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RESEARCH ARTICLE

Compass cues used by a nocturnal bull ant, *Myrmecia midas*

Cody A. Freas*, Ajay Narendra and Ken Cheng

ABSTRACT

Ants use both terrestrial landmarks and celestial cues to navigate to and from their nest location. These cues persist even as light levels drop during the twilight/night. Here, we determined the compass cues used by a nocturnal bull ant, *Myrmecia midas*, in which the majority of individuals begin foraging during the evening twilight period. *Myrmecia midas* foragers with vectors of ≤5 m when displaced to unfamiliar locations did not follow the home vector, but instead showed random heading directions. Foragers with larger home vectors (≥10 m) oriented towards the fictive nest, indicating a possible increase in cue strength with vector length. When the ants were displaced locally to create a conflict between the home direction indicated by the path integrator and terrestrial landmarks, foragers oriented using landmark information exclusively and ignored any accumulated home vector regardless of vector length. When the visual landmarks at the local displacement site were blocked, foragers were unable to orient to the nest direction and their heading directions were randomly distributed. *Myrmecia midas* ants typically nest at the base of the tree and some individuals forage on the same tree. Foragers collected on the nest tree during evening twilight were unable to orient towards the nest after small lateral displacements away from the nest. This suggests the possibility of high tree fidelity and an inability to extrapolate landmark compass cues from information collected on the tree and at the nest site to close displacement sites.

KEY WORDS: Ants, Orientation, Landmarks, Path integration, Nocturnal navigation

INTRODUCTION

Animals need an external compass reference to walk in a straight line (Cheung et al., 2007). Solitarily foraging ants derive compass information from terrestrial landmarks and path integration to head toward their goal (e.g. Wehner, 2003; Collett et al., 2006; Collett, 2012; Schultheiss et al., 2016). To obtain compass information from landmarks, ants first acquire visual information around the goal (Nicholson et al., 1999; Narendra et al., 2007; Baddeley et al., 2011; Zeil et al., 2014a; Fleischmann et al., 2016) through a carefully orchestrated series of learning walks that occur in different compass directions around the goal. When returning to the goal, ants move to match their current view to the memorized nest-oriented image to head toward the goal (Wehner et al., 1996; Collett et al., 2001; Graham and Cheng, 2009; Wystrach et al., 2011a,b; Zeil, 2012; Narendra et al., 2013a). Ants also obtain compass information from multiple celestial cues, most notably the pattern of polarized

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skylight derived from the sun (e.g. Zeil et al., 2014b). The polarization information is acquired through a specialized dorsal region of the ant's eyes (e.g. Zeil et al., 2014b; Narendra et al., 2016b) and is processed via polarization-sensitive optic lobe neurons (Schmitt et al., 2015). This directional information is coupled with distance information, which the ant accumulates as it travels away from the nest. To return home, ants integrate these two sources of information and compute the shortest home vector (e.g. Collett and Collett, 2000; Wehner and Srinivasan, 2003).

These vision-based navigational abilities have been widely studied in diurnal ants, which are active when visual cues are easy to distinguish (Wehner et al., 1996; Fukushi, 2001; Beugnon et al., 2005; Cheng et al., 2009; Bühlmann et al., 2011). Nocturnal foragers have the challenge of navigating during twilight or at night when ambient light levels drop significantly and visual cues become increasingly difficult to detect (Warrant, 2008; Warrant and Dacke, 2011). Yet, little is known about navigation in ant species that forage at low light intensities.

The ant genus *Myrmecia* allows for interesting comparisons of navigational behaviour, as animals travel from the nest to forage on nearby *Eucalyptus* trees. Several species within this genus are active in discrete temporal niches, experiencing different light levels and different navigational information (Narendra et al., 2010, 2011; Jayatilaka et al., 2011; Reid et al., 2011, 2013). These ants have adapted remarkably well to their temporal niches, and this is most evident in their visual system, where the eyes of each species and each caste have evolved suitable adaptations for specific light environments (Greiner et al., 2007; Narendra et al., 2010, 2016a). Previous navigational work in this genus has shown that both diurnal and nocturnal *Myrmecia* species depend heavily on terrestrial visual cues. The day-active *Myrmecia croslandi* navigate primarily using landmark information, and typically rely on path integration only when familiar terrestrial cues are absent (Narendra et al., 2013a). The night-active *Myrmecia pyriformis* use both landmarks and celestial cues while navigating between the nest location and nest-specific foraging trees (Reid et al., 2011).

Here, we investigated the compass cues used by a nocturnal bull ant, *Myrmecia midas* Clark 1951. First, we determined their daily activity schedule. Next, we tested whether foragers use a home vector to orient in the absence of familiar landmark information, by displacing foragers to unfamiliar locations. We then asked how ants weight their home vector and terrestrial cues by creating a conflict between the two cue sets. To explore the possibility that foragers navigate using cues beyond the surrounding landmarks, we displaced foragers locally near the nest with the surrounding landmark panorama obscured. Finally, we compared the navigational knowledge of ants when they had to travel different distances (0.3–14.0 m) from the nest to reach their foraging tree.

