at the feeder, the ants put more weight on terrestrial versus celestial directional cues (compare Fig. 4C and D). Evidence concerning the familiarity or similarity of the release sites comes from the scatter of headings of zero-vector ants released at these two locations (Fig. 4A,B): the group released closer to the feeder had smaller directional scatter (Fig. 4A). If the more familiar view is associated with a more reliable directional signal (Wystrach and Graham, 2012), this pattern accords with Bayesian principles (Cheng et al., 2007; Friedman et al., 2013; Körding, 2007), at least qualitatively: the weight assigned to a cue is in accord with its reliability.

In experiment 1, when cues conflicted by 180 deg, the zero-vector ants were not oriented. Two interpretations are plausible. One is that the ants were not engaged in any systematic heading at all, but started a systematic search pattern, which might start in any direction. This would presumably be caused by the large mismatch between the dictates of celestial and terrestrial cues. The other interpretation is that the ants were actually averaging the highly discrepant cues. But variable weights across animals accorded to the two types of cues resulted in a random distribution. We can report that under some conditions, *M. bagoti* ants do integrate different celestial cues at 180 deg discrepancy (Wystrach et al., 2014). This took place in full-vector ants when the position of the sun, one of the celestial cues, was mirror-reflected by 180 deg. According to theoretical derivations based on Bayesian principles, however, cues that are too discrepant should not be averaged, because the chances are that they are associated with two different objects or sources rather than one (Körding et al., 2007). Perhaps this species of ants integrate multiple cues no matter how discrepant they are, large discrepancies being rare in their lives.

The way in which celestial cues are used may differ between the artificial arena environment used in the first experiment and the natural environment used in the second. In the arena, rotation of terrestrial cues provided by the panorama had no significant effect on full-vector ants (see supplementary material Fig. S1, Table S1). We assume that, in this case, the artificial terrestrial cues were relatively weak and the path integration system based on celestial cues dominated the search for the full-vector ants. Nevertheless, the performance of these ants suggested the averaging of two directional dictates. As full-vector ants, they would have a directional dictate from path integration pointing in the feeder–nest direction, at 0 deg. The reward contingencies, however, favour an initial heading at 270 deg, where the exit was located. We could interpret the behaviour of these ants as an integration of these two dictates. But such an interpretation is fraught with uncertainties because when we observed the ants in training, none of them took a direct path from the feeder to the exit. A 270 deg initial heading had never been

rewarded because it was not executed. The ants ended up quite efficient at getting out of the arena with their not fully recalibrated initial heading. They would bias their searching to the left (towards the exit) when they got to the wall. This is just as likely a single incomplete recalibration process as a process of integrating conflicting dictates. In a different setup, over open field, North African ants (*Cataglyphis fortis*) performed similarly (Wehner et al., 2002). These ants did not have much, if anything, by way of terrestrial cues because their habitat was an open salt pan. They were repeatedly displaced from a feeder, as full-vector ants, before their return journey. *C. fortis* too did not recalibrate fully – the vector derived from the outbound path dominated, even after much training. They too, biased their search pattern in the direction where the nest was expected based on their training experience. *C. fortis* ants displaced radially (along the feeder–nest axis) in a channel also adjusted their search patterns according to their training experience (Cheng and Wehner, 2002).

Functionally, it actually makes sense to err reliably – in the limit always – to one side of a small goal. When the traveller gets to the wall, it ‘knows’ the direction that it has to turn to get to the exit. If one aims directly at small opening and misses, one would not know which way to turn to begin the systematic search for the exit. Indeed, *C. fortis* ants compensate reliably for navigational errors by aiming slightly downwind from a food source, at a site with a constant wind direction (Wolf and Wehner, 2005). In this fashion, they can be sure to catch the odour plume and then navigate to the food source.

By contrast, rotation of terrestrial cues provided by the panorama in the arena resulted in cue integration by zero-vector ants. When the path integration system was inactivated for the zero-vector ants, both celestial and terrestrial cues were used by the view-based system and the information was integrated when the cues conflicted. In the natural environment used in experiment 2, full-vector ants integrated celestial-based path integration and terrestrial information, suggesting that the natural terrestrial cues were sufficiently salient to exert some control, even when path-integration information was present (Fig. 4D,E). However, celestial cues were not used to orient in the absence of path integration (zero-vector ants, Fig. 4A,B), presumably because the natural terrestrial cues were salient and had dominated during learning. Similar dominance of terrestrial cues for zero-vector ants that had learned a natural panorama was found in a study by Graham and Cheng (Graham and Cheng, 2009): they suggested that it was the learning conditions that mattered. Graham and Cheng replaced the natural panorama on tests with a black arena approximating the skyline at the feeder. This impoverished test panorama had full control of the orientation of zero-vector ants when it was rotated. Further research is needed to verify these hypotheses.