MATERIALS AND METHODS
Field site and study species
Experiments were conducted from April 2015 to October 2016 on four *M. midas* nests located at the northern end of the Macquarie

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University campus in Sydney, Australia (33°46′11″S, 151°06′40″E). All testing was conducted on nests that were located within a 100 m area (Fig. 1). The area's vegetation consisted of stands of *Eucalyptus* trees with largely barren understories interspersed with grassy areas. *Myrmecia midas* nests were located at or close to the base of a tree (25.6±4.36 cm mean±s.d., *n*=20), and a portion of the nest's nightly foragers (~30%) travelled straight up the nest tree while the remaining foragers travelled to one of the surrounding trees (observations: C.A.F., three *M. midas* nests). We used headlamps with red filters to observe the ants at night, and forager behaviour suggests they were not affected by this light. Working with this ant species requires no animal ethical approval within Australia. All collection and testing procedures were non-invasive and we witnessed no adverse effects either in the tested individuals or in their nests during or after testing. All displacement collection and testing procedures occurred during the evening or morning twilight.

Foraging activity

We carried out a 24 h observation at one *M. midas* nest on 2 April 2016 to establish the species' activity pattern. We set up a 60 cm diameter reference marker around the nest entrance and filmed ants departing and entering the nest with an HD C920 webcam (Logitech International). A lamp covered with a red filter was placed 50 cm away from the nest entrance. Filming was carried out for a 24 h period from 1 h before sunset. Astronomical data were obtained from calculations in the Astronomical Almanac (http://asa.usno.navy.mil).

Orientation in an unfamiliar location

We investigated the use of the home vector by asking whether foragers with a home vector of 2.0 m (nest 1), 2.5 m (nest 2), 4.0 m (nest 3) and 5.0 m (nest 4) were oriented towards their fictive nest when familiar terrestrial cues are unavailable. Full-vector foragers from four nests were collected at the base of their foraging trees and displaced to one of four distant sites, each being 100 m away from the nest site. Displacement sites were chosen to put the true nest direction opposite to the home vector direction and, as such, would be in a 180 deg conflict with the vector cue. Displacement sites were devoid of an overhead tree canopy to allow unobstructed access to the overhead sky. For all displacement tests, collected foragers were tested on a 40 cm diameter goniometer painted on the surface of a

wooden board. The testing surface of the board was raised 5 cm off the ground with plastic legs. The goniometer was segmented into 24 sectors each encompassing 15 deg of the horizontal plane. The wooden board had a 2 cm diameter hole in the centre of the goniometer, which was attached to a 4 cm-long darkened plastic tube underneath the board surface. Foragers were deposited into the tube and allowed to climb up onto the board and exit. This method was employed because *M. midas* has a tendency to immediately run off a board when dropped on the surface. The ant's heading upon release was recorded at 20 cm from the release point. After foragers were tested, they were collected and marked, ensuring each ant was tested only once, and then returned to the nest site.

We displaced full-vector foragers in three conditions. In the first, we collected inbound homing ants at the base of the tree during the morning twilight as they travelled down the foraging tree (inbound 100 m condition). In the second condition, we collected individuals during their nightly outbound trip at the base of the foraging tree, held them for 15 min with a small amount of honey and released them at the distant displacement site (outbound 15 min delay 100 m condition). In the third condition, we collected outbound foragers during the evening twilight, offered them a small amount of honey and stored them overnight (11–12 h). These foragers were tested in the morning twilight (outbound overnight delay 100 m condition). After testing and marking, foragers were returned to the nest site. Foragers' orientations did not differ between nests and results were pooled across all four nests.

Additionally, we determined whether the length of the home vector itself affected the ability to orient using path integration. For this test, we studied a subset of ants from nest 1 and nest 3 that foraged on a tree 14.0 and 12.8 m from the nest entrance, respectively, which were the maximum observed foraging distances at any nest at this site, distances we term long vector. We again collected outbound foragers at the base of the foraging tree, but only during the evening twilight. Foragers were held for 15 min, and given a small amount of honey, then released at the 100 m displacement site (outbound long vector 15 min delay 100 m condition), where we recorded their initial orientations. Tested foragers were marked and returned to the nest site. Foragers' orientations from the two nests were pooled.

The overnight and morning inbound testing conditions were chosen so as to mirror the typical inbound activity patterns of this species, with foragers being released during the pre-dawn twilight when motivation to return to the nest should be high. Our 15 min delay conditions were chosen as this holding period allows time for the forager to feed in the collection tube while still being tested during twilight, as the navigational abilities of nocturnal *Myrmecia* have been shown to suffer after twilight ends (Narendra et al., 2013a,b).

Cue conflict

Next, we asked how ants weight path integration and landmark cues while returning home to the same four nests. We addressed this by capturing ants at the base of their foraging tree under identical collection conditions to the unfamiliar displacements and then displacing them locally. We first tested foragers in a control condition without creating a conflict between the home vector direction and the local landmarks. For this, we released outbound ants during the evening twilight after a 15 min holding period on the goniometer at the base of their foraging tree (outbound 15 min delay on-route condition). We then tested a separate group of foragers with conflicting terrestrial landmarks and the home vector by displacing foragers away from the nest site and perpendicular to the foraging route. The displacement site was 90 deg clockwise from
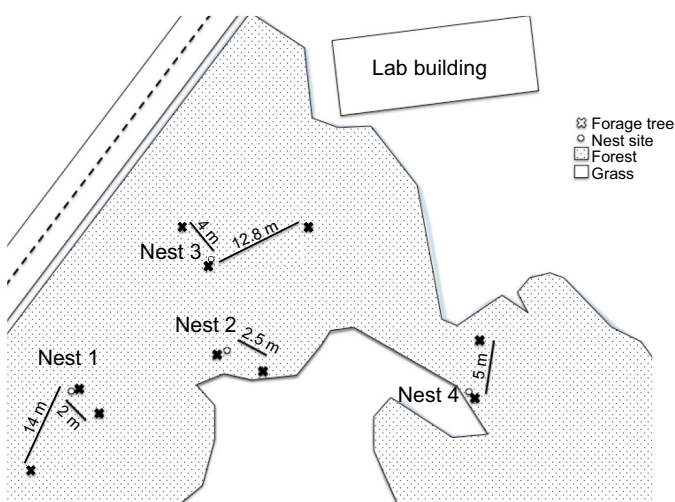

Fig. 1. The study site for the nocturnal ant *Myrmecia midas*. The field site was located on the Macquarie University campus. The four nests were located within 100 m of each other.
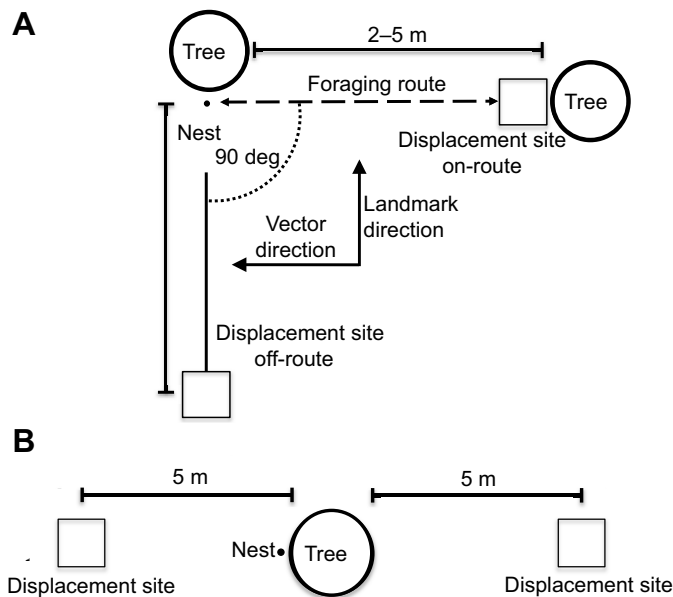
Fig. 2. Schematic diagram of the experimental setup. (A) Diagram of collection and displacement conditions for the experiments on cue conflict. (B) Diagram of collection and displacement conditions for the nest-tree foragers.

the individual's foraging tree with respect to the nest location and 5 m from the nest entrance (Fig. 2A).

Foragers were tested in three separate conditions, similar to the procedures for testing the use of path integration. In the first condition, incoming foragers were collected during the morning twilight at the base of their foraging tree (inbound off-route condition) and then tested. In the second and third conditions, foragers were collected on their outbound trip as they reached one of the foraging trees 2–5 m from the nest; foragers were either held for 15 min (outbound 15 min delay off-route condition) and then tested or held until morning twilight (outbound overnight delay off-route condition) and then tested. At the testing site, under all conditions the forager's initial orientation was recorded using the goniometer at 20 cm. After testing, individuals were marked and returned to the nest entrance. We analysed foragers' initial orientations toward both the true nest direction and the vector from path integration. Orientations were pooled across all four nests.

Again, we determined whether the length of the home vector itself affected the weighting of the home vector. For this test, we studied a subset of ants (n=30) from nest 1 that foraged on a tree 14.0 m from the nest (outbound long vector 15 min delay off-route

condition). We collected outbound foragers at the base of the foraging tree, but only during the evening twilight. Foragers were held for 15 min, given a small amount of honey, and then released at a displacement site 90 deg clockwise from the foraging tree with respect to the nest and 5 m from the nest entrance (Fig. 2A). At the release site, we recorded initial orientations, and then tested foragers were marked and returned to the nest site.

Landmark-blocking experiment
Next, we asked whether foragers successfully orient to the nest direction after local displacement in the absence of familiar terrestrial landmark cues. For this test, we collected outbound foragers at the base of their foraging tree 2–5 m from nest 1, nest 3 or nest 4 during the evening twilight (landmark blocking 15 min delay). Foragers were held for 15 min, offered a small amount of honey, and then released at a displacement site 90 deg clockwise from the foraging tree with respect to the nest and 5 m from the nest entrance (Fig. 2A). At the release location, a 38 cm high plastic sheet was used to block the landmark cues below 50 deg. The plastic sheet was erected into a 55 cm diameter circle around the goniometer. This sheet was raised 5 cm off the ground to allow any potential scent cues to reach foragers at the centre of the goniometer. After releasing the forager onto the goniometer, we recorded the orientation of animals at 20 cm. After testing, individuals were marked and returned to the nest.