The integration of cues is found across diverse taxa (Cheng et al., 2007), reflecting perhaps some convergent evolution driven by functional Bayesian principles. Among hymenopterans, various pieces of evidence suggest that honeybees integrate multiple kinds of cues, for instance in their dance behaviour (von Frisch, 1948; von Frisch, 1949) and in striking a heading after displacement (Menzel et al., 1998). Recent models of insect navigation accommodate cue integration well (Collett, 2012; Cruse and Wehner, 2011; Wystrach et al., 2013). Although these models posit a great deal of modularity, with different subsystems devoted to processing different cues for particular tasks, they suggest that the different ‘modules’ feed into a common centre that integrates the cues and directs motor output. Computationally, this provides a locus at which weights may be assigned to different cues. This process of assigning weights, however, should not be interpreted too mentalistically as some decision process strategically parcelling out weights. It might

somehow emerge from more basic neurobiological processes of information processing. Indeed, much work is needed to discover the neurobiological bases of cue integration and to elucidate how widespread it is in insect navigation.

MATERIALS AND METHODS

Field site and study species

Experiments took place ~10 km south of Alice Springs town centre, at a site filled with tussocks, mostly of the invasive buffel grass (*Cenchrus ciliaris*), with a scattering of bushes, trees, and some buildings. Nests of red honey ants (*M. bagoti*) are found in the area, and one nest was used for each experiment ($N=362$ in experiment 1 and $N=132$ in experiment 2). The species is highly thermophilic (Christian and Morton, 1992): when tested in a closed flask with 3 mm of soil, its critical thermal maximum was determined to be ~56.7°C. It forages solitarily in the heat of the day during the summer months on a range of dead animals and plant materials (Muser et al., 2005; Schultheiss and Nooten, 2013).

Training setup for arena experiment

Ants were trained to visit a feeder provisioned with pieces of cookies and mealworm. To encourage the ants to visit the feeder, we set up an enclosure that surrounded both nest and feeder. The enclosure was constructed of plastic-coated particle boards of 10 cm width, with the plastic side inwards. This prevented most ants from exiting the enclosure and encouraged them to visit the feeder. We sank the boards into the ground so that the tops were at ground level. We dug a trench on the inside and filled it with debris. This allowed ants unobstructed views of the panorama as they travelled to and from the feeder. Otherwise, we cleared the enclosure of debris and vegetation regularly during experimentation.

The ants had to enter a circular arena to get to the feeder (Fig. 1). The outside entrance to the arena was marked with two short black cylinders. These cylinders were not visible from inside the arena (see Fig. 1B). The training arena (diameter=1.5 m, maximum height=50 cm) was made out of 1 mm Lexan™ polycarbonate resin thermoplastic (colour: flat green). We placed it in the field so that its centre was 9.1 m South of the nest (compass bearing to the nest: 350 deg). The location of the arena entrance/exit was located at a compass bearing of 270 deg relative to the nest entrance, which was dug under the wall (Fig. 1A). The arena had irregular height in its walls (see Fig. 1B,C). The feeder was a plastic box (~15 cm square and ~9 cm deep). We coated it with fluon to prevent ants from climbing out. During training, we placed sticks in the feeder as exit ramps for the ants. Around the feeder, we placed a goniometer made out of the same Lexan material (30 cm radius). This goniometer was transparent and lightly dusted with sand to mimic the natural substrate. We set up a second circular arena with the same pattern of wall height for testing. This arena had no exit and no feeder. The goniometer in the test arena did not have a hole where the feeder would be. It was divided into 24 sectors of 15 deg each. We drew a circle of 30 cm radius on the goniometer.