Nest-tree foragers
Observations of forager activity at the nest indicate that a portion (∼30%) of *M. midas* foragers do not travel farther than 30 cm to reach their foraging tree. To determine the navigational knowledge of these foragers, we displaced outbound full-vector ants 5 m away from the nest, either directed toward the nest entrance (nest-side condition) or directed toward the tree (tree-side condition; see Fig. 2B). Foragers were collected on their outbound trip as they reached the base of the foraging tree. These individuals were fed, held for 15 min, and then released on the goniometer at one of the displacement sites. Foragers' orientations at 20 cm were determined at nests 1, 2 and 3. After testing, individuals were marked and returned to the nest.

Statistical analysis
For statistical analysis, a minimum of 30 foragers were collected for each condition. In each testing condition, foragers were collected as they were discovered and assigned a condition within that experiment group randomly. Data were analysed with circular statistics (Batschelet, 1981; Zar, 1998) using the statistics package Oriana version 4 (http://www.kovcomp.co.uk/oriana/index.html).
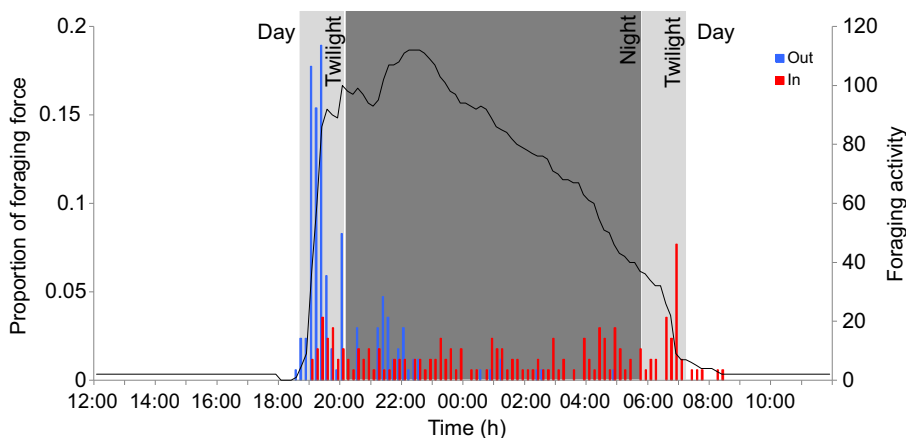

Fig. 3. Nightly foraging activity pattern of *M. midas* foragers. The observation period began on 2 April 2016 with sunset at 18:49 h. Bars indicate outgoing foragers (blue, n=171) and incoming foragers (red, n=169). The black line indicates the number of individuals actively foraging away from the nest site during this period. Data are shown in 10 min bins.

Table 1. Statistical results for all displacement experiments

Condition	n	Mean vector (deg)	95% CI (deg)		Rayleigh test		V-test at 0 deg	
			Minus	Plus	Z	P	V	P
Inbound 100 m	40	301.96	224.14	19.788	1.03	0.36	0.085	0.225
Outbound 15 min delay–100 m	45	205.23	128.61	281.84	1.06	0.35	−0.139	0.906
Outbound overnight delay–100 m	37	309.15	209.78	48.506	0.63	0.54	0.083	0.24
Outbound long vector 15 min delay–100 m	34	354.86	308.49	41.225	2.81	0.059	2.361	0.009
Inbound off-route	45	6.63	352.76	20.511	22.55	<0.0001	0.703	<0.0001
Outbound 15 min delay off-route	40	1.97	345.28	18.662	16.89	<0.0001	0.626	<0.0001
Outbound 15 min delay on-route	42	346.54	313.77	19.304	5.48	0.004	0.351	<0.0001
Outbound overnight delay off-route	41	6.05	345.78	26.325	12.72	<0.0001	0.554	<0.0001
Outbound long vector 15 min delay off-route	30	5.40	344.78	26.02	11.54	<0.0001	0.618	<0.0001
Landmark blocking 15 min delay	32	244.70	102.68	26.73	0.311	0.736	−0.04	0.631
Nest-tree foragers – tree-side	40	119.56	324.75	174.368	0.262	0.771	−0.04	0.101
Nest-tree foragers – nest-side	40	356.38	269.29	83.474	0.823	0.442	0.143	0.639

n, number of ants; CI, confidence interval.

Watson and Wheeler F-tests were used to compare mean vectors between testing conditions. A Rayleigh's test was conducted on each displacement condition, to test whether the data met the conditions of a uniform distribution ($P>0.05$). We used a V-test, with α set at $P=0.05$, to determine whether the initial orientation of ants at the release site was significantly towards the nest direction or to the home vector. During displacement tests where foragers did not orient to the nest direction, V-tests were conducted across all 15 deg sections of the goniometer to test whether foragers were oriented to directions other than the nest. All data are available upon request.

RESULTS
Foraging activity
The onset of foraging activity occurred just before sunset, with most foragers heading out from the nest in the evening twilight (Fig. 3). A small number of foragers left the nest after twilight ended. Foragers began returning during the evening twilight, with some individuals carrying insect prey. Foragers returned throughout the night, and a burst of incoming foragers arrived at the nest during the morning twilight and just after sunrise. Activity was greatly reduced after this burst, and activity ceased completely after 1–2 h from sunrise.

Orientation in an unfamiliar location
Ants released on the goniometer would initially remain motionless while within the tube. After emerging from the tube and onto the wooden goniometer board, ants typically scanned the environment along the horizontal axis and then headed off in a chosen direction. The nest-tree distance varied between nests (Fig. 1; nest 1, 2 m; nest 2, 2.5 m; nest 3, 4 m; nest 4, 5 m), and we found no difference between nests in forager orientation to a home vector.

Ants with home vectors ranging from 2 to 5 m when displaced to distant locations did not successfully orient in the direction indicated by their path integrator under any collection condition. Foragers' initial orientations met conditions of a uniform distribution and they were not significantly oriented in the direction of their home vector at 0 deg (Fig. 4A–C, Table 1). Foragers from all three conditions were not oriented in any direction on the goniometer, even when using V-tests ($P>0.05$, orientation data binned at 15 deg). Further observations of individuals with 5 m vectors after distant displacement indicate that they do not continue in this initial direction but loop back to the release point, a behaviour indicative of systematic search.

In contrast, ants that travelled long distances to their nest-specific tree (nest 1 and 3) and were then displaced to the distant site within

15 min of capture were significantly orientated toward their home vector and away from the true nest location when analysed using a V-test. Yet, forager orientations also met the conditions of a uniform distribution when analysed using a Rayleigh's test, indicating random initial headings and no orientation. As results from nest 1 and nest 3 did not differ (V-test, $P<0.05$; Rayleigh's test, $P>0.05$) and had similar mean vectors (Watson and Wheeler F-test, $P>0.05$), they were pooled (Table 1, Fig. 4D). At the end of each experiment, foragers were returned to the nest vicinity, and all foragers immediately searched for and entered the nest.

Cue conflict
For local on- and off-route displacement tests, full-vector ants were displaced either back at their foraging tree or off-route (Fig. 2A). Outbound forager orientations when displaced back on-route after 15 min were non-uniform and were directed towards the nest (outbound 15 min delay on-route condition; Fig. 5A, Table 1). The orientation of full-vector ants, displaced (5 m off-route) to create a conflict between the vector and local landmark cues, was non-uniform. These ants were significantly oriented toward the nest (Fig. 5B–D) and not toward the home vector at 270 deg (inbound off-route condition, V-test, $V=0.776$, $P=0.22$; outbound 15 min delay off-route condition, V-test, $V=0.844$, $P=0.34$; outbound overnight delay off-route condition, V-test, $V=−0.771$, $P=0.779$). In all local displacement conditions, mean vector did not significantly differ between conditions (Watson and Wheeler F-test, $P>0.05$).

The orientation of long-vector foragers, accumulating 14 m vectors to their nest-specific tree (nest 1), then displaced locally with a 90 deg cue conflict within 15 min of collection (outbound long vector 15 min delay off-route condition) was also significantly non-uniform and oriented toward the true nest direction and not the vector at 270 deg (Table 1, Fig. 5E). Different holding durations did not have an effect on the heading directions in all off-route conditions (Watson and Wheeler F-test; $P>0.05$).

Landmark-blocking experiment
The initial orientation of foragers displaced locally without access to familiar landmark cues within 15 min of collection met the conditions of a uniform distribution, and ants were not significantly oriented in the direction of their nest at 0 deg (Table 1, Fig. 5F).

Nest-tree foragers
Full-vector ants, when displaced 5 m either side of the nest (Fig. 2B), did not orient significantly toward the nest direction.
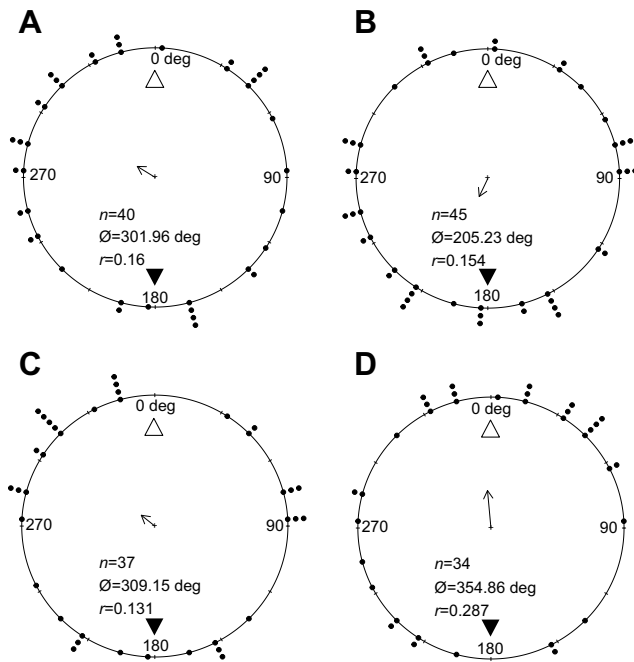
Fig. 4. Circular histograms of initial headings of individual _M. midas_ foragers at 20 cm in unfamiliar location experiments. Nest direction indicated by path integration was at 0 deg and the actual nest site direction was at 180 deg. Histograms show orientation data in 15 deg bins. The arrow in each histogram denotes the length of the mean vector and the direction of the average orientation. The filled triangle in each histogram indicates the true nest direction and the open triangle indicates the vector direction. (A) Foragers were collected during their morning inbound trip and displaced to a site 100 m away (inbound 100 m condition; _V_-test at 0 deg; _V_=0.085, _P_=0.225). (B) Foragers were collected during the outbound trip, at the base of the foraging tree, 2–5 m from the nest entrance, held for 15 min and then released 100 m from the nest site (outbound 15 min delay 100 m condition; _V_-test at 0 deg; _V_=−0.139, _P_=0.906). (C) Foragers were collected during their outbound trip, held for 12 h, and displaced to a site 100 m away (outbound overnight delay 100 m condition; _V_-test at 0 deg; _V_=0.083; _P_=0.240). (D) Foragers were collected during the outbound trip with the longest observed vectors (12.8–14.0 m), held for 15 min and displaced to a site 100 m away (outbound long vector 15 min delay condition; _V_-test at 0 deg; _V_=2.361, _P_=0.009). _n_, number of individuals in each condition; Ø, mean vector; _r_, length of mean vector.