Procedure for arena experiment

On first arrival at the feeder, we marked each ant with a dot of acrylic paint on the thorax or abdomen to mark the day on which it started training. We left ants to forage naturally for at least 1 day before testing. On a test, we allowed an ant to enter the feeder, take a bit of food and head home. Just before the ant entered the nest, we captured it and placed it in the centre of the goniometer in the testing arena. In four different testing conditions, we rotated the test arena 0, 90, 180 or 270 deg relative to the training arena. We tested each ant only once, always in the afternoon between 13:00 h and 18:00 h local time. We only tested ants that held on to a piece of food, to ensure motivation for homing. Data gathered consisted of the sector that the tested ant crossed at 30 cm after being released at the centre.

Full-vector ants came from the same nest, and were trained in the same way as the zero-vector ants. After at least 1 day of training, a full-vector ant holding on to food was taken directly from the feeder to the test arena. It was released in the middle of the goniometer for a single test. The sector at which it crossed at 30 cm was noted. The same four conditions of testing were effected and the ant faced the replica of the training arena rotated by 0, 90, 180 or 270 deg.

Training setup for nest-site experiment

We again made an enclosure using particle boards, this time consisting of a circle around the nest (1.5 m diameter) with a long extension down one side (Fig. 2A). We removed the board joining the circle to the long extension when training ants. Otherwise, we kept the ants within the circle surrounding their nests to limit their visual experience. We used the same kind of feeder as in experiment 1, this time placed 11 m South of the nest (direction from feeder to nest).

Procedure for nest-site experiment

We again marked ants at the feeder with one dot of paint to identify the day at which they started training. After at least one full day of training, we tested ants in one of six conditions. In three of the conditions, we captured ants directly from the feeder for testing on the three release points (RPs; full-vector ants). In the other three conditions, we captured ants very close to the nest entrance for testing on the three release points (zero-vector ants). All tested ants were loaded with food to ensure homing motivation.

We tested full-vector and zero-vector ants at the same three different release points, whose panoramic views are displayed in Fig. 2B. RP3 is a long way from the feeder, making its view unfamiliar, and we expected the ants to rely completely on celestial information for navigation at this site. RP1 and RP2 are closer, and at these release points, celestial information based on path integration and terrestrial information based on view-matching were in conflict. At each release site, we set up a wooden goniometer divided into 24 sectors, and we released the ant at the centre of the goniometer. Again, we noted the sector which the ant crossed at 30 cm from the starting point. In this experiment, we tested each ant twice, at two of the three release points, chosen at random and balanced across ants.

Data analysis

We conducted circular statistics following Batschelet (Batschelet, 1981), using either Oriana software (Kovach Computing Services, Anglesey, Wales, UK) or MATLAB (MathWorks, Natick, MA, USA). We used Rayleigh tests to examine whether ants were significantly oriented in any direction in a condition, and the Watson–Williams test to examine whether the mean direction in two conditions differed. We set the alpha level at $P=0.05$.

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors made significant contributions to the conceptualization of this project, including the precise question of study, design of the experiments, and analysis and interpretation collected data. All authors also made significant contributions to the preparation and editing of this manuscript. E.L. and A.W. conducted the two experiments presented in this paper and collected all relevant research data.

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Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.107862/-/DC1>

References

Alais, D. and Burr, D. (2004). The ventriloquist effect results from near-optimal bimodal integration. *Curr. Biol.* **14**, 257–262.

Batschelet, E. (1981). *Circular Statistics in Biology*. New York, NY; London: Academic Press.

Cheng, K. (2000). How honeybees find a place: lessons from a simple mind. *Anim. Learn. Behav.* **28**, 1–15.

Cheng, K. and Wehner, R. (2002). Navigating desert ants (*Cataglyphis fortis*) learn to alter their search patterns on their homebound journey. *Physiol. Entomol.* **27**, 285–290.

Cheng, K., Shettleworth, S. J., Huttenlocher, J. and Rieser, J. J. (2007). Bayesian integration of spatial information. *Psychol. Bull.* **133**, 625–637.

Cheng, K., Narendra, A., Sommer, S. and Wehner, R. (2009). Traveling in clutter: navigation in the Central Australian desert ant *Melophorus bagoti*. *Behav. Processes* **80**, 261–268.

Cheng, K., Schultheiss, P., Schwarz, S., Wystrach, A. and Wehner, R. (2014). Beginnings of a synthetic approach to desert ant navigation. *Behav. Processes* **102**, 51–61.