Their initial orientation had a uniform distribution at both displacement sites (nest-side condition, Fig. 6A; tree-side condition, Fig. 6B; Table 1). Foragers from both conditions were not oriented in any direction or toward any other tree (_V_-tests, _P_>0.05). At the end of the experiment, foragers were returned to the nest vicinity, and all foragers immediately searched for and entered the nest.

DISCUSSION

In the current study, we show that nocturnal ants with short home vectors of ≤5 m when displaced to distant unfamiliar locations did not orient toward the fictive nest. But ants with longer home vectors of 12.8–14.0 m weakly oriented toward the fictive nest, suggesting vector length influences cue strength. Additionally, when foragers were displaced locally with conflicting vector and landmark cues, they oriented exclusively using landmark cues and ignored any potential home vector regardless of vector length. When the landmark panorama (up to 50 deg elevation) around the displacement site was obscured, foragers could no longer orient to the nest direction, suggesting terrestrial cues are critical to homeward orientation. Finally, we found that the nest-tree forager
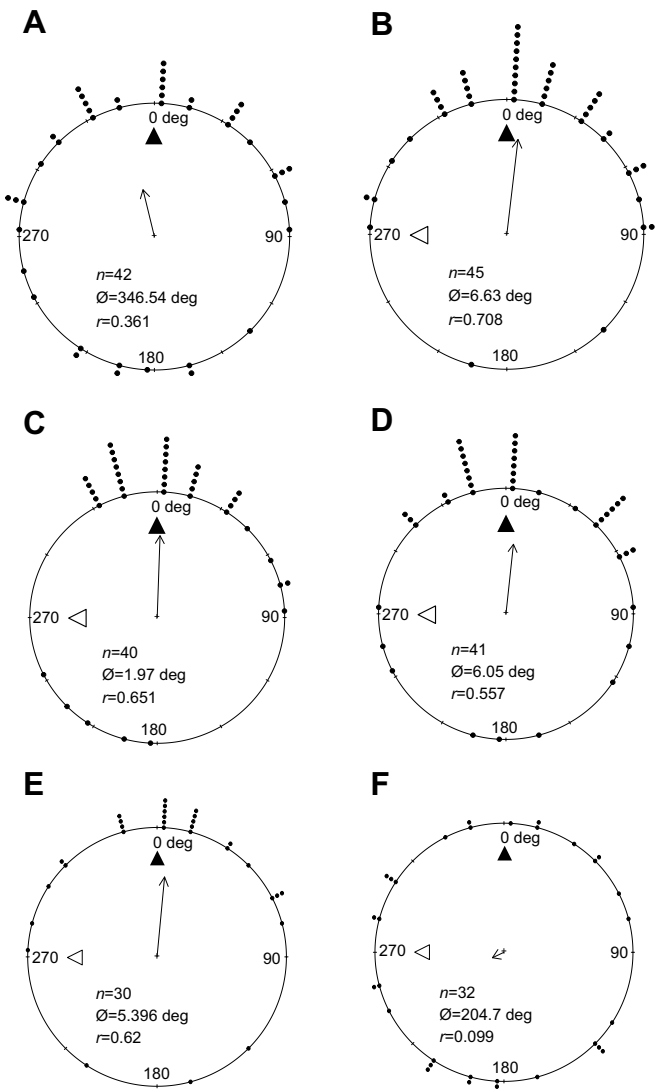