Christian, K. A. and Morton, S. R. (1992). Extreme thermophilia in a Central Australian ant, *Melophorus bagoti*. *Physiol. Zool.* **65**, 885–905.

Collett, M. (2012). How navigational guidance systems are combined in a desert ant. *Curr. Biol.* **22**, 927–932.

Collett, T. S. and Collett, M. (2000). Path integration in insects. *Curr. Opin. Neurobiol.* **10**, 757–762.

Cruse, H. and Wehner, R. (2011). No need for a cognitive map: decentralized memory for insect navigation. *PLoS Comput. Biol.* **7**, e1002009.

Deneve, S. and Pouget, A. (2004). Bayesian multisensory integration and cross-modal spatial links. *J. Physiol. Paris* **98**, 249–258.

Ernst, M. O. and Banks, M. S. (2002). Humans integrate visual and haptic information in a statistically optimal fashion. *Nature* **415**, 429–433.

Friedman, A., Ludvig, E. A., Legge, E. L. G. and Vuong, Q. C. (2013). Bayesian combination of two-dimensional location estimates. *Behav. Res. Methods* **45**, 98–107.

Gepshtein, S. and Banks, M. S. (2003). Viewing geometry determines how vision and haptics combine in size perception. *Curr. Biol.* **13**, 483–488.

Graham, P. and Cheng, K. (2009). Ants use the panoramic skyline as a visual cue during navigation. *Curr. Biol.* **19**, R935–R937.

Kohler, M. and Wehner, R. (2005). Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: how do they interact with path-integration vectors? *Neurobiol. Learn. Mem.* **83**, 1–12.

Körding, K. (2007). Decision theory: what “should” the nervous system do? *Science* **318**, 606–610.

Körding, K. P., Beierholm, U., Ma, W. J., Quartz, S., Tenenbaum, J. B. and Shams, L. (2007). Causal inference in multisensory perception. *PLoS ONE* **2**, e943.

Legge, E. L. G., Spetch, M. L. and Cheng, K. (2010). Not using the obvious: desert ants, *Melophorus bagoti*, learn local vectors but not beacons in an arena. *Anim. Cogn.* **13**, 849–860.

Menzel, R., Geiger, K., Joerges, J., Müller, U. and Chittka, L. (1998). Bees travel novel homeward routes by integrating separately acquired vector memories. *Anim. Behav.* **55**, 139–152.

Muser, B., Sommer, S., Wolf, H. and Wehner, R. (2005). Foraging ecology of the thermophilic Australian desert ant, *Melophorus bagoti*. *Aust. J. Zool.* **53**, 301–311.

Narendra, A. (2007a). Homing strategies of the Australian desert ant *Melophorus bagoti*. I. Proportional path-integration takes the ant half-way home. *J. Exp. Biol.* **210**, 1798–1803.

Narendra, A. (2007b). Homing strategies of the Australian desert ant *Melophorus bagoti*. II. Interaction of the path integrator with visual cue information. *J. Exp. Biol.* **210**, 1804–1812.

Narendra, A., Gourmaud, S. and Zeil, J. (2013). Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. *Proc. R. Soc. B* **280**, 20130683.

Reid, S. F., Narendra, A., Hemmi, J. M. and Zeil, J. (2011). Polarised skylight and the landmark panorama provide night-active bull ants with compass information during route following. *J. Exp. Biol.* **214**, 363–370.

Schultheiss, P. and Cheng, K. (2011). Finding the nest: inbound searching behaviour in the Australian desert ant, *Melophorus bagoti*. *Anim. Behav.* **81**, 1031–1038.

Schultheiss, P. and Cheng, K. (2013). Finding food: outbound searching behavior in the Australian desert ant *Melophorus bagoti*. *Behav. Ecol.* **24**, 128–135.

Schultheiss, P. and Nooten, S. S. (2013). Foraging patterns and strategies in an Australian desert ant. *Austral Ecol.* **38**, 942–951.

Schultheiss, P., Wystrach, A., Legge, E. L. G. and Cheng, K. (2013). Information content of visual scenes influences systematic search of desert ants. *J. Exp. Biol.* **216**, 742–749.

Schwarz, S. and Cheng, K. (2011). Visual discrimination, sequential learning, and memory retrieval in the Australian desert ant *Melophorus bagoti*. *Anim. Cognit.* **14**, 861–870.