Fig. 5. Circular histograms of initial headings of individual _M. midas_ foragers at 20 cm in local displacement experiments. Nest direction indicated by the home vector was at 270 deg and the actual nest site direction was at 0 deg. Histograms show orientation data in 15 deg bins. The arrow denotes the length of the mean vector direction. In all off-route conditions, the filled triangle indicates the true nest direction and the open triangle indicates the vector direction. (A) Outbound foragers were collected at the base of the foraging tree, held for 15 min and then released at the base of the tree on their foraging route (outbound 15 min delay on-route condition; _V_-test at 0 deg; _V_=0.351, _P_<0.001). (B) Inbound foragers were collected during their morning inbound trip and displaced to a site 5 m away (inbound off-route condition; _V_-test at 0 deg; _V_=0.703, _P_<0.001). (C) Outbound foragers were collected at the base of the foraging tree 2–5 m from the nest entrance, held for 15 min and then released at the 5 m displacement site (outbound 15 min delay off-route condition; _V_-test at 0 deg; _V_=0.580, _P_<0.001). (D) Foragers were collected during their outbound trip, held for 12 h and then displaced to a site 5 m away (outbound overnight delay off-route condition; _V_-test at 0 deg; _V_=0.554, _P_<0.001). (E) Outbound foragers were collected at the base of their foraging tree 14 m from the nest entrance, held for 15 min and then released at the 5 m displacement site (outbound long vector 15 min delay off-route condition; _V_-test at 0 deg; _V_=0.618, _P_<0.001). Nest direction indicated by path integration was at 270 deg and is marked with an open triangle. (F) Outbound foragers were collected at the base of their foraging tree 2–5 m from the nest entrance, held for 15 min and then released at the 5 m displacement site with the surrounding landmarks blocked from the forager's view (landmark blocking 15 min delay condition; _V_-test at 0 deg; _V_=−0.04, _P_=0.631).
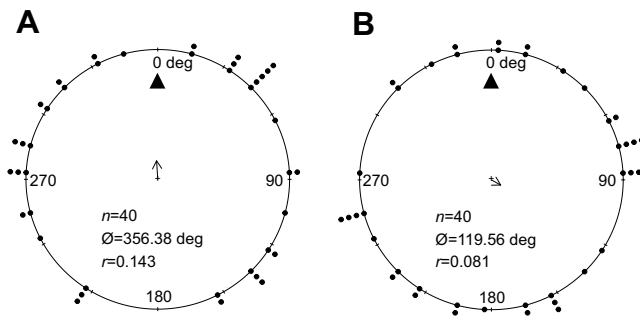
Fig. 6. Circular histograms of initial headings of individual *M. midas* foragers at 20 cm in nest-tree experiments. Nest location for both conditions was set at 0 deg. Histograms show orientation data in 15 deg bins. The arrow denotes the length of the mean vector direction and the filled triangle designates the true nest direction. Outbound foragers travelling up the nest-tree were collected at the tree base and displaced 5 m from the nest site either (A) on the nest side of the tree (nest-side condition; *V*-test at 0 deg; *V*=−0.04, *P*=0.101) or (B) with the tree in between the nest site and the displacement site (tree-side condition; *V*-test at 0 deg; *V*=0.143, *P*=0.639).

subset did not orient to local landmark cues when displaced, and these animals appear to have reduced navigational knowledge compared with their nest mates that travel away from the nest to forage.

In our distant displacement tests, full-vector ants did not orient toward the fictive nest when displaced to an unfamiliar site and showed a random distribution of heading directions indicative of search behaviour (Müller and Wehner, 1994; Schultheiss and Cheng, 2011; Schultheiss et al., 2015). Such a distribution was present across all three conditions when the foragers were tested with home vectors ≤5 m, representing typical foraging distances for this site. Continued observations of foragers with ≤5 m vectors displaced to distant locations suggest that foragers begin systematic searching behaviour. The lack of any significant orientation in these short-vector groups suggests that these individuals either were not path integrating, suppressing the homeward vector when presented with unfamiliar landmarks, or do not accumulate sufficiently reliable vectors at foraging distances of 5 m or less. Motivation is unlikely to be the reason, especially in the groups released in the morning twilight when foragers would typically be returning down the foraging tree to the nest. Additionally, after testing, when foragers were returned to the area around the nest, they willingly entered the nest, suggesting nest-ward motivation. Lack of motivation would also seem to conflict with our local displacement results with identical collection procedures where individuals oriented correctly towards the nest site using exclusively landmark information (see below).

We also tested foragers with the maximum accumulating vectors we could find at the field site (12.8–14.0 m), as the weighting of vector cues when in directional conflict with familiar terrestrial cues has been shown to increase with vector length, as a result of reduced directional uncertainty (Wystrach et al., 2015). Thus, foragers travelling to trees farther away may accumulate sufficiently strong or sufficiently reliable vectors to orient in unfamiliar locations. When displaced to a distant site, these individuals appeared to orient toward the home vector and away from the true nest location, but the strength of the orientation was weak (Fig. 5D), statistically showing both orientation to the vector and a uniform distribution of headings. This statistical conflict may be indicative of the liberality of *V*-tests as a statistical test, returning many significant results. Weak orientation away from the true nest direction and to the vector direction suggests that path integration is in use during inbound

segments of the foraging journey, but at smaller distances, the home vector may be suppressed by dominant landmark cues or may be insufficiently reliable for orientation. It is also possible that the great majority of foragers at this field site, which make short (≤5 m) foraging trips, are not path integrating during foraging. Alternatively, long-vector forager orientations could be the result of foragers orienting to other directional cues such as the wind direction, shown to be a directional component of path integration in desert ants (Müller and Wehner, 2007; Wehner et al., 2016), or distant landmark cues that do not update with large displacements (see Fig. S1). It is currently unknown whether (and if so what) celestial cues are used by *M. midas* foragers during vector-based orientation, but previous work in other nocturnal ant species suggests the use of the polarization pattern in the sky (Reid et al., 2011) and the position of the moon (Klotz and Reid, 1993; Reid et al., 2013). The potential use of these cues in this ant species warrants further study.