Sommer, S., von Beeren, C. and Wehner, R. (2008). Multiroute memories in desert ants. *Proc. Natl. Acad. Sci. USA* **105**, 317–322.

Srinivasan, M. V. (2011). Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics. *Physiol. Rev.* **91**, 413–460.

von Frisch, K. (1948). Gelöste und ungelöste Rätsel der Bienen. *Naturwissenschaften* **35**, 38–43.

von Frisch, K. (1949). Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzern der Bienen. *Experientia* **5**, 142–148.

Warren, W. H., Jr, Kay, B. A., Zosh, W. D., Duchon, A. P. and Sahuc, S. (2001). Optic flow is used to control human walking. *Nat. Neurosci.* **4**, 213–216.

Wehner, R. (1994). The polarization-vision project: championing organismic biology. *Fortschr. Zool.* **39**, 103–143.

Wehner, R. (1997). The ant's celestial compass system: spectral and polarization channels. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 145–185. Basel: Birkhäuser Verlag.

- Wehner, R.** (2003). Desert ant navigation: how miniature brains solve complex tasks. *J. Comp. Physiol. A* **189**, 579-588.
- Wehner, R.** (2009). The architecture of the desert ant's navigational toolkit (Hymenoptera: Formicidae). *Myrmecol. News* **12**, 85-96.
- Wehner, R. and Müller, M.** (2006). The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. *Proc. Natl. Acad. Sci. USA* **103**, 12575-12579.
- Wehner, R. and Srinivasan, M. V.** (2003). Path integration in insects. In *The Neurobiology of Spatial Behaviour* (ed. K. J. Jeffery), pp. 9-30. Oxford: Oxford University Press.
- Wehner, R., Gallizzi, K., Frei, C. and Vesely, M.** (2002). Calibration processes in desert ant navigation: vector courses and systematic search. *J. Comp. Physiol. A* **188**, 683-693.
- Wehner, R., Boyer, M., Loertscher, F., Sommer, S. and Menzi, U.** (2006). Ant navigation: one-way routes rather than maps. *Curr. Biol.* **16**, 75-79.
- Wolf, H. and Wehner, R.** (2005). Desert ants compensate for navigation uncertainty. *J. Exp. Biol.* **208**, 4223-4230.
- Wystrach, A. and Graham, P.** (2012). What can we learn from studies of insect navigation? *Anim. Behav.* **84**, 13-20.
- Wystrach, A. and Schwarz, S.** (2013). Ants use a predictive mechanism to compensate for passive displacements by wind. *Curr. Biol.* **23**, R1083-R1085.
- Wystrach, A., Beugnon, G. and Cheng, K.** (2011a). Landmarks or panoramas: what do navigating ants attend to for guidance? *Front. Zool.* **8**, 21.
- Wystrach, A., Schwarz, S., Schultheiss, P., Beugnon, G. and Cheng, K.** (2011b). Views, landmarks, and routes: how do desert ants negotiate an obstacle course? *J. Comp. Physiol. A* **197**, 167-179.
- Wystrach, A., Beugnon, G. and Cheng, K.** (2012). Ants might use different view-matching strategies on and off the route. *J. Exp. Biol.* **215**, 44-55.
- Wystrach, A., Schwarz, S., Baniël, A. and Cheng, K.** (2013). Backtracking behaviour in lost ants: an additional strategy in their navigational toolkit. *Proc. R. Soc. B* **280**, 20131677.
- Wystrach, A., Schwarz, S., Schultheiss, P., Baniël, A. and Cheng, K.** (2014). Multiple sources of celestial compass information in the Central Australian desert ant *Melophorus bagoti*. *J. Comp. Physiol. A* **200**, 591-601.