Often, when ants have access to both a home vector and terrestrial landmark compass cues, the terrestrial landmark information suppresses information from the path integrator (Wehner et al., 1996; Narendra et al., 2007a,b). However, when these cues are in conflict with each other, some ant species in some conditions choose a direction that is a compromise between the information dictated by the terrestrial cues and the path integrator (Narendra, 2007a,b; Collett, 2010; Legge et al., 2014; Wystrach et al., 2015; Wehner et al., 2016). Under the testing conditions in which *M. midas* foragers were displaced to an off-route site 5 m from the nest, either during the morning inbound section of the foraging trip or after a holding period of 15 min or overnight, individuals oriented toward the terrestrial cue direction and showed no effect of the conflicting vector even when tested with the largest (14 m) vector observed at the site (Fig. 6A). These results are consistent with the behaviour of foragers released back at the foraging tree after 15 min with no conflicting vector, as these foragers were also oriented correctly to the nest. Additionally, when the terrestrial landmark panorama around the forager was blocked, individuals were no longer able to orient to the correct homeward direction after local displacement. These results suggest that terrestrial visual cues are critical to successful orientation in foragers of this species and that other potential directional cues, such as scent or vibration, are unlikely to be sufficient to allow successful orientation. Furthermore, the results of the landmark-blocking experiment suggest that the critical terrestrial cues are under 50 deg elevation and that potential landmark cues above this elevation, such as the overhead canopy (Hölldobler, 1980; Reid et al., 2011), are not used for orientation in *M. midas*. Our results indicate that within a local area around the nest, foragers are predominantly directed towards the nest site by terrestrial landmark cues when these cues are present. From the initial orientations, it appears that in a familiar environment, home vector information was suppressed by the terrestrial cues present at the displacement site for each nest.

Our final testing conditions concerned the subset of the nest's worker force that forages on the nest-tree. Individuals displaced while climbing these trees <30 cm from the nest entrance did not orient toward the nest site when displaced 5 m away. The uniform nature of the initial headings indicated searching behaviour similar to that of foragers displaced to unfamiliar locations. These results correspond with unpublished observations (observations: C.A.F.) of *M. midas* released in unfamiliar territory, where individuals perform looping search paths heading away from and then returning back to the release point. The random distribution of initial headings was observed regardless of the position of the displacement site. As with

the lack of orientation in the initial distant displacements, it is not possible to know with certainty the nest-ward motivation for these individuals as they may be hunting for insect prey after consuming the honey. Again, we believe this is unlikely, as foragers displaced locally off-route after 15 min and individuals displaced distantly with 12.8–14.0 m vectors appear to be motivated to return home. Additionally, once foragers were returned to the nest vicinity, they immediately searched for the entrance and voluntarily entered. Our conclusion is that these individuals have reduced navigational knowledge when compared with foragers travelling away from the nest site. Observations of all *M. midas* foragers over multiple nights suggest a high degree of tree fidelity for foragers, with marked tested individuals travelling to the same tree each night, although no long-term study of forager tree fidelity has been conducted. Because of this foraging consistency, some foragers may be ecologically restricted to the horizontal area immediately around the nest entrance and thus have never experienced large changes in the landmark panorama, resulting in reduced visual knowledge of the area surrounding the nest. These results suggest nest-tree foragers may show similarities to non-foraging digger ants in *Cataglyphis bicolor*, which do not leave the nest area and show reduced navigational knowledge compared with foragers displaced locally (Wehner and Menzel, 1969). These nest-tree foragers may be unable to extrapolate views of the panorama around the nest without having experienced the displacement site, even if the landmark panorama is very similar, and may need to accumulate views between the nest site and any site away from the nest on an outbound path to be able to accurately orient toward the nest from that site. This theory is supported by our unpublished experiments where nest-tree foragers correctly orient while vertical on their foraging tree. This navigational limitation would support a recognition-triggered response model for navigation in these foragers. The recognition-triggered response model predicts that nest-tree foragers with no previous experience away from the nest site, either during previous foraging trips or on the outbound segment of the current foraging trip, should be unable to successfully orient home (Gaussier et al., 2000; Graham et al., 2010). It is currently unknown whether these individuals spend their whole lifetime foraging at this tree or whether they switch to another foraging tree. Nest-tree foragers may be younger foragers that switch to foraging trees farther away from the nest as they age, or these foragers may be restricted to the nest tree for their whole lifetime. Further investigation into this species' foraging ecology and this subset of foragers is warranted.

Conclusions

Myrmecia midas appear to detect and use both path integration and the surrounding terrestrial landmarks to navigate at low light levels during twilight. Our results suggest that vector cue strength is weighted by vector length, as only at the longest observed vector lengths did foragers orient successfully in unfamiliar surroundings. The lack of orientation to smaller (2–5 m) home vectors in the presence of unfamiliar terrestrial cues and the very weak orientation at the maximum observed natural vector (12.8–14.0 m) coupled with the lack of orientation to all vector lengths (2–14 m) when in the presence of conflicting familiar terrestrial cues indicates that vector cues may be weakly integrated during orientation and can be overridden by stronger terrestrial cues or that these foragers do not path integrate over short distances. Additionally, terrestrial visual cues appear critical to successful homeward orientation, as blocking these cues in local environments results in foragers no longer being oriented to the nest direction. Finally, our results show that a portion of *M. midas*' foraging force lacks the ability to navigate back to the nest site using landmark cues over short distances, and that these ants may be unable to extrapolate beyond the nest site panorama even over short distances if they have only experienced the terrestrial panorama immediately surrounding the nest site.

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