Full Vector Ants

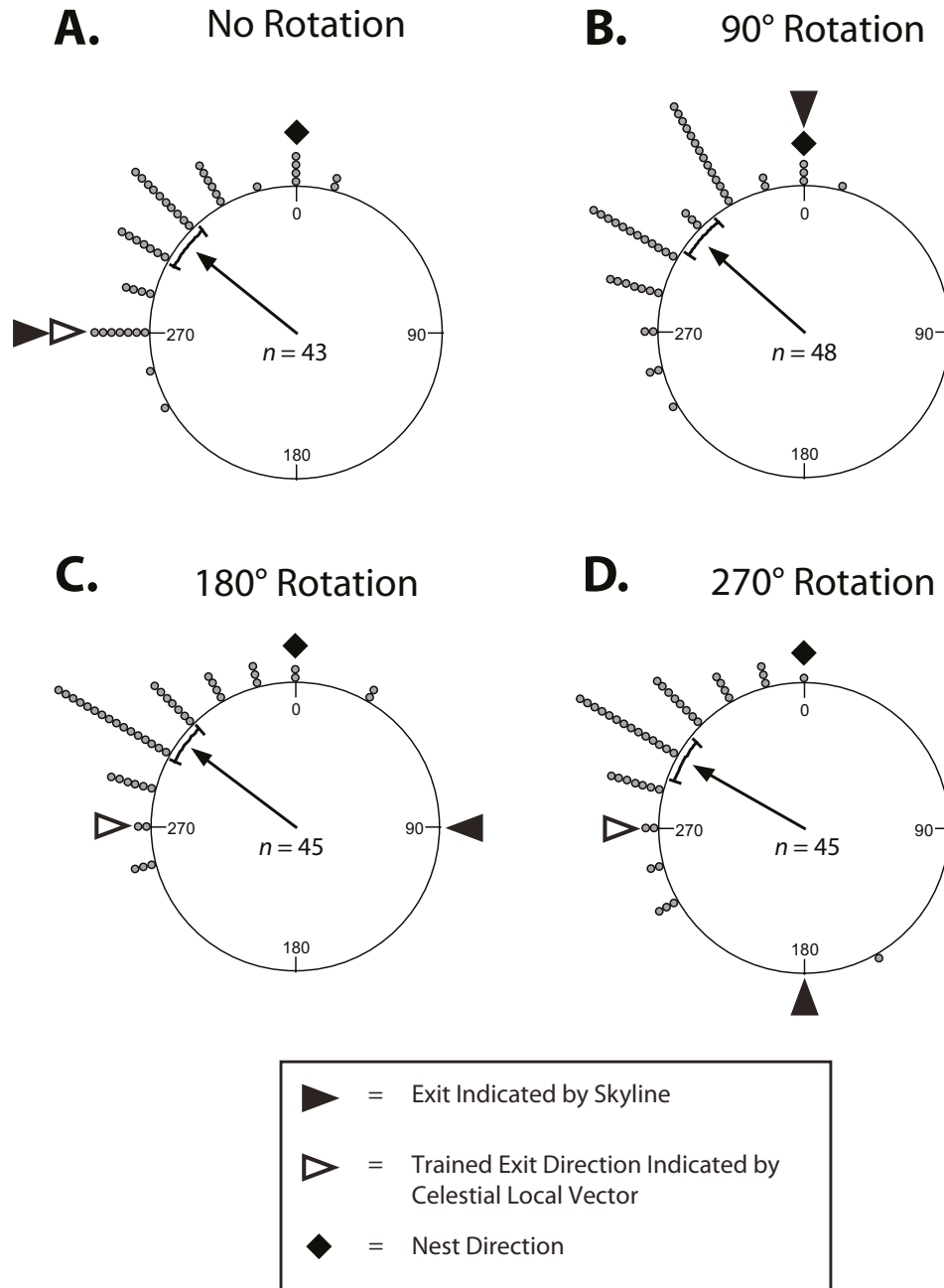


Fig. S1. Full-vector ant directional bearings for each test in Experiment 1. Grey circles indicate ants' directional bearings after crossing a marked goniometer line 30cm from a central release location. Open triangular markers indicate the nest direction. Black diamond markers indicates the trained exit direction of the arena according to a learned route (celestial local vector). Closed triangular markers indicate the exit direction according to ants' view-based navigational system (terrestrial panorama). Headers above each circular plot indicate the degree the testing arena was rotated relative to the orientation of the training arena. The 95% confidence interval of the mean is displayed as a curved marker on the inside of the circular plot.

Table S1. Mean heading direction and scatter of full-vector ants in each arena

Arena rotation	Mean heading direction	Circular SD (scatter)
0 deg	308.76 deg	0.15
90 deg	311.61 deg	0.16
180 deg	307.29 deg	0.13
270 deg	299.60 deg	0.15
